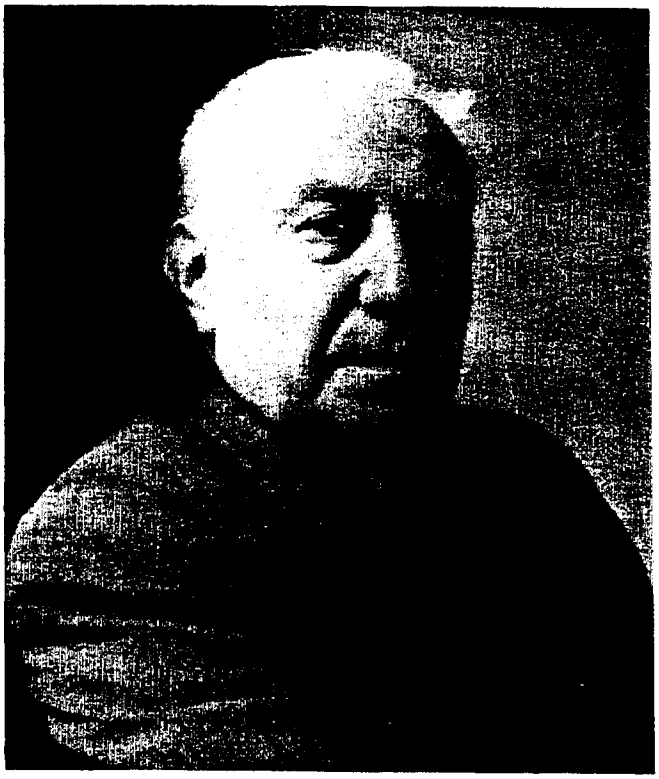
UNIT V.
BLOOD GROUPS

Karl Landsteiner (1869–1943)
Courtesy National Library of Medicine



Ludwig Hirszfeld (1884–1954)
Courtesy Col. Frank R. Camp, Jr. and Army Medical Research Laboratory, Fort Knox, KY

Fritz Schiff (1889–1940)
Courtesy Col. Frank R. Camp, Jr., and Army Medical Research Laboratory, Fort Knox, KY

Alexander S. Wiener (1907–1976)
Courtesy National Library of Medicine

Prof. Leone Lattes (1887-1954)
Courtesy Edizioni Minerva Medica and National Library of Medicine

Prof. Dr. Otto Prokop (1921-)
Courtesy Vergesellschaft Otto Spatz and National Library of Medicine

Dr. Ruth Sanger (1918-) and Dr. R. R. Race (1907-)
Courtesy Col. Frank R. Camp, Jr., Dr. R. R. Race and National Library of Medicine

SECTION 18. INTRODUCTION TO THE FORENSIC APPLICATIONS OF GENETIC MARKER SYSTEMS TO IDENTIFICATION AND DISPUTED PARENTAGE PROBLEMS

There are two major application areas of forensic or medicolegal serology and biochemistry: (1) disputed parentage testing; and (2) identification testing of bloodstains and secretions, usually in criminal matters. Although both these areas make use of the same genetic marker systems, they tend to be separate subspecialties in practice. It is exceptional, at least in this country, to find laboratories engaged in both kinds of testing. In parentage and affiliation testing applications, any genetic marker system for which the mode of inheritance has been firmly established can be employed, at least in theory. In blood and secretion stain analysis, there is an additional dimension: the blood or body fluid materials in the stains or spots must be identified before genetic markers and typed. Analysis, therefore, consists of identification tests followed by individualization tests (genetic marker system typing). Typing of the various systems in dried blood and secretion materials, and in post mortem tissues and fluids, presents problems that are not encountered with freshly taken samples. This book was prepared with stain analysis work foremost in mind, and it is organized accordingly. Units II through IV have discussed identification issues. Units V through VIII are devoted to individualizing markers. Emphasis has been given to blood and body fluid stain analysis, but parentage testing applications are discussed in connection with the various systems. In this section, the general principles and considerations involved in parentage testing and in stain identification analysis are discussed briefly as an overall introduction to the units on genetic marker systems. Genetic marker systems may be divided into five major classes: blood groups, isoenzymes, serum group systems, hemoglobin, and HLA. They are discussed in the units which follow. A sixth class that would include "other" genetic markers, such as the polymorphic proteins of saliva and hair, could be added for completeness.

Professor Dodd, in her presidential address to the British Academy of Forensic Sciences (Dodd, 1980), has given an excellent and thoroughly readable overview of both the civil and criminal aspects of forensic serology.

18.1 Disputed Parentage Testing

Disputed parentage cases usually involve disputed paternity. The mother is assumed to be the genetic mother in these cases.The legal issue is usually the support of a dependent child. Disputed affiliation cases can also arise in connection with immigration matters and citizenship claims, inadvertent baby mix-ups, kidnapping, disputes over the inheritance of money or property, and divorce cases.

In theory, any genetic marker system for which the inheritance patterns are straightforward and well established can be employed in disputed affiliation cases. It must also be clear that the genetic marker system is expressed in the child according to its own genotype. Probably over 60 systems could be listed as potentially applicable at the present time (Chakraborty *et al.*, 1974; Lee, 1975; Joint AMA-ABA Guidelines, 1976). As a practical matter, far fewer systems are normally used in casework. Some systems are much more informative than others. Some require reagents which are not widely available. Others requre special equipment or special training and expertise which every laboratory does not have. Cost is a consideration as well, since it must usually be borne by the defendant. It is clear that laboratories must be highly skilled in the typing of all the systems they use in casework, and such skill is usually developed through a combination of background knowledge and considerable practical experience. Lee (1975) reviewed the status of parentage testing, and gives comparisons of the various combinations of systems used in different countries. Polesky and Krause (1977) discussed the capabilites of American laboratories.

Genetic marker systems employed in disputed parentage tests must follow well known and established rules of inheritance. Thus, a child cannot have a gene which both parents lack, and must inherit one of a pair of chromosomes from each parent (if the system is controlled by a single locus, the chromosome will carry one allele; if the system is controlled by a series of linked loci, the chromosome will carry a haplotype). Further, a child cannot have a pair of genes unless both parents have the gene, and a child must inherit a genetic marker allele for which either parent is homozygous. Apparent violations of these rules are the basis for exclusions of parentage. Apparent violations of the first two rules, i.e. (1) a child has an allele which is absent in both parents, or (2) in a multiple allelic system, a child lacks both alleles which are found in the alleged father, are called direct exclusions (or "first order" or "primary" exclusions). With some extremely rare exceptions, these can be accepted with great confidence. Such exclusions are based upon the presence or absence of genetic markers demonstrable by direct examination. Apparent violations of the second two rules, i.e., (1) a child is homozygous for a marker allele which is not present in both parents, or (2) a child lacks a marker for which the alleged father is homozygous, are known as indirect exclusions (or "second order" or "secondary" exclusions). They are based on the inference of homozygosity detected by a negative reaction in a particular test. These exclusions are interpreted with great caution, particularly if

there is a single second order exclusion (one system). Each system has its own rarities and peculiarities, and one must be familiar with them and take them into consideration when interpreting the results of a parentage investigation.

The probability (PE) that a particular system will exclude a falsely accused father *a priori* can be calculated, and depends on the gene frequencies of the particular system in the population of interest. For most systems, the PE can be calculated quite easily (see Walker, in AABB, 1978). For some of the more complex systems, like Rh, Gm and HLA, the calculations are correspondingly more complicated. Tables of values have been published for many systems, however, for easy reference (e.g. AABB, 1978). The accuracy of the PE value depends on the accuracy of the gene frequencies used to perform the calculation. The cumulative probability of exclusion (CPE) for a series of genetic marker systems can be computed from the individual PE values according to: $CPE = 1 - (1 - P_1)\ (1 - P_2)\ (1 - P_3)\ \ldots\ldots (1 - P_n)$, where P_1 is PE for the first sysem, P_2 is PE for the second, etc., and P_n is PE for the n^{th} system, and where n systems have been used. A list of genetic marker systems that are applicable in forensic serology is shown in Table 18.1. The table shows the approximate PE for each system for Black and White populations. To the extent that data were available, the values are applicable to most U.S. populations. HLA (section 46) is the most powerful system by far. The CPE for all the systems in Table 18.1 would exceed 99%.

If a panel of systems has been employed for paternity testing, and no exclusions have been found, it may be of interest to calculate the probability of paternity. This value can be calculated by a number of different methods, some quite simple and some quite complicated (see Walker in AABB, 1978). The value will depend on which systems were used in the tests, and genotypic and phenotypic distributions. In some countries, this information is routinely calculated. In this county, the rules of evidence vary in different jurisdictions. In some states, blood grouping evidence is inadmissable in paternity cases unless it is exclusionary. In other states, courts will admit inclusionary evidence and estimates of the probability of paternity. Many genetic marker systems have rare alleles. These are seldom encountered, but can be very informative in parentage cases if found in a child and a putative parent. The incidence of rare alleles at 43 enzyme loci was discussed by Harris *et al.* (1974).

Sometimes, special situations arise that call for somewhat different approaches. PE values for cases involving relatives were discussed by Salmon and Brocteur (1978). Asano *et al.* (1978a and 1978b) discussed procedures and interpretation when all the parties to the case are not available for testing (e.g. deceased putative father).

Dodd and Lincoln (1978 and 1979) presented results on over 1,500 cases of disputed parentage investigated by blood grouping.

18.2 Blood and Body Fluid Stain Individualization

In criminal cases, individualizing genetic marker systems are used for comparisons of stains or spots with the known blood or body fluids of people known or thought to be involved. Firm conclusions can be drawn only in cases of nonidentity, that is, when the person suspected of having deposited the stain is excluded because he or she lacks a genetic marker found in the questioned material.

If samples are compared and found to be identical in one or more systems, it means only that the person tested is included in the subset of the population having the particular set of types found. If the frequencies of occurence of the types for the systems employed are known for the population in question, then the expected frequency of occurrence of the type or set of types can be calculated, and may be informative. The figure obtained in these calculations depends on the gene frequencies for the systems. The less accurate the gene frequency estimates, the less accurate will be the resulting estimate of frequency of occurrence of certain types. In general, the gene frequency estimate improves as more and more people are typed. In a population as heterogeneous as in the United States, it is sometimes difficult to decide how the "population in question" should be defined.

The ability of a genetic marker system to distinguish between individuals in a population is clearly related to how well the types are distributed. The probabilities of identity and discrimination can be calculated for genetic marker systems, and this subject was discussed in section 1.2.8. The principles are also covered in Selvin *et al.* (1979). In Table 18.1 is shown the DI for most of the systems. DI is a measure of the power, or value, of a system in distinguishing between individuals selected at random from a population. Comparisons of DI, and combined DI values for several systems, can help examiners and laboratories in choosing the panel of systems they will use in particular cases or in all cases. There are other important considerations in choosing systems as well.

In general, fewer systems can be used in stain analysis that in the typing of fresh materials. Techniques for typing stains are not worked out for every system. There are perhaps 20 systems that could theoretically be used in stain analysis. It is unlikely that so many systems would ever be applicable at a practical level because of the many other constraints imposed by casework samples. In many cases, the age of the stains is a problem. In others, the sample size is very limited. There are cost considerations (in comparison with the benefit to be derived) in adding new systems to the routine analysis scheme. In individual laboratories, resources may be rather scarce, and this will be a decisive factor as well. Nonetheless, a greater degree of individualization of stains is possible now than ever before. As new systems are added to the list of those applicable to this kind of work, the situation promises to improve even further in the years ahead.

Table 18.1 Genetic Marker Systems in Human Blood

System	Number of Common Phenotypes	Discrimination index		Reference(s) to Typing in Bloodstains	Probability of Exclusion of a Falsely Accused Father		Reference(s) to Typing in Parentage Cases
		Caucasian	Black		Caucasian	Black	
Blood Groups				See in Unit V			See in Unit V
ABO	4 (Note 1)	0.62	0.64	See Section 18	0.13	0.17	Wiener, 1943
Rh	7 (Note 2)	0.79	0.89	Bergagna and Pereira, 1967; Lincoln and Dodd, 1968b; Martin, 1977	0.27	0.18	Wiener and Wexler, 1958
MNSs	9	0.83	0.78	Pereira, 1963a; Fiori et al., 1963; Lincoln and Dodd, 1968b and 1975a,b	0.31	0.32	Sussman, 1976
Kell	2 (Note 3)	0.15	0.04	Douglas and Stavely, 1969; Lincoln and Dodd, 1975a,b	0.03	0.005	Boorman et al., 1977
Duffy	3 (Caucasian) 4 (Black) (Note 4)	0.62	0.56	Lincoln and Dodd, 1975a,b	0.18	0.04	AABB, 1978
Kidd	3	0.61	0.55	Lincoln and Dodd, 1975a,b	0.18	0.15	
Red Cell Isozymes				See in Unit VI			See in Unit VI
PGM	3 (Note 5)	0.52	0.47	Culliford, 1967; Berk et al., 1976; Sutton and Burgess, 1976	0.15	0.12	Monn, 1968c; Herbich and Pessendorfer, 1969
ACP	6 (Note 6)	0.66	0.54	Brinkmann, 1971; Wraxall and Emes, 1976	0.22	0.15	Fuhrmann and Lichte, 1966; Fiedler, 1967; Speiser and Pausch, 1967
AK	2 (Note 7)	0.13	0.04	Culliford and Wraxall, 1968	0.04	0.007	Prokop and Göhler, 1976; Dykes and Polesky, 1976; Boorman et al., 1977
ADA	2 (Note 8)	0.18	0.10	Culliford, 1971; Brinkmann and Dirke, 1971	0.05	0.03	Wust, 1971; Bauer and Herbich, 1972
ESD	3	0.35	0.27	Blake and Sensabaugh, 1974 and 1975; Parkin and Adams, 1976	0.08	0.08	Prokop and Göhler, 1976; Dykes and Polesky, 1977
GLO	3	0.61	0.62	Wraxall and Stolorow, 1976; MPFSL, 1978	0.18	0.16	Brinkmann and Püschel, 1978; Jakliński and Koziol, 1979
CA_{II}	3 (Black) (Note 9)	—	0.33	Hughes, 1978	—	0.09	—
Gd	See Section 33.1			Culliford, 1971	—	—	—
PGD	2	0.09	0.13	Culliford, 1971; Brinkmann, 1971	0.01	0.03	Brinkmann, 1971; Dykes and Polesky, 1976
GPT	3	0.53	0.48	Welch, 1972b	0.18	0.13	Radam and Strauch, 1972; Welch and Dodd, 1974
PEPA	3 (Black) (Note 10)	—	0.21	Culliford, 1971; MPFSL, 1976; Parkin, 1978	—	0.06	—
GALT	3 (Note 11)	—	—	—	0.068	—	Eriksen and Dissing, 1980
Serum Groups				See in Unit VII			See in Unit VII
Gm and Km	Many (Note 12)	—	—	Planques et al., 1961; Görtz, 1969; Nielsen and Henningsen, 1962; Kipps, 1978	0.27	0.39	Ellis et al., 1973; Sebring et al. 1979; van Loghem and Nijenhuis, 1979
Hp	3 (Note 13)	0.62	0.72	Culliford, 1971; Stolorow and Wraxall, 1976; Blake and Sensabaugh, 1978b	0.18	0.15	Galatius-Jensen (1962); Giblett (1963); Dykes and Polesky, 1976
Gc	3 (Note 14)	0.57	0.33	Nerstrom and Skarte Jensen (1963); Tumanov and Il'ina (1974); Wraxall and Stolorow, 1976; Zajac and Grunbaum, 1978	0.16	0.08	Chakraborty et al., 1974; Dykes and Polesky, 1976; Hirschfeld and Helken, (1963); Bütler et al., (1963); Holzhausen et al., (1964); Reinskou, (1966b); Bütler, Kühni, et al., 1978; Hoste, 1979
Tf	See Section 42				0.01 (Note 15)	0.05	Mauff et al., 1975; Dykes and Polesky, 1976; Thymann, 1978; Hoste, 1979
Pi	4-6 (Note 16)	0.36	0.06	—	—	—	—
Other							
Hemoglobin (Hb)	See Section 38	—	0.2	Pollack et al., 1968; Huntsman and Lehmann, 1962; Culliford, 1964 and 1971; Wiggins, 1976	—	0.04	Chakraborty et al., 1974
HLA	Many	—	—	Newall, 1978; Hodge et al., 1979	0.8	0.8	AABB, 1976; Terasaki et al., 1978

1. 6 if A_1 and A_2 subgroups are distinguished.
2. There are many less common phenotypes.
3. Three phenotypes can be distinguished with anti-K and anti-k, but KK individuals are rare; a number of further phenotypes can be distinguished if anti-Kp^a, anti-Kp^b, anti-Js^a and/or anti-Js^b are used.
4. Fy (a-b-) is common in the Black population, but exceedingly rare in Caucasians.
5. Three phenotypes, determined by two alleles, are detected by common electrophoretic methods; ten phenotypes, determined by four alleles, are detected by isoelectric focusing. The DI is substantially increased if the subtyping is done and so is the theoretical PE in parentage cases. DI for Europeans would be about 0.77.
6. The C phenotype is rare, as are the RA and RB types.
7. AK 2 is comparatively rare.
8. ADA 2 is comparatively rare.
9. CA_{II} is not polymorphic in Caucasians.
10. PEPA is not significantly polymorphic in Caucasians.
11. Heterozygotes for 'Duarte' and 'Los Angeles' can be distinguished; some workers consider $GALT^D$ and $GALT^{LA}$ together as "$GALT^2$" as was done in the paternity study cited.
12. The number of phenotypes which can be distinguished depends upon which antisera are available for use; antisera for many of the specificities are very rare.
13. A modified kind of Hp 2-1, called Hp 1-2M, occurs relatively frequently in Black populations. Hp 0 occurs in Black populations as well. See in Section 40.
14. The DI is significantly increased by Gc' subtyping (section 41.3); the PE for a falsely accused European father is also significantly increased (section 41.6.1).
15. The PE for a falsely accused European father is increased to 0.13-0.15 by Tf C subtyping.
16. There are at least 12 phenotypes of Pi, but most are relatively rare; DI values have been estimated based upon European and African population distributions.

SECTION 19. THE ABO AND SECRETOR SYSTEMS

19.1 Origins and Earlier Studies

The ABO blood group system was discovered in 1900 by Karl Landsteiner. In a footnote to a paper, devoted primarily to other matters, he said:

> The serum of healthy humans not only has an agglutinating effect on animal blood corpuscles, but also on human blood corpuscles from different individuals. It remains to be decided whether this phenomenon is due to original individual differences or to the influence of injuries and possible bacterial infection. I observed this behavior as especially pronounced in the case of blood from severely ill patients. This phenomenon could be related to the dissolving capacity of serum for blood corpuscles in the case of various diseases, as it was described by Maragliano (10th Congress of Internal Medicine, 1892)

The full account of Landsteiner's observations appeared in 1901. The essential finding was that the sera of healthy persons agglutinated the red cells of certain other healthy persons. The data allowed the sera of the individuals tested to be placed into one of three groups: in one, called "A", the serum reacted with the cells of another group, called "B", but not with the cells of group "A"; the serum of group "B" reacted with group "A" cells; in the third group, called "C", the serum agglutinated the cells of both groups "A" and "B", but the red cells of group "C" were not reactive with sera from either group "A" or "B". Landsteiner said that there must be at least two agglutinins present, one in the group "A" serum, another in the "B" serum, and both together in the serum of group "C". There was a body of opinion at the time which held that the phenomena were a function of certain disease states, but Landsteiner said that this thinking was not in accord with his findings. In some placental sera examined, the agglutinins appeared to be absent. It was noted that sera dried for two weeks on linen still gave the observed reactions, and that this fact might very well be exploited for medico-legal purposes. Landsteiner concluded by noting that these characteristics of human blood permitted an understanding of the consequences of various kinds of transfusions. The phenomenon of agglutination of human cells by human sera was called isoagglutination, and the agglutinins in serum were referred to as isoagglutinins, after the suggestion of Ehrlich and Morgenroth. In 1902, von Decastello and Sturli conducted further studies on the phenomenon, and could fully confirm all of Landsteiner's observations. In addition, it was found that the blood of 4 out of 155 persons behaved differently from the previously described three groups, i.e., the serum contained no isoagglutinins, and the cells were agglutinated by the sera of all the other groups. The isoagglutination patterns showed no dependence on any pathological condition. Differences in the titer of isoantibodies in different individuals were noted as well, along with individual differences in agglutinability of cells with a serum of constant strength. In some newborn blood samples, the isoagglutinins were not observed. In cases where they were observed, they were of lower titer than in adult bloods, and it appeared that there was variable development of receptors on the cells of newborns as well. Halban and Landsteiner (1902) conducted a number of immunohematological studies comparing maternal with neonatal blood, and obtained results very like those of von Decastello and Sturli with respect to isoagglutination.

In 1907, Jansky published a paper in a rather obscure Bohemian journal, written in the Czechoslovakian language, in which he examined isoagglutination using the cells of 99 persons with the sera of 30 others. The tests involved over 3100 individual agglutination tests, and he recognized the existence of four isoagglutination blood groups from his data. Peculiarly, he did not mention or cite Landsteiner's work, though he cited other German literature in the field, including von Decastello and Sturli's paper. Jansky classified the isoagglutination groups according to Roman numerals, Groups I through IV. In 1910, Moss examined blood from 100 persons and noted four isoagglutination groups as well. He, likewise, classified them according to a Roman numeral designation. Unfortunately, Moss group I corresponded to Jansky group IV, and conversely, (Moss, 1910a and 1910b). The adoption of one or the other systems of nomenclature by various different authors and institutions led to quite a bit of confusion in the literature for quite a few years. Von Dungern and Hirszfeld [sometimes spelled Hirschfeld] conducted extensive experiments on the isoagglutination groups beginning around 1910, and concluded that the four groups could be accounted for by the presence or absence of two isoagglutinogens, which they called A and B. The agglutinins were designated α and β (von Dungern, 1910; von Dungern and Hirschfeld, 1910a, 1910b and 1911). Guthrie and his colleagues in this country used upper case letters to designate agglutinins and corresponding lower case letters to designate agglutinogens (Guthrie and Huck, 1923). Lattes (1932) said that such usage was not defensible, because it was clear by then that the agglutinogens were inherited as Mendelian dominant characteristics. There were periodic calls in the literature for standardization of the nomenclature at the time (e.g. Verzár, 1927; Aldershoff, 1927). In this country, the American Immunological, Pathological and Bacteriological Societies called for the universal adoption of the Jansky system in 1921. In 1928, Kennedy published a survey of the nomenclature usage in American

hospitals. At that time, 72% were using the Moss system exclusively, 16% the Jansky system, and the rest two or more systems simultaneously. The 1921 recommendation of universal adoption of the Jansky system had not resulted in any significantly increased use of the system, the survey found. Finally, more than half the hospitals surveyed did not favor a standardized "compromise" system, and about ⅔ felt that such a system would only lead to additional confusion. The matter was finally settled in 1930 when the Permanent Standardization Commission of the League of Nations Health Organization reported the work of its Laboratory Conference on Blood Groups (League of Nations Health Organization, 1930). A resolution to adopt essentially the scheme of von Dungern and Hirszfeld was adopted at an April 1928 session of the Permanent Standardization Commission held in Frankfurt-am-Main. The test sera for groups A and B were to be called anti-A and anti-B, respectively. A comparison of the Moss, Jansky and International systems appears in Table 19.1. The use of α to denote anti-A, and β to denote anti-B is still encountered, and does not lead to any confusion.

In 1931, Kennedy published a paper with the stated purpose of making available in the more accessible literature the information contained in the original paper of Jansky (1907). A careful analysis of the data was carried out, and it was argued that Jansky had been the first person to observe and categorize all four blood groups from a single set of isoagglutination tests all in the same paper. He said that Shattock (1900) had actually been the first investigator to publish an observation of isoagglutination in humans. Most authorities think that Shattock was actually seeing rouleaux formation, and in any case, his experiments were carried out with other things in mind, and the notion of isoagglutination groups did not occur to him. Moreover, he was studying blood from sick patients. Zinsser and Coca (1931) published a few remarks on Kennedy's paper. They said that Shattock had undoubtedly been looking at rouleaux formation. More to the point, they seemed anxious to emphasize that the credit for the discovery of the blood groups blonged to Landsteiner, without detracting from Jansky's contribution. Kennedy had not made an explicit issue of the matter of primacy, though one could gain the impression from reading his paper that he regarded Jansky's report of all four groups in the same paper as more significant than the separate reports of Landsteiner, and of his collaborators, von Decastello and Sturli. Kennedy said that, as far as he could tell, Guthrie and Huck (1923) were among the few American workers who read Jansky's paper, and the only ones who had actually analyzed the data. It is quite clear, however, that Moss (1910a and 1910b) had seen at least an abstract of the paper after his own were in press. In an added note, he gave Jansky due credit, and noted the differences in their Roman numerical designations of the blood group.

There is no longer any question about the significance and priority of Dr. Landsteiner's contribution. Landsteiner himself did not regard the description of all four groups in one and the same paper as the principal issue. Von Decastello and Sturli were in fact his collaborators, and their report, describing what was later called group AB, was considered a logical extension of the previous work. Speiser (1961) reproduced an interesting letter from Landsteiner to Sturli on this point. The letter, written February 12, 1921, was rendered in English in Prokop and Uhlenbruck (1969):

12.2.21

The Hague
van Slingelandstr. 39, Holland
Dear Dr. Sturli,

I was very pleased to receive your card. I should like to ask you this: Isoagglutinins, which we have already studied, have achieved great importance in America, as they do many transfusions and for this purpose a blood group determination is always made. I do not know if you are aware of this. You will find something about this, for example, in the first number of the Journal of the American Medical Assoc. of this year. The Americans always say that I have found only 3 groups, as if the few cases of the fourth group were the main thing. Only recently you and Decastello were cited as the authors of this fourth group, earlier it was usually Moss or Jansky who have not done anything new at all. It would be important for me to be able to say, when the occasion arises, that when you were working with me as my pupil or collaborator, you undertook with Decastello the continuation of my work. That was the way things happened. It does not harm, or rather it does not make any difference to your position and I would appreciate it, as other people could not then accuse me of an error as they have until now. I myself saw at the time the necessity for more numerous investigations and found, however, that it was desirable that you should do these. I should be glad if you would let me have a line about this. Have you seen the new book on Immunity by Bordet? . . . .

Yours, Landsteiner

In 1930, Landsteiner received the Nobel Prize for Physiology or Medicine "for his discovery of human blood groups". In his Nobel lecture, he mentioned the medicolegal applications of blood grouping. Until the blood groups were discovered, he noted, ". . . .forensic medicine knew no way of distinguishing between blood stains of different persons. Since the isoagglutinins and the corresponding agglutinogens will also keep for a considerable time in a dried condition, the problem can in certain cases be solved, in particular when the bloods in question, e.g. that of the accused and that of the victim, belong to different groups. . . . . .according to a report by Lattes, who was the first to use it in forensic cases, [the test] has proved useful in a number of cases, and has been the basis of court verdicts and of the acquittal of accused persons." Landsteiner's contributions to immunology and serology extend far beyond his discovery of the blood groups. Landsteiner was born in 1868, and died in 1943. He spent the last 21 years of his life at the Rockefeller University in New York. His scientific life was

Table 19.1 Historical and Present ABO Nomenclature

Moss	Jansky	International
I	IV	AB
II	II	A
III	III	B
IV	I	O

extraordinarily active, 346 publications having appeared between 1892 and 1943. A full bibliography may be found in the *Journal of Immunology* **48**: 1-16 (1944), and some biographical information about him appears in the introduction to the Landsteiner Centennial held at the New York Academy of Sciences (*Ann. N.Y. Acad. Sci.* **169**: 1-293, 1970).

Landsteiner (1901) had indicated that his observations on what later became known as blood groups A, B and O could be explained by the presence or absence of two agglutinins in human serum. Hektoen (1907) supposed that there were three agglutinins and three agglutinogens, and he arrived at this conclusion from a number of absorption experiments. While noting that O cells absorb no antibody, he appeared to be saying that he did get absorption of both α and β by both A and B cells, a most peculiar result (except see section 19.7.2). He also observed a blood which belonged to group AB. In 1910, Moss attempted a systematic explanation of the four blood groups which, as noted above, he designated I through IV (Moss, 1910a and 1910b). He too thought that there were three agglutinogens and three agglutinins. Letting agglutinogens be designated A, B and C, and their corresponding agglutinins by a, b and c, there were two possible arrangements, he said, which would explain the agglutination data: (1) Group I A,o; Group II B, a + c; Group III C, a + b; Group IV O, a + b + c; or (2) Group I A + B + C, o; Group II B + C, a; Group III A + C, b; and Group IV O, c. In these representations O stands for no agglutinogen and o stands for no agglutinin. In Moss nomenclature, groups I through IV represent what we now call AB, A, B and O, respectively. Thus, according to either scheme, AB serum agglutinates no cells, but AB cells are agglutinated by A, B or O serum; A sera agglutinate AB or B cells, and A cells are agglutinated by B or O serum; B sera agglutinate A or AB cells, and B cells are agglutinated by A or O serum; and lastly, O sera agglutinate A, B or AB cells, but O cells are not agglutinated by any sera.

Von Dungern and Hirszfeld took up studies on this subject around 1910, and put forward a systematic explanation of the blood groups based on the original notions of Landsteiner and of von Decastello and Sturli. The groups were explained by the presence or absence of two agglutinogens, A and B, and two agglutinins α and β. The groups were designated according to the agglutinogens possessed by the red cells: group A has A cells, β in serum; group B has B cells, α in serum; group AB has A and B on cells, and no serum agglutinin; and group O lacks agglutinogens, but has both α and β in serum (von Dungern and Hirschfeld, 1910b and 1911). It was noted that different human sera may vary considerably in their agglutinin titer, and that red cell agglutinogens from different persons can likewise exhibit very different activities toward their corresponding agglutinins. Extensive studies on the occurrence of agglutinins for human red cells in animal sera were carried out as well. Selective

absorption experiments by many workers, including Koeckert (1920), Schütze (1921), Hooker and Anderson (1921) and Dyke (1922b), helped to establish the acceptance of the two agglutinogen-two agglutinin scheme. Group A cells absorbed only the α from group O serum, while group B cells would selectively absorb the β. Group AB cells absorbed both agglutinins from group O serum, or α and β separately from B or A sera. Hooker and Anderson (1921) prepared heterologous antisera against human red cells in rabbits, and, by appropriate selective absorption of the antisera, could render some of them group specific.

While the four group scheme was fairly widely accepted by 1920, apparent exceptions to the results expected on the basis of the theory had been observed. There were cases where agglutination between a serum and cells was expected but failed to occur, other cases in which cross-agglutination was observed between persons belonging to the same group, and cases where O cells had been observed to agglutinate. The experiments which may have attracted the most attention in this regard were those of Guthrie and his collaborators. Based on extensive observations, they postulated at first one additional agglutinogen-agglutinin pair (Guthrie and Huck, 1923; Huck and Guthrie, 1924), and later several more agglutinogen-agglutinin combinations (Guthrie and Pessell, 1924a and 1924b; Guthrie *et al.*, 1924). They pointed out that the number of theoretically possible combinations, even using two antigens and two antibodies, was not four but nine, without violating the principle that agglutinins never occur in the serum of a person whose cells have the corresponding agglutinogen (Landsteiner's Rule). Thus, one could imagine (using present-day nomenclature) O$\alpha\beta$, Oα, Oβ, Oo, Aβ, Ao, Bα, Bo and ABo, where "o" indicates no agglutinins. They had in fact observed a blood in which the cells were of group B, but the serum had no agglutinins, i.e., behaved as an AB serum. Coca and Klein (1923a and 1923b) accepted the interpretation of Guthrie and coworkers that there were additional agglutinogen-agglutinin combinations and described one themselves which they called "X". Other workers confirmed the observations, and accepted the interpretation as well (e.g. Simson, 1926; Bunker and Meyers, 1927). Landsteiner and Witt (1924) at first thought that these observations might indeed indicate an additional pair of factors. They noted that two different types of group AB blood could be distinguished, one of which contained an agglutinin for certain group A cells, and the other of which did not. They later changed their opinion as to how this observation should be interpreted, as will be discussed below in connection with A subgroups. The Italian workers, Lattes and Cavazutti (1924) and Mino (1924) did not agree at all that it was necessary to invoke the existence of additional agglutinogen-agglutinin combinations to explain the observations of atypical isoagglutination. These could be understood, they thought, on the basis of quantitative differences in cell receptors and in agglutinin content of sera, as well as of varying avidity of agglutinin for receptor, while retaining the basic four group hypothesis. The matter will be discussed somewhat more in connection with subgroups.

19.2 Inheritance of the ABO Blood Groups

Langer in 1903 and Hektoen in 1907 noted in passing that mothers and their children could have the same blood groups. If they entertained the notion that the blood groups were inherited, they were not explicit on the point. In 1908, Epstein and Otterberg reported the blood groups of two families at a meeting of the New York Pathological Society, and suggested that the blood groups might be inherited. The clear and unequivocal demonstration of the heredity of the blood groups, however, came from the work of von Dungern and Hirszfeld (von Dungern, 1910; von Dungern and Hirszfeld, 1910b). The first experiments were carried out in dogs with immune isoagglutinins, but they soon looked at a collection of data from 71 human families, including 342 people, in Heidelberg. The data were interpreted to mean that the agglutinogens, and not the agglutinins, were inherited. A blood group agglutinogen could not be found in children if it was lacking in both parents. If both parents have an agglutinogen, most of the children have it, but a few may not. And in a family where the parents are AB and O, A and B children are possible. The hereditary factors determining the presence of the agglutinogen were believed to be dominant, those determining their absence, recessive. The factors behaved strictly according to Mendelian principles, and it was thought that A and B were determined at separate, independent genetic loci. This work formed the basis of hundreds of studies by many workers, including themselves. According to the von Dungern and Hirszfeld notion that the genetics of the system could be explained on the basis of two, independently inherited allelomorphic pairs, A being dominant to non-A, and B being dominant to non-B, nine genotypes and four phenotypes could be represented as shown in Table 19.2, where A and B represent the genes for agglutinogens A and B, and a and b represent the recessive genes for their respective absence. Hirszfeld (1928) reviewed in detail the experimental foundations for scheme, as well as numerous family and population studies with which it was consistent.

In 1924, the mathematician Felix Bernstein published the first of his several papers in which he formulated a theory of inheritance for the ABO groups based upon statistical genetic considerations, and inferences drawn from the rather extensive body of population data which existed by that time. He introduced the notion of multiple alleles at a single genetic locus for the system. There was precedent for multiple allelic systems in *Drosophila,* but not in human genetics. According to Bernstein, there are three genes, A, B and R, which can give rise to the genotypes RR, RB, BB, RA, AA and AB. The agglutinins corresponding to A and B were called α and β in conformance with the accepted usage, and he said that an agglutinin ρ would correspond to R. According to this scheme, a child inherits only one blood group

Table 19.2 Blood Group Genetics According to vonDungern and Hirszfeld

Genotype	Phenotype
aabb	O
AAbb, Aabb	A
aaBB, aaBb	B
AABB, AABb, AaBb, AaBB	AB

genetic factor from each parent, instead of two as postulated by von Dungern and Hirszfeld. Genotypically RR people belonged to group O, RB and BB people were of group B, RA and AA people were group A, and AB people were of group AB. Bernstein analyzed a considerable amount of the available population data using a statistical genetic model, and found that his model fit the observations much better than the two allelic pair idea. If p, q and r are taken to stand for the gene frequencies of genes A, B and R, respectively, then the occurrences of the genotypes AA, AB, AR, BB, BR and RR in the population are given by p^2, 2pq, 2pr, q^2, 2qr and r^2, respectively, where $p + q + r = 1$ and $p^2 + 2pq + 2pr + q^2 + 2qr + r^2 = 1$ (Bernstein, 1924 and 1925).

Bernstein elaborated upon his ideas somewhat further in 1930 (Bernstein, 1930a and 1930b). His work is considerably more involved both in bulk and in computational complexity than has been indicated here. Much of it consists of detailed statistical and probabilistic analyses of the then-existing population data to demonstrate the correctness of his genetic hypothesis in comparison with the two allelic pair idea, and to answer various objections that had been raised to his ideas.

The alternative theories of Furuhata (1927 and 1929) and of Bauer (1928 and 1929) may be mentioned for the sake of completeness. Furuhata's theory postulated two pairs of alleles, but said that they were closely linked. He thought, further, that the genes exerted control over the presence or absence of the agglutinins as well as of the agglutinogens. The chromosomal composition could be ab, Ab or aB, but never AB. The idea was similar to Bernstein's in that there were three "hereditary units", and to von Dungern and Hirszfeld's in that two pairs of genes were involved. He would not allow that AB could come about in one and the same chromosome, and thus in a single gamete, as the result of a crossover. For all practical purposes, Furuhata's theory turns out to yield results identical with that of Bernstein, except that the explanatory mechanism is different, of course. Bauer's idea was similar to Furuhata's, except that he allowed for crossing over in certain cases, giving rise to an

AB chromosome. Lattes (1932) noted that Kirihara and Haku in Japan had had this idea before Bauer.

Predictions of the blood groups of children from particular matings in large populations based on the two allelic pair hypothesis on the one hand, and on the multiple allelic hypothesis on the other, clearly favored the latter. There were a few observations in the literture, however, which appeared to contradict Bernstein's notion, these being reports of children having resulted from matings in which one or both of the parents was excluded by the theory. Most authorities eventually came to believe that such discrepant results were probably explainable on the basis of grouping errors or of unsuspected illegitimacy. Schiff and Boyd (1942) pointed out that the number of apparent exceptions to the rules of heredity dropped markedly after 1925, in part because of the fact that Bernstein's hypothesis had not been published, and investigators could not go back and re-examine all their groupings, and in part because of improvements in technique. There were 19.5 "exceptions" per 1000 families (13.2 per 1000 children) in the 1910–1925 period in material consisting of 973 families with 2270 children. In 1926, in 928 families with 2213 children, the comparable figures were 1.1 per thousand families and 0.47 per 1000 children. Comparably low figures were found in the data for 1927–1930 and 1935–1937 as well. These "exceptions" fall into two categories: A or B children being attributed to parents, neither of whom had either A or B; and O children attributed to parents, either of whom was AB, or AB children attributed to parents, either of whom was O. The possibility of errors in blood grouping cannot be overlooked either. In 1919, Pemberton, in his discussion of systemic transfusion reactions, noted that there were 12 such cases out of 1032 transfusions at the Mayo Clinic. In 9 of these cases, it was possible to do the blood grouping over again, and errors in the initial report of the blood group (primarily clerical) were found in every case. Lattes (1932) noted that there would always be a certain minimal number of apparently aberrant cases, because of errors and because of unsuspected illegitimacy. Furthermore, quantitative variations in antigen content, and the existence of weak subgroups of certain types, could lead to errors in routine group determinations on occasion. Such errors today would be entirely unacceptable, and these comments, it must be kept in mind, were made over 40 years ago.

It is clear that, at least by 1930, most of the major authorities had come to accept the Bernstein multiple allelic hypothesis as the correct explanation for the inheritance of the blood groups. With possible minor modifications, because of the possible occurrence of rare alleles in the system, the basic multiple allelic hypothesis is widely accepted today.

19.3 Subgroups in the ABO system

19.3.1 Subgroups of A

19.3.1.1 A_1 and A_2. In 1911, von Dungern and Hirszfeld noted that not every example of anti-A serum from B persons agglutinated every example of A cells. Their studies were carried out primarily with three anti-A sera. Two of these agglutinated a greater number of A cells than did the third. A small number of bloods, which reacted with the first two sera, did not react with the third one at all, and these were said to possess the characteristic "little A". In addition, if this third anti-A serum were absorbed with particular A cells, an agglutinin remained in the serum which would agglutinate certain A cells, but not others. It appeared, therefore, that there could be two types of agglutinins in the sera of group B (or O) people directed against A cells. Similar observations were made by Guthrie and Huck (1923) and by Coca and Klein (1923b), although they interpreted the results as indicating the presence of an additional agglutinogen-agglutinin combination in addition to A/α and B/β, and by Schütze (1921). Landsteiner and Witt (1924) made the same observation and initially agreed with the interpretation that indicated the presence of an additional pair of factors. Lattes and Cavazutti (1924) and Mino (1924) did not agree with this interpretation (although the observations were fully confirmed). The observations could be explained, the Italian investigators said, on the basis of quantitative differences, i.e., on the basis of differences in the number of receptors on the cells, and in the variability of titer and avidity of the agglutinins. In 1926, Landsteiner and Witt extended their observations and could, by suitable absorption experiments, establish quite unequivocally that the isoagglutinins which act on A cells could be separated into two qualitatively different fractions, which were called α and α_1. Similarly, A and AB cells could be distinguished on the basis of their agglutinability with α and α_1, the receptor for α_1 being named A_1. It was also found that the sera of AB people could sometimes contain α_1, the A_1 then not being present in the cells. In the same year, Landsteiner and Levine (1926b) suggested that the factor on A cells which is not recognized by α_1 be called A_2. They did not think at the time that A_1 and A_2 were probably controlled by separate genetic factors, and they noted too that their data indicated the presence of a receptor on O cells, which was not common to all bloods, but bore some similarity to A_2. The A_1–A_2 nomenclature has persisted to the present time. In 1930, Thomsen *et al.* put forward the idea that Bernstein's three-allele hypothesis for the inheritance of the ABO system should be modified to include two A factors, namely A_1 and A_2. This idea was supported by a number of studies on families (Friedenreich and Zacho, 1931). Thus, the system could have 10 genotypes, which give rise to six distinguishable phenotypes if anti-A, anti-A_1 and anti-B grouping reagents are used (Table 19.3). It is generally thought that A_1 blood has two antigenic receptors, A and A_1, while A_2 blood has only the A receptor. Anti-A from the serum of a B person generally has both anti-A and anti-A_1. Race and Sanger (1975) have noted that it is somewhat unfortunate that the term "anti-A" is used in two senses, i.e., to mean the antiserum on one hand, and to mean one of the antibodies contained in the antiserum on the other.

A number of different methods have been used for the discrimination of A_1 and A_2, and these are discussed by Prokop and Uhlenbruck (1969). The topic merits some dis-

cussion though, in part because the resulting classification can depend on the method used, but particularly because, as will be discussed below, there are some cases which do not clearly fall into one of the two categories. Von Dungern and Hirszfeld (1911) used a saturation method for the preparation of anti-A_1 serum, in which B-serum was simply saturated with A_2 cells, and the remaining antibody constituted the desired reagent. Thomsen *et al.* (1930) used a saturation technique as well, but in this case a B-serum was absorbed with a particular amount of unknown blood cells, and the titer of the remaining antibody against A cells was then determined. The process is repeated several times, and the data can be plotted to yield so-called "saturation curves". These can then be compared with the results obtained using measured amounts of known A_1 and A_2 cells. The finding that pepsin possesses A blood group characteristics has been exploited for the production of anti-A_1 serum (Ottensooer and Zurukzoglu, 1932; Schiff, 1933a). Prokop and Uhlenbruck (1969) noted that not all examples of pepsin are suitable for use with B sera in the production of anti-A_1 serum, and that the specificity of the reagent does not persist beyond a few days, at which time the pepsin treatment must be repeated. It is best, with all these methods, to choose a B-serum which had a high anti-A_1 titer to begin with. The data of Olbrich and Walther (1941) indicated that the anti-A_2 titer of a B-serum tends to be higher as the anti-A_1 titer is higher also. So-called "irregular" antibodies can be used as anti-A_1 reagents. These are anti-A_1 which occur in the sera of A_2 and A_2B people (Lauer, 1928; Friedenreich, 1931). The anti-A_1 titer varies with the temperature, the antibodies being detectable in more of the sera at lower than room temperature. Additionally, anti-A_1 is more often found in A_2B than in A_2 sera. Taylor *et al.* (1942) found α_1 in about $\frac{74}{283}$ (26.15%) of A_2B and in about $\frac{62}{3871}$ (1.6%) A_2 bloods in the U.K. Juel (1959) reported 12 A_2B bloods out of 40 (30%) as having anti-A_1 at 5°, and 9 (22.5%) at 18°. Lenkiewicz and Sarul (1971) said that they found about 14% of A_2 bloods and 51% of A_2B bloods to have anti-A_1 at 10° in a large number of cases examined in the Warsaw area. Prokop and Uhlenbruck (1969) cite various values from several authors, the numbers ranging from about 21 to 40% of A_2B bloods and from about 2 to 8% of A_2 bloods. Other techniques have been proposed for discriminating A_1 from A_2, based on blood group substances in saliva (see in subsequent section) and on

Table 19.3 ABO System Genetics with A_1 and A_2 Subgroups

Genotype	Phenotype	Reaction With		
		Anti-A	Anti-A_1	Anti-B
A_1A_1 A_1A_2 A_1O	A_1	+	+	−
A_2A_2 A_2O	A_2	+	−	−
BB BO	B	−	−	+
A_1B	A_1B	+	+	+
A_2B	A_2B	+	−	+
OO	O	−	−	−

the use of various animal sera. Perhaps the simplest technique is the use of A_1 specific phytagglutinins, one of the most common being from *Dolichos biflorus* (Bird, 1951 and 1952). Phytagglutinins are discussed more fully in subsequent sections.

It should be pointed out that Lattes in 1932 still did not accept the notion of qualitatively different subgroups of A as having been established. The data could be explained, he thought, on the basis of avidity differences in antibodies. The issue is still apparently not fully settled (Juel, 1959; Mäkelä *et al.*, 1969; Race and Sanger, 1975).

19.3.1.2 Subgroup A_3. In 1936, Friedenreich described an example of A cells which reacted more weakly than A_2 cells (Friedenreich, 1936a and 1936b). This type of behavior was characteristic of certain families, was apparently rare, and no transitions could be observed between this weak A and A_2. Six unrelated people, out of about 4000, were found to have the weak A cells, and were investigated along with members of their families. Since the results showed that the cells were of a distinct type of A, they were designated A_3. A_3 cells reacted with anti-A reagents at least as weakly as do A_2B cells (which react more weakly than A_2 cells), if not more so. Sera which gave strongly positive reactions even with A_2B cells gave only weak reactions with the A_3 cells. It was noted that these A_3 cells formed a few rather large, fragile agglutinates amongst a large number of free cells. This characteristic agglutination picture was later referred to by Dunsford (1959) as "mixed field agglutination." With weak antisera, the A_3 would be missed altogether, i.e., grouped as O. With sera of too high titer, the A_3 might be misclassified as an A_2, but Gamelgaard (1942) noted that, because of the characteristic and unusual agglutination pattern, this should not be much of a problem. The sera of A_3 people contain a normal quantity of β-agglutinin, and no unusual α_1-agglutinins, except for a trace of "cold α_1-agglutinin" in a few cases. The absorption capacity of A_3 cells for anti-A was found to be intermediate between A_2 and A_2B. Three A_3B bloods were noted, their absorption capacity being less than that of A_2B. Friedenreich suggested that the A_3 characteristic was almost surely the result of an additional allele at the ABO locus, recessive to both A_1 and A_2. Gammelgaard (1942), in his extensive and important study on the weak A receptor types, looked at 170 persons of type A_3 and 33 of type A_3B. The agglutination picture with A_3, as originally described by Friedenreich, was confirmed. The absorption characteristics, and the fact that the weak A_3 agglutination does not decrease as rapidly upon serum dilution as does A_2B agglutination with anti-A reagents, were also confirmed. In the material studied by Gammelgaard, the incidence of A_3 was about 1:1000 A persons. The A_3 cells appeared to consist of a spectrum of red cells of varying receptor strength, from relatively agglutinable to non-agglutinable. Subsequent studies on A_3 have not unequivocally proven, but have not excluded the possibility that A_3 is an allele of the system (Race and Sanger, 1975). Race and Sanger also note that the diagnosis of A_3B is not an easy one, and that family evidence is really needed for confirmation. Dguchi *et al.* (1978) could isolate a population of A_3 cells by affinity chromatography on a Sepharose column, to which was bound lima bean anti-A lectin, which were completely inagglutinable by anti-A. Studies with eel serum anti-H showed that these cells had a large number of H sites, but only about 9% of the A sites that would be found on an A_1 cell. The A structure was believed to be the same on those A_3 cells as on A_1 cells, but the density of A sites too low to permit agglutination by lima bean anti-A lectin.

19.3.1.3 Further subgroups of A. The other weak A receptors which have been described are principally weaker than A_3. They have been given a variety of designations, and the relationships between the different ones are not always clear. They are all quite rare. In 1935, Fischer and Hahn described a blood from a patient which had a very weak A receptor. The cells were agglutinated by some group O sera, but only weakly or not at all by group B sera. Sheep immune anti-A agglutinated the cells weakly. The cells were designated A_x. They absorbed less anti-A than either A_1 or A_2 cells, but the anti-A which was absorbed could be more easily eluted from the A_x cells than from the A_1 or A_2 cells. The patient's serum contained anti-B, an anti-A_1 activity at 6–8°, and an anti-A_2 activity at "ice bath" temperature. In 1940, Gammelgaard and Marcusson described a blood with a very weak A receptor, which was not A_3, and which they called A_4. They supposed that it was due to an additional allele in the ABO system. The investigated family of 64 persons had 24 A_4 members. The incidence of A_4 was put at 1:60,000. The original designation was changed by Gammelgaard in 1942 to A_5, because some other cases had been found which were better referred to as A_4. These were slightly weaker than A_2B but did not give the characteristic A_3 agglutinates. A trace of A substance could be identified in the saliva of some of the A_4 persons, but not enough to try to distinguish between secretors and non-secretors (see in subsequent section). The A_5 cells were weaker than A_4, and this was the reason for the redesignation. Gammelgaard speculated that Fischer and Hahn's A_x was most likely an A_4. Gammelgaard described one odd blood, which he called A_x, and which did not have the characteristics that would indicate it should be called "A_6", that is, the cells did not agglutinate with "anti-O" serum (see below) nor was there a decreased secretion of A substance in saliva, as is observed A_1 through A_5.

In 1948, Jonsson and Fast reported a blood in which the cells had a very weak A, but it differed somewhat from previously reported variants, and was called A_6. Some of the A_6 sera had an α_1-agglutinin, but no A was found in saliva. Dunsford (1952) reported a blood which behaved very much like the A_4 of Gammelgaard. Further examples of A_4 were reported in an English family, one of whose members also had a very rare Rhesus type in addition to the A_4 (Dunsford and Aspinall, 1952). Estola and Elo (1952) found a weak A blood that was similar to Gammelgaard's A_5, and this was called A_z. Grove-Rasmussen *et al.* (1952) reported a case of a weak A receptor, which they called A_o. Ellis and Cawley found another case of an A_o in 1958. Salmon *et al.* (1965) reviewed A_x and presented studies on 42 cases in 8 families.

By A_x in this case, they were referring to the original designation of Fischer and Hahn. For the most part, the results of the family studies indicated that A_x could be accounted for by a rare ABO allele, but in one case, an A_2B parent (the other parent was not A or AB of any kind) had six A_x children. The inheritance of many of the weak A forms is, therefore, not always that clear. As mentioned, some of the family studies can be explained quite simply by postulating the presence of rare allele. In other cases, though, such as the one of Salmon *et al.* just mentioned, and in others to follow, the inheritance does not seem to follow that simple pattern. Cahan *et al.* (1957) have described a family in which an A_x father had an A_2B son who, in turn had an A_x daughter. The wife of the A_2B son was an A_1, and in 1962, another child was born to these parents who was A_2B (Fisher and Cahan, 1962). Likewise, van Loghem and van der Hart (1954) found three A_4 children from an O x O mating. They noted that this "A_4" could be Fischer and Hahn's A_x or Grove-Rasmussen *et al.'s* A_o. Cahan *et al.* (1957) had suggested using the designation A_x for all the variants, except A_1, A_2 and A_3, until the situation became a little clearer. Thus, A_4, A_5, A_6, A_o, A_z and A_x could all be grouped under the "A_x" heading, to give an indication of the priority of discovery by Fischer and Hahn. Dunsford, in his 1959 review, rather agreed with this suggestion. Even in 1975, Race and Sanger, who were co-authors of the Cahan *et al.* (1957) paper, thought that this practice should be continued. It should be noted that Wiener (1953) was of the opinion that these weak A variants discussed so far in section 19.3.1.3 were not A bloods at all, but examples of blood group C (see below). This opinion was still held in 1973 (Wiener *et al.*, 1973).

In 1956, Wiener and Gordon described a somewhat different variant of A, which was called A_m. These cells were not agglutinated by anti-A from B-serum or from O-serum, nor by immune anti-A. The serum had normal quantities of anti-B, however, and A substance could be found in the saliva. The blood did not behave like other weak A variants, such as A_o, A_4, etc., and was not, they said, an example of blood group C. Examples of other family material showing the characteristic were described by Weiner *et al.* (1957), Salmon *et al.* (1958) and Hrubiško *et al.* (1966). A_m cells are not agglutinated by any kind of anti-A, i.e., they group like O cells. But the serum contains anti-B, and A substances can be secreted into saliva. Sometimes the serum contains an irregular anti-A_1 antibody, and the non-agglutinability of the cells can depend on the IgM content of the anti-A serum used (Hrubiško *et al.*, 1966). There is variation within the phenotype. Hrubiško *et al.* (1966) found two probable A_m homozygotes, and in their material, the A_m behaved as if it were due to a rare allele. On the other hand, Weiner *et al.* (1957) could show that their A_m person was genotypically A_1O, and that an A_mB person was genotypically A_1B. The A_m cells reacted with anti-H (see further on) and A and H substances were secreted. The cells would absorb anti-A. The A_mB cells did not absorb anti-A, but the saliva contained A, B and H substances. Weiner *et al.* postulated the existence of a Mendelian pair of modifying genes, called Yy, to explain these A_m cases. According to this idea, A_m people would be homozygous recessive at the Yy locus (i.e., yy), and the absence of Y would lead to impaired development of the A antigen on red cells, but not in saliva (see Race, 1957). A_m cells are very rare. Because of its unusual characteristics, A_m should not be considered in the same category with A_x, A_o, A_4, etc. (Dunsford, 1959; Race and Sanger, 1975).

19.3.1.4 So-called intermediate A (A_{int}, A_i). As noted in 19.3.1.1, it is possible to classify the vast majority of A cells as either A_1 or A_2. Cells are occasionally encountered, however, which appear to be intermediate between A_1 and A_2 and cannot be unequivocally placed in either category. This phenomenon was first noted by Landsteiner and Levine (1926b), and was further studied by Friedenreich and Zacho (1931). The cells react with anti-A_1 reagents, but also with so-called anti-A_2, usually more with one than with the other. They react with *Dolichos* anti-A_1 and *Ulex* anti-H lechtins as well. Dahr (1942b) described examples of the "intermediary A" blood. The diagnosis of A_i may vary with the technique used. Gmyrek (1962) pointed this out, noting that identification of A_i was best done on the basis of reactions with anti-A_1 and anti-H, rather than by the absorption methods. He also pointed out that the strength of A and H in a series of A bloods, if judged by the titer with anti-A_1 and anti-H, do not vary inversely in a linear fashion. He also noted that there can be identified a graded series of A types, in between A_1 and A_2 in reactivity. Grundbacher and Summerlin (1971) also made the point that the antigenic strength of A_1 and H on cells does not appear to vary inversely in these cases, as would be expected if H were a precursor substance for A (see below). They confirmed the fact that the incidence of A_i is much higher in Black people (36 of 114 A types) than in Caucasians (5 of 199 A types) in a Richmond, Virginia population. Wiener (1950) had found the same thing in New York, where 5 A_i were identified in 200 bloods from Black people, and only 3 were found among 846 Caucasian bloods. Race and Sanger (1975) examined two A_i samples and found that they reacted more strongly with anti-H than do A_2 blood cells. If A_i were truly intermediate between A_1 and A_2, the anti-H reaction would be expected to be intermediate as well, and they thought that A_i might, therefore, be a distinct class of some kind. Bird (1964) reported that Moharastrian blood donors (India) showed the A_i type in about 2% of all A cells. Brain (1966) found the incidence to be almost 14% of A cells in the South African Bantu.

19.3.1.5 Additional subgroups of A. A number of other variants have been described, and named, e.g., A_{end}, A_{el}, A_{bantu}. These are discussed and references given to the sources in Race and Sanger (1975) and in Prokop and Uhlenbruck (1969). They are all very rare.

19.3.1.6 Quantitative approaches. The red cell antigen-isoagglutinin reaction has been the subject of physico-chemical and quantitative studies. The ABO system has been looked at by a number of workers in an effort to understand the subgroups. The quantitative and physico-chemical

approaches will be discussed subsequently. Gibbs and Akeroyd (1959) used a technique for evaluation of the A receptor strength which had been developed by Wilkie and Becker (1955). The latter had devised the procedure for the study of B antigen-antibody reactions, based on the studies of the Filitti-Wurmser *et al.* group (see below). If one quantitates agglutination by measuring the number of cells remaining unagglutinated under a standard set of conditions with different concentrations of antisera, and plots the percentage agglutination against the log of the antiserum concentration, a sigmoidal curve is obtained. With suitable computational transformations, the sigmoidal curve can be fitted to a line, the slope of which is related to antigen strength. Gibbs and Akeroyd (1959) found that the slope decreased (antigen strength decreased) as $A_1 > A_1B > A_2 > A_iB > A_2B > A_3$. Grundbacher (1964 and 1965) devised a hemolytic assay for antigen strength, using an immune anti-A hemolysin serum which had been absorbed with B and O cells. Under carefully controlled test conditions, hemolysis, which can be readily quantitated colorimetrically, becomes a measure of antigen strength. The results of the application of this technique to the study of A_1, A_2 and A_i were mentioned in 19.3.1.4 (Grundbacher and Summerlin, 1971). Cohen and Zuelzer (1965) used an immunofluorescence technique, and found that there was a continuum of antigen strength, running from A_1 to the weakest forms. The data, they said, supported a quantitative basis for the differences in receptor strength, and they suggested that most of the weak forms be grouped under a single designation (they suggested A_w). Wiener and Karowe (1944) suggested that the difference between A_1 and A_2 might have a structural basis. If the antigenic receptor was the same in both cases, but if in the case of A_2 it was attached to the cell surface by a shorter "stalk", as it were, i.e., not as accessible to the antibody, this might explain the weaker reactivity. The strength of the receptor is thus explained by steric hindrance to antibody binding. Carrying the argument a step further, A_2B has a weaker reaction than A_2 because the neighboring B receptors are attached by the longer (A_1-like) stalks, and can thus interfere sterically with antibody molecule approach to the A_2 receptor. This view was expressed again quite recently (Wiener and Socha, 1974). The studies of Bar-Shany *et al.* (1970) were interpreted to mean that the A_1 and A_2 reactivity differences might very well have a steric basis. As expected, steric hindrance would give rise to greater differences in reactivity on red cell surfaces than in the soluble, secreted blood group substances. Economidou *et al.* (1967) used ^{125}I-labelled antibodies to determine the number of A and B receptors in A_1, A_2, A_1B and A_2B cells. The number of A sites decreased as $A_1 > A_1B > A_2 > A_2B$. B cells had more B sites than A_1B cells. The differences in equilibrium constant and dissociation rate with A_1 and A_2 cells using anti-A were consistent with slight differences in the molecular structure of the antigen.

Cartron *et al.* (1974) determined the A antigen site density on a series of samples representing a number of the "weak A" phenotypes, including A_3, A_x, A_{end}, A_m and A_{el}. They used a rabbit IgG anti-A which was labelled with ^{125}I. Site density was found to decrease in the order $A_1 > A_2 > A_3 > A_x > A_{end} > A_m > A_{el}$. While A_3 cells had about 30,000 sites per cell, A_{el} cells had only about 700, compared with 850,000 for A_1 and 240,000 for A_2.

19.3.2 Variants of B

Variants of B are rarer than those of A, and there is considerably less literature on them. Many of the weak B types have been observed in only one or a few kindreds. As with weak A types, the relationships between the different weak-B types, given a variety of designations by different authors, are not always clear. One of the earliest observations of weak B forms was that of Moskow (1935). He distinguished what he called B_1 and B_2. Following his work, the area was not very active for a number of years, but a number of papers began appearing in the mid-1950's. The variants have been well reviewed in Prokop and Uhlenbruck (1969) and in Race and Sanger (1975), and references to the individual observations may be found in these classic works. Race and Sanger (1975) classify the B variants into three categories, based on whether the individual secretes B substance or H substance in saliva or not, and on whether there is an anti-B in the serum. Table 19.4 gives a summary of the weak B variants.

19.4 Antibodies of the ABO System

19.4.1. Anti-A and anti-B

Ordinarily, human serum contains anti-A, anti-B, or both or neither, corresponding to the antigen(s) that is (are) absent. Absence of an expected agglutinin nearly always means that there is something unusual about the person's blood. The isoantibodies develop in most cases around the age of 3–6 months, and the titer increases for a number of of years, after which it declines steadily (Morville, 1929; Thomsen and Kettel, 1929). Infant serum contains some 10% of the IgM levels found in adults, and occasionally anti-A or anti-B is present in this fraction. It was thought for a long time that the isoagglutinins of newborns were primarily derived from the mother, but this need not always be the case, because isoagglutinins against maternal cells can be detected in some cases (Toivanen and Hirvonen, 1969a). Infant serum contains IgG antibodies derived from the maternal circulation. The initiation of IgM synthesis in the newborn precedes that of IgG by several weeks, IgM biosynthesis beginning within a few days of birth (West *et al.*, 1962).

The origin of anti-A and anti-B have occupied much attention, because their "natural" occurrence in human sera appears, on the face of it, to be in violation of the fundamental immunological principle: No antibodies without an antigenic stimulus. A major discussion of this subject was given by Thomsen in 1936. Furuhata (1927) thought that the isoantibodies were under direct genetic control, and that the genes controlling them were allelic to the genes responsible for the isoagglutinogens (see in section 19.2). This

Table 19.4 Summary of Some Weak B Types

Notation	Anti-B in Serum	B in Saliva	H in Saliva	Race and Sanger Category
B_v	+	(+)[1]	−	1
B_w; B_x; B_m	−[2]	+	−	2
B_3; B_x; B_{weak}	−	−[3]	+	3

[1] Some kind of B in saliva
[2] Or weak cold anti-B
[3] Or doubtful B in saliva

idea is no longer widely accepted, but the concept of genetic control of isoantibody biosynthesis has persisted in the Japanese literature for a long time, and the issue is not completely settled. Grundbacher (1976) recently determined the anti-A and anti-B agglutinin (and hemolysin) titers in 401 group O people from 74 families. The titers were generally higher in Blacks than in Whites. Whites also generally showed higher α than β titers, and antibody levels were generally higher in women than in men. In Blacks, the agglutinin titers tended to be equal, and sex differences were not apparent. Statistical analysis of data from related persons suggested that 20 to 30 percent of the variation could be accounted for by genetic variation. Schiff and Adelsberger (1924) believed that group A people had a small amount of B present, just enough to stimulate anti-B production, but not so much as to absorb the antibody formed. The stimulating agents were termed "isoreagins". Dupont (1934) first suggested that isoantibodies do arise in response to immunization, not with red cell antigens, but with closely related antigens from various things in the environment. The A-like and B-like antigenic stimuli could come from a number of different sources, e.g., bacteria inhabiting the gut, or on dust particles which are inhaled, or on various materials ingested as food. This notion has been called the "cryptogenic immunization" theory. There is some experimental support for the idea from the work of Springer *et al.* (1959). White Leghorn chickens form an anti-B-like agglutinin for human red cells when they are quite young, but this agglutinin does not form if the chicks are kept in a germ-free environment. The idea that some external stimulus is required for the production of these antibodies is more satisfying in terms of present-day immunology. Kabat (1956) was inclined to accept the view that a stimulus is needed. Burnet's clonal selection theory (Burnet, 1959) can explain isoantibody formation. In these terms, the antibodies are understood as an immunological tolerance phenomenon. The plasma cells forming molecules that can act as anti-A or anti-B (or anti-H) are influenced very early in embryonic life by the presence of an antigen. If an antigen is present, it has the effect of destroying those cells producing antibodies to it, i.e., the antigen is recognized as "self". Thus, in an A person, the anti-A machinery is shut down very early on, and only anti-B develops. There have been other theories proposed too, which are reviewed by Prokop and Uhlenbruck (1969). The work of Filitti-Wurmser *et al.* (see below) has strongly suggested that isoantibody formation does have some sort of genetic basis, a position with which a number of authorities agree.

A distinction is often made between "natural" and "immune" antibodies, the former being present in the serum of a person who has had no known exposure to the corresponding antigen. The best examples of so-called "naturally occurring" antibodies are the iso-anti-A and anti-B, although

"natural" antibodies to other blood group system determinants can be found as well. If it turns out that the anti-A and anti-B isoantibodies are, in fact, immune, i.e., the result of exposure to antigen then the distinction will have little meaning. The distinction is based on the fact that there are differences in the serological behavior of the two kinds of antibodies. Reepmaker and van Loghem (1953) have mentioned a number of these differences, which include: (1) the immune sera have a higher titer at 37° than at 4°; (2) the immune antibodies are more thermostable; (3) immune antibodies often give a higher titer in proteinaceous or viscous media; (4) immune sera usually react weakly with A_2 cells in saline; and (5) immune antibodies are more difficult to neutralize with soluble A or B substance. When a person who already has isoantibodies is exposed to antigen by injection, by a pregnancy with an incompatible fetus, etc., the term "hyperimmunization" is sometimes applied, and the antiserum referred to as "hyperimmune". A very good discussion of natural and immune isoantibodies may be found in Mollison (1972). Lopez *et al.* (1979) studied the reactivity of IgM and IgG anti-A and anti-B toward the "weak" forms of A and B cells. A_x and B_x cells were agglutinated better by IgM than IgG antibodies, but the two types of antibodies behaved similarly with A_3, A_{end} and B_3 cells.

19.4.2 Anti-H and "Anti-O"

An understanding of anti-H and anti-O is closely connected with the problem of blood group O. From the beginning, it was wondered whether blood group O could be positively characterized, that is, whether there was an "O receptor". Isoantibodies to group O are not found in human beings, and Bernstein thought that A and B were completely dominant over O. It has been known for quite a long time, though, that O cells can be positively characterized in some way, since sera, especially animal sera, were found which preferentially agglutinated O cells. Schiff (1927b) found that some cattle serum, absorbed with AB cells, preferentially agglutinated O cells. With one fairly potent serum, he got no reaction at all with a number of examples of A, B, and AB cells—a strange result, as Prokop and Uhlenbruck (1969) pointed out, in view of our present knowledge of H substance and its relationship to A and B substances (see further below). In any case, Schiff thought that this antibody was detecting the antigenic product of Bernstein's R gene. Other animal sera, such as dog, guinea pig, cat and chicken have agglutinins acting on O cells as well (Dahr, 1938). Hooker and Anderson (1921) produced anti-O sera in rabbits by immunization with human O cells. Matta (1937) did likewise in both rabbits and goats. Jadin (1934) found that Schiff's "anti-O" reacted with A and B cells, and thought that this would provide a way of distinguishing between homozygous and heterozygous A and B types, a possibility that was also pursued by Dahr (1938) and others around the same time. Moureau (1935b) found, however, that the "anti-O" reacted with A and B cells from persons who were known to be homozygous, and he said that the serum could not distinguish AA from AO nor BB from BO. It was found, too, that some AB cells could react with the so-called "anti-O", as well as with Eisler's Shiga immune serum. Eisler (1930) had found that serum from goats immunized with Shiga bacilli had agglutinins for the bacteria as well as for human red cells. Absorption with red cells did not remove the bacterial agglutinin, but absorption with the bacilli removed all the agglutinins. The common antigen was not identical to the Forssman antigen (Eisler, 1931). Thomsen (1932) thought that erythrocytes did have a specific agglutinogen O, which was the product of the O gene. The reactions of "anti-O" with other than O cells were explained by either heterozygosity or by different degrees of dominance. Moureau (1946) again denied the possibility of detecting the genotype of A or B persons using cattle "anti-O" serum, and said that this serum was detecting not a product of the O gene, but a heterogenetic antigen which was not specific to humans. Lambert (1941) did not believe that A and B people could be genotyped using the cattle serum, and said that the O element being detected with the serum decreased progressively in cells from type O to type AB. This finding was in complete agreement with Hirszfeld's so-called "pleiade theory" (Hirszfeld and Kostuch, 1938; Hirszfeld, 1947). According to this view, O is the primary evolutionary form, and the oldest antigen. A and B forms, and their so-called subgroups, are mutants of O in various mutational stages, the final one of which is called "complete". Mutation is occurring along two separate, parallel lines, one leading toward "A complete" and the other toward "B complete". The cells of people are classified according to how much of the "residual" O is present, and those having comparable amounts constitute a "pleiade". The subgroups represent different pleiades, and the notion of subgroups, Hirszfeld suggested in 1947, should be dropped, and replaced by this idea. At the end of the mutational line, where residual O substance is absent altogether, an "anti-O" in human serum is a possibility, but since this condition is so rare, "anti-O" antibodies are very rarely found in human sera. Lambert (1941) could confirm that the "anti-O" component of the goat *Shiga dysenteriae* serum is removed by absorption with the bacteria, as is also the "anti-O" of cattle serum. The O component of human cells is thus immunologically related to the bacterial antigen, and Lambert suggested that it was in fact a heterogenetic antigen, analogous to the Forssman antigen.

In 1948, Boorman *et al.* described a serum from a "Mrs. G.", who was A, Rh+, which reacted with all O cells, many A and B cells, a few AB cells, but not at all with cells of group A_1B. The serum was thought to be specific for O and A_2. The results obtained with 500 blood samples were in close agreement with what was predicted if the serum were reacting with the product of the O gene. The animal sera, on the other hand, were thought to be detecting a different substance, which corresponds to Hirszfeld's basic factor. In consultation with Dr. Morgan, it was proposed to call the basic substance being detected by the animal sera "H substance", and the reagents which detected it "anti-H". The "H" stands for "heterophile". Morgan and Watkins (1948)

conducted a number of experiments using "Mrs. G" serum and a few other sera which had become available. In addition, a cattle serum absorbed with A_1B cells was studied, along with a rabbit immune serum prepared by injecting rabbits with a purified human H substance. This last had been obtained by Morgan and van Heyningen (1944) from ovarian cyst fluid from group O women and purified by Morgan and Waddell (1945). This purified material inhibited the reaction of O cells with a rabbit antiserum prepared against it, and it also inhibited the reaction of absorbed cattle serum with O cells. The experiments of Morgan and Watkins (1948) indicated that certain of the human anti-O sera were, in fact, reacting with the O gene product. The serum could be inhibited in its reaction with O cells by soluble substances from human ovarian cyst fluids and erythrocytes. These soluble substances were suggested to be O gene products, and were said to be secreted only rarely. Those sera which detect H, and the cattle and immune rabbit and goat sera, are inhibited by the so-called "O substance" secreted in saliva and body fluids of all secretors regardles of blood group. The specific human "anti-O" sera, however, are only inhibited by the rare soluble substance which appeared to be the O gene product. The previously designated "anti-O" sera should, therefore, be termed "anti-H", and the designation "anti-O" reserved for reagents like "Mrs. G" serum. It was suggested that Hirszfeld's theory could be modified to accommodate this-information by considering H as the primary gene which gives rise to basic H substance. H substance is then an evolutionary precursor of O, A and B substances, and H gene is in the process of mutation to pure, or complete, O, A or B. Thus, most red cells come from transitional forms, and have variable amounts of H substance on red cells.

In 1949, Grubb obtained antisera from chickens by immunization with "O substance" from group O secretors, or with Shiga bacilli, which reacted preferentially with group O red cells. These had all the characteristics of anti-H reagents, and Grubb said that these should be considered as anti-H (Grubb, 1949 and 1950). Jonsson (1944) had discovered an eel serum with a high titer for O cells, and this was one of the best anti-H reagents available, according to Grubb's results. Eel anti-H was very useful in discriminating group O secretors from nonsecretors.

There are a number of plant seed extracts which have anti-H activity, such as those of certain *Ulex*, *Cystisus*, *Lotus* and *Laburnum* species (Renkonen, 1948; Cazal and Lalurie, 1952). There are usually called "lectins". The discussion of group substances will be concluded in section 19.9, dealing with the nature and biosynthesis of these materials. Our present understanding of the biochemical genetics of the ABO system (section 19.9) makes it quite clear that the *O* gene is, in fact, silent, in the sense that it does not make a product that is responsible for the synthesis of any ABO (H) substance. However, the gene may make a product, which is immunologically related to the products of the *A* and *B* genes (see in section 19.9.3).

The material in section 19.4.2 is excellently reviewed and enlarged upon by Watkins and Morgan (1955).

19.4.3 Isoagglutinins in body fluids other than serum

In 1919, Bond carried out experiments in which he looked for isoagglutinins in a number of body fluids, but, based on some observations he had made on blood, he was operating under the assumption that drying samples down, pulverizing them and then reconstituting them, or else simply exposing them to mechanical friction had some enhancement effect on the isoagglutinin content. The results with body fluids were not very clear-cut. Kirihara (1924) reported finding isoagglutinins is pleural and pericardial fluids, but not in cerebrospinal fluid. In 1928, Yosida conducted an extensive investigation of isoagglutinins in body fluids and secretions, and found them to be present in many cases. In most examples of tears, saliva, seminal plasma, and in pleural and pericardial fluids, isoagglutinins were found. They could be found in urine too, if it were first concentrated to ⅕ to ⅐ or its original volume (Yosida, 1928a and 1928b). Schwartzmann (1928) found isoagglutinins in vaginal secretions, and in ovarian and vaginal cyst fluids. In milk and colostrum were found isoagglutinins corresponding to those in the serum. Happ (1920) said that isoagglutinins occurred in human milk and corresponded to those in the serum. Heim (1926) agreed with this finding. Hara and Wakao (1926) reported that milk and colostrum can contain a nonspecific general hemagglutinin in some cases, and they found isoagglutinins corresponding to the serum in about half the samples examined. Hirszfeld (1928) found that isoagglutinins were present in the milk of group O mothers, but lacking in about half group A or group B mothers.

Yosida's observations on the presence of isoantibodies in the saliva (1928a and 1928b) have been confirmed, but with some modifications. Some of the Japanese investigators have tended toward the view that the isoagglutinins were inherited, or that the ability to secrete them in saliva was inherited. Prokop (1961) indicated, however, that "nonsecreting" parents could have "secreting" children, and that the presence of isoantibodies in saliva is much more frequent in group O people than in A or B people. In addition, the antibodies from O subjects can be of the cross-reacting type in some cases (Prokop, 1963). Putkonen (1930) had observed that isoagglutinins were most frequent in group O salivas as well. These observations which have been confirmed by others do not support a genetic basis for the phenomenon. Boettcher (1967c) found agglutinins in a significantly higher percentage of O salivas than in A or B ones, and a higher incidence of anti-B in A_2 than in A_1 saliva. Jakobowicz *et al.* (1966) got similar results. Schlesinger and Osińka (1964) found the very same thing, and in addition, they reported that a lower percentage of pregnant women showed aggutinins in saliva. Bell and Fortwengler (1971) found anti-A and anti-B in the whole salivas of most group O males. The titer of the agglutinins increased upon immunization with A and B substances. They thought that the agglutinin activity was due primarily to secretory IgA (see

below). There was not a good overall correlation of saliva with serum titer in the same person, and it was said that the secretory system is probably independent of serum titer. As Prokop (1961) showed, there are sometimes correlations between serum and salivary agglutinin titers, so that they do not seem to be completely independent, yet people with high serum titers can lack salivary agglutinins altogether. Matsuzawa *et al.* (1972) noted agglutinins in about ⅔ of the O, A and B subjects they examined, but said this percentage is much higher if the agglutination test is carried out in colloidal media, such as PVP.

The first report of isoagglutinins in tears appears to be that of Hegner (1916). He found the antibodies in 3 of 20 people suffering from relapses of typhoid fever, but there were no agglutinins in 20 healthy people who had been immunized against typhoid, although their serum showed normal isoagglutinin activity. Putkonen (1930) found isoagglutinins in the tears of 5 out of 21 O, A and B subjects, in whom he induced the lachrymal response with onion slices or with bromacetone. Prokop *et al.* (1963) looked for isoagglutinins in tears by placing strong test cell suspensions into the subjects' eyes, and examining the lower lid area for microscopic agglutination. Isoagglutinins did not always occur, but those which did corresponded to the serum content except that, as in saliva, O persons may sometimes have either anti-A or anti-B and not both.

Isoagglutinins have been found in cervical mucus as well. Gershowitz *et al.* (1958) reported that 17 of 77 women showed isoagglutinin activity in their cervical mucus, and 15 of these were group O. This work was expanded upon (Solish *et al.*, 1961) in studies on 128 subjects. When multiple samples were collected from the same subject, the percentage showing agglutinins in at least one of the specimens was 63.4. The occurrence of antibodies was much greater in persons of group O. The presence of the agglutinins was not dependent on the phase of the menstrual cycle, nor on the secretor status of the subject with respect to blood group substances. Parish *et al.* (1967) confirmed the presence of isoantibodies in cervical mucus, and found immune type anti-A hemolysins in some samples as well.

IgA is the most abundant immunoglobulin in saliva and colostrum, in which it is dimerized and connected to a smaller "secretory" component. The anti-A and anti-B of these fluids is most probably made up of secretory IgA, which has been detected in urine and nasal mucus as well. Secretory IgA differs from serum IgA in containing the "secretory piece" (Mollison, 1972; Zmijewski and Fletcher, 1972).

19.5 Quantitative and Physicochemical Approaches

Much of the work on the physical chemistry of the isoagglutination reaction was first carried out by the Filitti-Wurmser group in France, and reported in a lengthy series of papers beginning around 1947. It could be established that the isohemagglutination reaction is a reversible, equilibrium reaction, amenable to the usual kinds of mass action and kinetic treatment. Filitti-Wurmser and Jacquot-Armand (1947) carried out a series of experiments on the agglutination of B cells by anti-B (A serum), in which a known number of cells were incubated with a constant amount of antiserum, and percentage agglutination scored by determining the number of free cells remaining after the reaction had achieved equilibrium. The same yield of agglutination was obtained when the reaction was carried out at 37°, or in two stages, the first being at 4°, and the second at 37°. Similarly, the same yield was obtained when dilution was used as a means of dissociating the agglutinates. In studies on the temperature dependence of agglutination, it was observed that the maximum number of cells agglutinated at a particular temperature varied directly with the total number of cells present, and that the number of agglutinated cells present decreased as a function of temperature between 4° and 37°. The maximum number of cells agglutinated at 4° was designated N_4, and the same value obtained at 37°, all other conditions being identical, was designated N_{37}. It could be shown that sera differ in their ability to bring about agglutination as measured by the N_4/N_{37} ratio. Anti-B sera from A_1O, A_1, A_2 and O individuals differed in this property (Filitti-Wurmser *et al.*, 1950 and 1954). In a somewhat more complicated measurement, designed to give a measure of the dissociation constant for anti-B with B cells, differences between the four different sources of anti-B were also apparent (Filitti-Wurmser *et al.*, 1952). The heats of reaction for the various combinations were determined as well, and differences in this parameter were found to be significant. The reaction is exothermic (Filitti-Wurmser *et al.*, 1952 and 1953a). Measurements conducted with the ultracentrifuge indicated that $\beta(00)$, $\beta(A_1A_1)$ and $\beta(A_1O)$ differed in s_{20} (see in section 1.1.3.4) as well, corresponding to molecular weights of about 177,000, 300,000 and 500,000, respectively (Filitti-Wurmser *et al.*, 1953a). The MW of the $\beta(A_1A_1)$ agglutinin was later revised to about 200,000 (Wurmser and Filitti-Wurmser, 1957). Other physicochemical measurements were carried out as well, all of which indicated differences between the anti-B antibodies. It could be shown further that then $\beta(A_1O)$ isoagglutinin did not behave like an equal mixture of $\beta(A_1A_1)$ and $\beta(00)$ (Filitti-Wurmser *et al.*, 1953b). These studies were reviewed by Wurmser and Filitti-Wurmser in 1957 in a paper written in English. In sum, the experiments indicated that the same isoagglutinin, with the same blood group specificity, differed considerably in its properties depending upon the genotype of the person from whom it was derived. Furthermore, the isoantibodies from persons of a given genotype showed considerable homogeneity in the studies, while it is known that antibodies obtained by immunizing animals show a heterogeneous antibody composition in the resulting antiserum. Kabat (1956) levelled a number of criticisms at the studies of the Filitti-Wurmser *et al.* group, based on some consultation he had had with physical chemists. Reasons were given why some of the data might be deceptive

because of the conditions under which the measurements were done, and thus, why it could have been misinterpreted. Filitti-Wurmser *et al.* did not accept most of the criticism of their results, and replied to it in a paper in 1960.

As noted above (section 19.3.1.5), Wilkie and Becker (1955) devised a modified quantitative hemagglutination assay based on the work of Filitti-Wurmser *et al.*, but which was regarded as simpler. This procedure can be used to test relative antigen strength with a particular antiserum, as was done by Gibbs and Akeroyd (1959) and discussed above. If percentage agglutination in a hemagglutinating system is plotted against antiserum concentration, a sigmoidal curve, not unlike a pH titration curve, is obtained. Since the curves do not always have the same shape, and present difficult computational problems, the data are usually transformed to give a straight line. The linear transformation is accomplished by converting the original function, percent agglutination, to some other function, usually logit or probit, and plotting this against the log of serum concentration. From such lines, the 50% agglutination value can be read or calculated, and the probit transformation is often preferred because it gives a line with the least statistical error. Using this kind of analysis, antisera may be compared as to their relative strength with certain cells, or else a series of cells could be compared using a particular antiserum, as for instance, the comparison of a series of a subtypes with an anti-A serum. The "50% hemagglutinating dose", as it is usually called (abbreviated HD_{50}), may be compared as simple ratios of a standard value to unknown values. Antiserum concentration is often expressed as "mℓ undiluted antiserum/mℓ final dilution". The reciprocal of the HD_{50} can be taken as a measure of antiserum strength. If, for instance, 0.01 mℓ antiserum in 1 mℓ final volume gives 50% agglutination, then one mℓ of that serum may be said to contain 100 HD_{50} units. Another way of expressing relative differences between standard and test samples is to determine the difference in the HD_{50} values and take its logarithm to the base 2. A $\log_2$ difference of 1.0 may be understood as a difference of one full serum dilution, or, in more familiar serological jargon, as a "one tube difference" with the usual doubling dilution technique. it may also be noted that, as a consequence of the sigmoidal dependence of serum concentration on percent agglutination, the percentage agglutination is obviously most sensitive to changes in the agglutinin concentration in the region of 50% agglutination (i.e., the "steep" part of the curve). Experiments at constant antiserum concentration, where maximal sensitivity is wanted, should, therefore, be carried out at serum concentrations close to HD_{50} value. An excellent and understandable review of this material may be found in Solomon *et al.* (1965).

The studies of Salmon and collaborators should be mentioned here. They have employed quantitative hemagglutination methods, essentially those of the Filitti-Wurmser group, to the study of subgroups. The results have given rise to somewhat different designations of the subgroups. In a series of rare weak B variants, for example, the percent cells agglutinated by a constant concentration of a particular anti-B is compared to the same parameter for a "normal" B (Salmon and Reviron, 1964; Salmon *et al.*, 1964). The variants are designated by numbers, representing those percentages relative to an ordinary B, which is set at 100%. Thus, an ordinary B is B_{100}, and the variants are B_{80}, B_{60}, etc. This kind of analysis is thought to be more precise, and the designations more informative than the more descriptive usages. Similar analysis has been applied to weak A types, as for example the case of an $A_{1(80)}$ described by Salmon *et al.* (1965). For reviews of this work, see Salmon (1965 and 1969) and Salmon *et al.* (1973). It may be noted, too, that these quantitatively-based designations fit in well with Hirszfeld's pleiade theory, discussed in section 19.4.2.

19.6 The Bombay Phenotype

In 1952, Bhende *et al.* described the blood of three people in Bombay, which possessed a most peculiar set of ABO system characteristics. The serum agglutinated every sort of cell tested with it, and was shown to contain anti-A, anti-A_1, anti-B and anti-H. Although they were first thought to be of group O, their cells were not agglutinated by any of the usual reagents, and the cells did not react like cells of any of the four basic blood groups. They did not secrete A, B, H, or Le^b in saliva, but they did secrete Le^a. The Lewis system will be discussed separately. It was thought that these people had some new rare allele at the ABH locus. Ceppellini *et al.* (1952) speculated that the explanation for this phenomenon might lie in inhibitory genes, operating at a different locus. Ceppellini's idea was shown to be fully applicable to a family described in 1955 by Levine *et al.* (1955b). The proposita was an American of Italian descent. Her blood behaved like the Bombay bloods, but the pedigree clearly showed that she possessed an unexpressed normal B gene. It was suggested that the phenotype first described in the Bombay persons be called "O_h", and this usage has persisted. In 1955, Watkins and Morgan wrote with considerable foresight that the presence of H substance might be under the control of an independent allelic pair of genes, *H* and *h*, and that the Bombay type bloods were rare examples of homozygous recessivity at this locus. With the support of the biochemical studies which have since been done, this notion has indeed become the basis of the current understanding of the ABO system. The subject will be returned to in a subsequent section. Further examples of O_h phenotypes were soon found, and it became clear that they could be manifestations of A_1 or A_2 suppression, as well as of B or H suppression. In cases where it could be discovered which allele was suppressed (through family studies), a superscript was added to the O_h designation. The person described above by Levine *et al.* (1955b) would thus be an O_h^B. Lanset *et al.* (1966) demonstrated in a French family with O_h members that the suppressed allele could be detected on red cells by absorption-elution technique. In this way, they detected an O_h^B and three O_h^{AB} people in a family without recourse to family studies. These results and others have to mean that the ABH substances are present on the red cell, in accordance with the genotype, in

some form, even though agglutination with anti-A, anti-B and anti-H cannot be observed.

Rare examples of bloods have been observed which behave in some ways like O_h, but are not identical. These have been referred to by Race and Sanger (1975) as "Para-Bombay phenotypes." The first example of this kind of blood was described by Levine *et al.* (1961d) in a healthy, young Czechoslovakian woman with no history of pregnancy or transfusion. Her cells grouped as A_2, and the serum contained anti-A_1, anti-B and anti-H. Her red cells were anti-H negative, but Le(a+). Le^a was secreted in saliva, but A, B and H were not. This case was interpreted as one of an incompletely suppressed A by a suppressor gene, and it was suggested that the phenotype be called "A_h" by analogy to O_h. If a case of an incompletely suppressed B were found, Levine *et al.* (1961d) said that it should be called "B_h". Examples of B_h have since been described (see Kitihama *et al.*, 1967). Cases are known, too, where cells of phenotype O or A_x did not react with anti-H, but the group substances were secreted in saliva. These have been designated O_m^h or A_m^h. It has been suggested that persons with suppressed red cell antigens, but with group substances present in saliva, be designated instead as O_{Hm}, O_{Hm}^A, O_{Hm}^B and O_{Hm}^{AB}. Race and Sanger (1975) agree with this newer notation, and additional information on these kinds of cases may be found in their discussion.

19.7 Some Other Complexities in the ABO System

19.7.1 So-called "Cis-AB"

In 1964, Seyfried *et al.* reported on a family in which an A_2B daughter of an O mother (father not tested) was married to an O man, and had two A_2B children. These were not ordinary A_2B types, in that the B was weak, and the serum contained some kind of anti-B (not autoagglutinating). A and weak B were secreted in the saliva of these people. Paternity could not be excluded by other systems, and there was every reason to believe that the children were legitimate. Obviously, A and B had been inherited in this family from a single parent. Another family was reported in Japan (Yamaguchi *et al.*, 1965) in which an A_2 and a weak B antigen (called A_2B_3) had been inherited from a single parent. A note was added to this report by Professor Komai, saying that the B gene was weakened in these cases by a position effect, the B gene giving a weaker antigen when in the "cis" position (AB/O) than in the usual "trans" position (A/B). The slash in these parenthetical designations stands for the homologous chromosome pair, and it is clear that a designation like "AB/O" carries with it the assumption that the ABO locus is complex, i.e, that A and B genes are not precisely allelic. Professor Komai had expressed this view in 1950 with respect to both the ABO and the Rh systems. There is ample precedent in classical genetics for very closely linked genes in *Drosophila* and in other organisms. Loci of this kind have been termed "semi-allelic," "partially allelic" and "pseudoallelic." The meaning is the same whatever the term used. Such loci, when carefully studied, turn out to consist of a series of very closely linked "alleles," hereditary units which are adjacent to one another on the chromosome. Crossing over among these "pseudoalleles" does occur; that is the way in which they are detected. Crossing over at such loci is, of course, exceedingly rare. Another family exhibiting the inheritance of A and B from one parent was described by Yamaguchi *et al.* (1966). An A_2B_3 mother and an O father had three A_2B_3 children. These workers referred to this unusual condition, following Professor Komai's suggestion, as "cis AB," in contrast to the usual "trans" AB. A number of "cis AB" A_2B bloods have been reported. They seem to be more common in Japan than in Europe (see Yamaguchi *et al.*, 1970). Reviron *et al.* (1967 and 1968) reported the first cis AB blood that was A_1B. Their results indicated that the B antigen in the cis A_1B complex is part of the normal B antigen. The anti-B in such people is directed against the remaining part of the antigen. Boettcher (1966) discussed modifying the original Bernstein hypothesis in terms of looking at ABO as a pseudoallelic locus, although he did not use that term. One of the possibilities he put forth was that the "alleles" A, A_1, and B are really pseudoalleles. This idea can satisfactorily explain cis A_2B and cis A_1B types by recombination. Understood in terms of what we now know about biochemical genetics, we would say that the ABO locus is "polycistronic." This explanation is very satisfying in biochemical genetic terms. It would be even more satisfying if the relationship between the gene (cistronic) product and the ABO antigen were a little clearer. It is to be noted that the phenomenon can be explained by postulating a rare allele, which gives rise to an antigen having A_1 or A_2 with weak B determinants, but, as Race and Sanger (1975) have rightly noted, this latter explanation is the far less exciting of the two. Finally, Moullec and Chevrel (1959) had reported a family in which an AB with a weak B and some kind of anti-B in serum was segregating in four generations. It seems probable in hindsight that these persons were of the cis AB phenotype. Valdes *et al.* (1978) reported an interesting case from Illinois, in which an A_2B mother had two group O children. She was apparently cis-AB, but with a normal B antigen, and no anti-B in her serum.

Badet *et al.* (1978) have studied the transferase activity (gene product activity) in the sera of a number of cis-AB people and their families (see in section 19.9). The transferase activities differed from those of ordinary people, and from one family to another, but tended to be similar within a family. They favored the explanation that cis-AB is the result of a mutation leading to an enzyme which could transfer both Gal and NAc-Gal-NH_2.

19.7.2 Cross reacting anti-A- and anti-B-like antibodies in group O serum—blood group C

Absorption of A cells with group O serum, and subsequent elution, yields an antibody which reacts with B cells in addition to the expected anti-A. Hektoen seems to have been observing this phenomenon in 1907 (see in section 19.1).

Landsteiner and Witt (1926) described it in detail for the first time. Thus, A cells yielded, upon elution, not only the expected anti-A, but also an antibody that reacted with B cells, after having been placed into group O sera. Similarly, B cells will yield up the expected anti-B, but some antibody that reacts with A cells as well. Dodd (1952) studied this phenomenon, and suggested that certain of the antibody molecules in O sera might have both A and B specificities. It was also found that immune sera, i.e., those O sera having an anti-A or an anti-B as the result of a known frank immunization, are a richer source of the cross-reacting antibodies. Bird (1953) largely agreed with Dodd's explanation and termed the behavior "dual receptor" antibody structure. Bird (1953 and 1954) also drew attention to the often observed asymmetry of the reaction, i.e, that with some O sera, A cells may absorb and yield anti-B upon elution, whereas B cells will not absorb and yield up any anti-A that is detectable. Equal mixtures of anti-A and anti-B do not exhibit the cross-reacting behavior. In 1955, Rosenfield noted that in a large sample, most mothers of incompatible infants were of group O, and it could be shown that the cross-reacting antibody crossed the placenta more easily than do ordinary anti-A from B serum or anti-B from A serum (Rosenfield and Ohno, 1955).

There is no completely satisfactory explanation for these observations, and there is certainly not general agreement on an explanation. The different hypotheses that have been given to account for the observations can be conveniently grouped into four categories (Race and Sanger, 1975). The explanation noted above, that some of the antibody molecules in group O sera can have anti-A as well as anti-B specificities, was put forth by Dodd (1952) and supported by Bird (1953 and 1954). Dodd (1957) gave further experimental support for the view, indicating that the cross-reacting antibodies (which were called anti-AB) showed dual specificity with animal cells possessing A- or B-like receptors. Jones and Kaneb supported this view as well, using a different technique designed to detect a minor population of red cells in a mixture. This technique was first applied to Rh antigen studies, and later to ABO. As an example, suppose one wanted to detect a minor population of A cells in a mixture of many O cells. Detector cells consisting of a cells sensitized with anti-A, but not agglutinated, would be added to the mixture. Agglutination upon addition would mean that A cells had been present in the mixture (Jones and Kaneb, 1959). The technique could be used to titrate the cross-reacting antibodies in O serum without absorption and elution procedure by linking A cells to B cells with antibodies in group O serum (Jones *et al,* 1959). The technique was applied to the study of cross-reacting antibodies (Jones and Kaneb, 1960) and they appeared to be reacting with an antigen homologous to A and B. The asymmetric behavior of the cross reacting antibodies was also noted.

A second explanation of the observations postulates the existence of a third antibody in group O serum, usually called anti-C, along with the notion that A, B and AB cells have the corresponding C agglutinogen. Moss (1910a and 1910b) formulated an explanation of the ABO system with three agglutinogens and three agglutinins, but the view was gradually abandoned as subgroups of A were disclosed. Koeckert (1920) did not agree with the Moss scheme based on his experiments. A number of the Japanese investigators have accepted the idea of a third agglutinogen-agglutinin pair for quite some time (Furuhata and Matsunaga, 1950). They believed that the isoagglutinins, including anti-C, were inherited as well. In this country, Wiener has supported the idea. It must be noted that Wiener had a slightly different immunological conception of the system (and of other systems, too, such as Rh and MN) than do others, and he has employed nomenclature devices to keep the distinctions clear. An "agglutinogen" is a definable substance on the red cell surface, and these are designated by ordinary Roman capital letters. Agglutinogens are antigenic, but they may have multiple antigenic determinants, i.e., they may give rise to more than one kind of agglutinin if injected into an animal. The individual antigenic specificities associated with an agglutinogen are called "blood factors," and are denoted by upper case boldface Roman letters. Agglutinogens in Wiener's view, therefore, are or can be complex antigens. Blood factors are not definite substances, but may be particular intramolecular arrangements which, under a specified set of conditions, are bound by an antibody recognizing the constellation and causing agglutination. Since the antibodies are regarded as being against the blood cell factors, they are designated with the upper case bold face symbols (anti-**A**, anti-**B**, etc.). The *genes* which code for the agglutinogens are denoted by *italic type,* as are genotypes. Wiener's conception of the ABO system is summarized in Table 19.5. According to this, O serum does not contain "cross-reacting" antibody, but anti-C, which reacts with the C blood factor in A, B and AB cells. The scheme has further implications. One can imagine four additional ABO blood groups, as indicated in Table 19.6. At the time when the first major paper on the subject came out (Wiener, 1952), Wiener thought that an example of blood group C (first row of Table 19.6) had been observed by Dunsford and Aspinall (1952). They had called their blood sample an A_4 (it is discussed in section 19.3.1.3), but Wiener said that the blood possessed all the features of a group C, i.e, the serum contained anti-A and anti-B while the cells reacted like group O to ordinary grouping sera. Her cells were strongly agglutinated by all group O sera, however. Some agglutination was seen with potent anti-A reagents. As for the hypothetical blood groups, Wiener said that they should be readily recognizable if encountered. The difficulty with anti-C was in obtaining it separately from anti-A and anti-B (Wiener *et al.,* 1953c). In 1973, Wiener *et al.* said that sera containing anti-C and anti-A could be produced by frank immunization of O persons with blood group A substance originating from pigs. Because of the anti-A content, these reagents were said to be useful for testing cells which had C but not A. Cells having A and C would have to be tested with a serum prepared by immunization of O people with B substance. Jones and Kaneb

Table 19.5 ABO System as Conceived by Wiener

Blood Group	Genotype	Red Blood Cell Agglutinogen	Red Blood Cell Factors	Serum Isoantibodies
O	*OO*	–	–	anti-A, anti-B, anti-C
A	*AA*	A	A + C	anti-B
	AO	A + O	A + C + O	anti-B
B	*BB*	B	B + C	anti-A
	BO	B + O	B + C + O	anti-A
AB	*AB*	A + B	A + B + C	None

(1960) thought that if the cross-reacting antibody were, in fact, a third agglutinin anti-C, that it should behave symmetrically toward A and B cells, and they used the asymmetric behavior as an argument against blood group C. Bird (1954) noted, however, that the asymmetry could be explained on the basis of differences in titer of the various antibodies in serum, and could be accommodated by the C/anti-C hypothesis. At the time, he did not regard blood group C as having been established, however.

Kabat (1956) stated a third explanation, based in part on the experiments and discussion of Owen (1954). Since there are close similarities in structure between A, B and O substances, then an individual of group O might form some antibodies of anti-A and anti-B specificity which recognize molecular constellations common to both A and B. Kabat believed this to be the most attractive explanation, and the most satisfying to an immunochemist. Schiffman and Howe (1965) agreed with this explanation. Franks and Liske (1968) interpreted the results of their studies on mixed agglutination between red cells and buccal epithelial cells with cross-reacting antibodies, and its inhibition by soluble A and B substances, to mean that the cross reacting antibody was recognizing a common portion of the A and B antigens, and that it had a lower affinity for A and B sites than do anti-A and anti-B from B and A people.

The fourth explanation is based on the observation that antibodies are said to be able to be absorbed in a nonspecific way by an antigen-antibody complex (Ogata and Matuhasi, 1960). Thus, β can be taken up, and subsequently eluted, from A cells to which anti-A is already bound, and conversely for α. Ogata and Matuhasi say that an equal mixture of anti-A and anti-B behaves exactly like the anti-A,B of an O serum in their experiments. The existence of cross-reacting antibodies is denied (Ogata and Matuhasi, 1962). The non-specific absorption has been called the Ogata-Matuhasi phenomenon. The existence of blood group C is denied by these authors on the basis of experiments in which fluorescent A or B cells do not form mixed agglutinates in the presence of anti-A,B, which, they say, should occur if a third agglutinogen were in fact present. The non-specific uptake of blood group antibodies by cells coated with specific antibody has been confirmed by Bove *et al.* (1973) using ^{125}I and ^{131}I labelled antibodies, but the coating of the cells with specific antibodies did not increase the non-specific uptake. While this study shed considerable light upon the Ogata-Matuhasi phenomenon, it did not specifically deal with the phenomena being discussed in this section.

Dodd *et al.* (1967) have shown that the "cross-reacting" antibody obtained from O mothers who have had A children is different from the one obtained from O mothers who have had B children. This must indicate that there are two types of cross-reacting antibodies, and they said that these appeared to be directed at a common portion of the A and B antigens, which includes the C-2 substituent of the terminal sugar (see further on in the discussion of the biosynthesis of ABH substances in section 19.9). Lincoln and Dodd (1969) have reiterated this theme in their subsequent studies on the phenomenon.

Table 19.6 Additional Blood Groups Predicted by the C/anti-C Hypothesis

Blood Group	Red Blood Cell Agglutinogen(s)	Red Blood Cell Factors(s)	Serum Isoantibodies
C	C	C	anti-A + anti-B
A′	A′	A	anti-B + anti-C
B′	B′	B	anti-A + anti-C
A′B′	A′ + B′	A + B	anti-C

19.7.3 Acquired B

Although acquired B appears to be a rare event, it is an important one in that it illustrates the close similarity of bacterial cell wall structures to that of the ABH blood group substances. Red cells, almost always in group A_1 people, can "acquire" B under certain conditions of old age or disease. The cells then type as A, B weak.

In 1959, Cameron *et al.* reported on seven bloods they had been examining over the course of four years. The people were all genotypically A or A_1O, and suffered from a variety of diseases, often cancer of the colon or rectum, or they were quite old, or both. Their serum had the expected anti-B, and the secretors secreted A and H but not B. The cells were agglutinated, though, by some anti-B reagents. It was suggested that the B-like receptor on the cells was acquired, not inherited, and it was noted as being significant that none of the seven were of group O. Giles *et al.* (1959) reported a completely similar case in a patient with carcinoma of the colon who was A_1A_2. Marsh *et al.* (1959) described a group A cancer patient who had acquired B (which was referred to as "pseudo B"). The B reactions had all but disappeared when the cells of this person were examined postmortem. They noted that a "pseudo B" could be produced *in vitro* by allowing cells to react with a bacterial enzyme (T-activating enzyme). This treatment rendered the cells polyagglutinable, so that the presence of the B-like antigen had to be detected by absorption and elution.

In 1956, Springer had noted that certain extracts of higher and lower plants contained substances that were A-like, B-like or H-like, in that they could inhibit the isohemagglutination reactions in much the same way as the secreted blood group substances. *Escherichia coli* O_{86} was found to possess a high B-like activity. It soon became clear that the B-like activity in the *E. coli* O_{86}:B7 resided in an extractable lipopolysaccharide fraction (Williamson and Springer, 1959). The soluble B-like substance from *E. coli,* as well as from a variety of other microorganisms, could be found in the culture media. Furthermore, these soluble substances, as well as the partially purified lipopolysaccharide and protein-lipopolysaccharide fractions from *E. coli* O_{86}:B7, could coat human red cells, thus rendering them sensitive to anti-B. The treatment did render the cells polyagglutinable, but the B-like activity could be detected by absorption and elution, and the treatment had no effect upon the genetically determined blood group antigen present on the cells. Both O and A cells could be coated (Springer and Ansell, 1960; Springer and Ansell-Hahn, 1960). Stratton and Renton (1959) had noted, in reporting a case of

acquired B by an A_1 cancer patient, that red cells take up *E. coli* polysaccharide *in vitro,* and are thus rendered B-like. They suggested that this was probably the mechanism of the acquired B phenomenon. Springer and Ansell-Hahn (1960) noted that some coating of red cells by the *E. coli* substances had occurred *in vivo* in sick children, but they thought that there might be some plasma factors which inhibit such uptake in healthy people. It is known that *E. coli* O_{86}:B7 have an A-like antigen as well as the B-like one (Pettenkofer *et al.,* 1960; Gonano *et al.,* 1961), but there are no cases of "acquired A." It is also quite well established (Springer and Ansell, 1960; Springer and Ansell-Hahn, 1960) that the B-like substances can coat O cells as well as A cells *in vitro.* Marsh (1960) could produce a B activity in A or O cells *in vitro* with a potent T-activating enzyme of bacterial origin, prepared by Dr. Friedenreich. The B-producing activity was separable from the T-producing activity, and the two had different thermostability properties. There have not, however, been reported cases of acquired B in persons of group O. The majority of acquired B cases were of group A_1, with a few being A_2. Marsh (1970) noted that A_1 cells, transfused into an A_1 individual who had acquired B, and then recovered, had themselves acquired the B within 48 hours. The transfused cells were recovered, incidentally, by using cells of a different MN type. If O cells were transfused into the same subject, and recovered, however, there was no acquired B antigen.

Iseki (1977) gave an excellent discussion of the ABH-like substances in living organisms other than human beings. Many vertebrate red cells react with anti-A, anti-B and/or anti-H, as demonstrated by Tumosa (1977a).

It can be said that acquired B is most often associated with old age and/or disease, frequently carcinoma, that it usually occurs in A_1 people, and that it is very rare *in vivo.* To clinical serologists, and those who deal with fresh blood, acquired B is primarily an instructive curiosity, seldom if ever encountered. In forensic blood grouping, and especially in body fluid grouping work, however, "acquired B" has a somewhat different meaning (at least in terms of coming about *in vitro* rather than *in vivo*) and must be taken much more seriously. This matter will be taken up under the heading of medico-legal applications of the ABO system (section 19.10).

19.8 The Secretor System

19.8.1 Group specific substances in body fluids

The occurrence of soluble A, B and H group specific substances in body fluids was recognized clearly in the 1920's. It was later found that the presence of these materials in body fluids is under discreet genetic control. In most populations, the majority of people are "secretors," having substantial amounts of soluble group specific substances in their body fluids. The "non-secretors" do not have the large amounts of these substances in their body fluids. The secretion property has had broad implications for the understanding of the ABO system, as will be discussed in the section on the nature and biosynthesis of the ABH substances. Since body fluids other than blood are often encountered in criminal cases, the presence of group specific substances in them has important medico-legal ramifications as well. Further, since the secretor property is inherited, it may be used as an additional genetic marker in cases of disputed affiliation. The medico-legal aspects are discussed in a subsequent section.

Credit for the first recognition of group specific substances in body fluids other than blood belongs to the Japanese investigator Saburo Sirai in 1925. He was mentioned in this regard by K. -I. Yosida (1928b). The reference to Sirai's studies is: *Hokkaido Igaku Zasshi* 3(2):25-73 (1925). Schiff (1924b) had observed soluble A substances in the serum of A and AB people by noting that anti-A red cell serum from rabbits gave a precipitin reaction and a positive complement fixation test with these sera. Dervieux (1921 and 1923) had done some experiments in which he showed that an anti-human semen serum gave a stronger precipitation reaction with the seminal plasma against which the antiserum had been raised than with samples from other individuals (see in Section 10.5.1). Sussmann (1925) could not confirm this finding, nor several other assertions that Dervieux had made. He did notice some individual-specific serological differences in seminal plasma, though, and speculated on the possible presence of group specific substances. In 1926, Yamakami established that seminal plasma, as well as saliva and vaginal secretions, contained group specific substances. Seminal plasma from a group A individual could inhibit anti-A activity in either O or B serum, and had no effect on A serum. Group AB semen could inhibit both anti-A and anti-B activity. Yamakami said that saline extracts of four month old dried seminal stains had the same specific hemagglutination inhibition properties, and that this fact should be significant for medico-legal investigations. Landsteiner and Levine (1926a) tested spermatozoa for the group specific substances, and found that they were present, and corresponded to the red cell group. The observations on seminal plasma were confirmed by Krainskaja-Ignatowa (1929) and by Steusing (1930). Brahn and Schiff (1929) found group specific substances in saliva, in stomach and duodenal material, and in urine if it were concentrated and dialyzed. Yosida (1928a and 1928b) confirmed the presence of the group specific substances in a wide variety of secretions, including saliva, semen, tears and pleural and pericardial fluids. Likewise, Thomsen (1930a and 1930b) reported group-specific substances in serum, organ cells, especially tumor cells, leucocytes and in urine if it were concentrated. Brahn and Schiff (1929) detected the A and B substances in human milk as well. In 1930, Lehrs conducted extensive studies on the group specific characteristics of saliva, working under the direction of Professor Schiff. The saliva of 40 different individuals from all four blood groups was studied by the inhibition technique. On occasion, O saliva slightly inhibited anti-A and anti-B, but in most cases not at all. Inhibition of the A reaction by A saliva, and the B reaction by B saliva, was shown to be quite strong and

completely group specific. Saliva from AB persons inhibited both reactions. There were considerable differences in the group specific substance content of saliva from different persons, as indicated by the differing degree of inhibition observed by titrating the antiserum against constant amounts of saliva, or by titrating the saliva with constant amounts of antisera. In a few cases, inhibition was very weak (the people were probably nonsecretors). There seem to have been fewer non-secretors in the population he studied than would be expected on the basis of what we now know about their distribution in the population. There was little variation in the same individual over about 5 months time. Immunization with saliva produced the corresponding group specific immune agglutinin.

Another major study on group specific characteristics in body fluids was carried out by Putkonen in 1930. Studies were conducted on the presence of isoaggutinins in body fluids (see in section 19.4.3). The presence of isoagglutinogens was established in saliva, urine, tears, semen and in the amniotic fluid (corresponding to the blood group of the child). The tests were done quantitatively, either with doubling dilution titration of antisera at constant body fluids concentration, or conversely. Serum dilutions were carried out to 1:512, and body fluid dilutions were carried out to as much as 1:4000 in some cases. The group specific substance content varied among different individuals. Further, it tended to be high in saliva, semen and amniotic fluid, low in tears and urine. Some variation was noted in samples collected from the same individual at different times. In about 14% of A, B and AB people, group specific substances were absent. In AB people, a majority showed similar amounts of A and B, but in six cases out of 32, the A and B content was unequal. Gibb (1965), using an improved technique, noted that there is more group specific substance in A and B secretor urine that Putkonen had thought. There were difficulties in assessing group O.

Likewise in 1930, another major study was published by Schiff in Berlin. The presence of group substances in urine, saliva, gastric and duodenal juices, bile, milk and in a number of organs was confirmed. Schiff concentrated on studying the nature of the group substances as well. Most of the secretions and organs contained these materials in water soluble form. But alcohol soluble fractions could be found in red cells and in a number of organs. The water soluble group substances from saliva and urine particularly were thermostable and nondialyzable. They did not give positive reactions with simple chemical tests for protein, carbohydrate or "lipoid". Both water- and alcohol-soluble fractions of A substance inhibited A-specific hemolysis, but they showed differences in behavior in the complement fixation test. It was not clear whether the two fractions were chemically different, or whether the differences reflected the chemical environments in which they were located.

In 1937, Friedenreich put forward the idea that group substances in the body fluids were synthesized locally, in the cells of the organ secreting them. A "nonsecretor" individual was one in whom this synthesis did not take place. This notion contradicted Schiff's original idea that the group substances were present on red cells in alcohol-soluble form and that they were excreted into body fluids, being altered to a water-soluble form in the process. High concentrations of water soluble group substances were found in saliva, gastric juices, bile and seminal plasma, and in the mucous membranes and glands of the digestive tract in Friedenreich's studies. The parotid gland, liver and colon had relatively little group substance, and the lowest concentrations were found in brain and testis. The alcohol soluble antigen content of various organs was studied, and the results indicated that the alcohol soluble substances were quite distinct from the water soluble ones. Secretions do not have the alcohol-soluble substances. The content of alcohol soluble group substance in organs is independent of secretor status, and was said to be independent, therefore, of the secretor system. Friedenreich and Hartmann (1938) carried on these studies. They tested a variety of body fluids for antigen content (the highest doubling dilution of the body fluid which would inhibit an appropriate antiserum with a titer of 1:4), and they tested serum for group specific substances as well. The idea behind the experiments was to see whether there was enough A, B or H substances in circulation to account for their presence in body fluids by simple transfer, and there was clearly not enough. The inevitable conclusion was that the group substances in body fluids had to be synthesized by the cells secreting these fluids. A number of organs from corpses were investigated in this study, and a correlation was found between the group substance content of an organ and the content in its secretion. Studies on this subject were pursued by Grethe Hartmann, and the results communicated in a lengthy monograph (Hartmann, 1941).

19.8.2 Inheritance of the secretor characteristic

By 1932, it was clear from the work of Brahn and Schiff (1929), Putkonen (1930), Sasaki (1932) and others, that the secretion characteristic was a relatively consistent feature in an individual, but that there were individuals within all the blood groups in whom group substances appeared to be absent in body fluids. In 1932, Schiff and Sasaki established that the trait was indeed hereditary. "If one chooses an experimental arrangement which deliberately neglects minor differences, one obtains two sharply distinct types for the A and also for the B secretors and nonsecretors," they wrote. Similar behavior was found with AB people, and, using cattle serum absorbed with AB cells (which they called anti-O, but which we would now call anti-H—see in section 19.4.2), a secretor-nonsecretor distinction could readily be observed in group O salivas as well. In 50 families, it was clear that secretor children had to have at least one secretor parent, and that nonsecretor parents did not ever have secretor children. In 51 pairs of twins, of whom 21 were identical, 7 discordant pairs were found, and these exclusively in fraternal twins. The property of secreting was dominant, and designated "S", while its allele was called "s". *SS* and *Ss* persons are secretors; *ss* persons are nonsecretors. The original German words for secretor and nonsecretor

(*Ausscheider* and *Nichtausscheider*) have sometimes been translated as "eliminator" and "noneliminator" in the older literature. Among the 369 people studied, 30.9% were nonsecretors. The gene frequencies were 0.444 for *S*, and 0.556 for *s*. Additional family studies over the years which followed fully confirmed the mode of inheritance of the secretor characteristic postulated by Schiff and Sasaki. Wiener (1943e) had collected together the data of a number of other workers, including Schiff and Sasaki, for 185 families with 486 children. Of these, 18 families showed the informative nonsecretor x nonsecretor mating, and all 42 children were nonsecretors. Andersen (1951) studied 154 matings with 370 children, this material containing six nonsecretor x nonsecretor marriages, and fully confirmed the mode of inheritance. Prokop and Uhlenbruck (1969) collected the data from 252 additional families and these results were also in complete accord with the postulated inheritance pattern. They gave the references to the family studies of others. They also reported another study by Kerde in 1961 on 105 additional families with 3 informative matings. The designations *S* and *s* were originally applied to the secretor genes. This usage persisted in the literature for about 20 years. In 1947, a new blood group factor was discovered by Walsh and Montgomery in Australia, and found to be associated with the MN system. The new anti-body was named anti-S by Sanger and Race (1947) after some consultation with Prof. Fisher and Dr. Ford (see Section 21.2). Race and Sanger (1950) said that in assigning the symbol "S" to the new MN-associated factor, they had simply overlooked the fact that the designation was being used for "secretor". The duplication apparently caused no confusion, but in 1955, Levine *et al.* called the secretor alleles "*Se*" and "*se*" (Levine *et al.*, 1955b), Race and Sanger adopted this usage in 1958, and it has persisted since that time. It is now known that the *Se/se* locus is linked to the Lutheran blood group system locus. The linkage was discovered by Mohr (1951a and 1951b) who thought, however, that *Lu* was linked to Lewis. The relationship of the Lewis system to secretion will be discussed in the section on the Lewis system. The *Lu*:*Se* linkage has since been confirmed (Greenwalt, 1961; Lewis *et al.*, 1977).

19.8.3 Further studies on group substances in body fluids

19.8.3.1 Saliva. Considerable attention has been focused on group substances in saliva because it is so easy to obtain. Once it had been recognized that the secretor characteristic had a definite genetic basis, there was interest in using it as a genetic marker. A number of the studies which have been carried out indicate that the secretor characteristic may not be quite as simple and straightforward as it first seemed to be, at least in some cases. It is quite likely too that variations in findings from different laboratories may be attributable, at least in part, to differences in technique.

Wiener and Kosofsky (1941a and 1941b) conducted a series of quantitative studies on A and B group substances in saliva. They showed that the inhibition characteristics of secretor salivas were very much dependent on the antiserum being used. This fact was observed with anti-A as well as with anti-B reagents. There was little variation in group substance content from secretor saliva of the same person over time, and not very much difference from one secretor to another. Little difference was found in the amount of A secreted in saliva by persons whose cells were of subgroups A_1, A_2 and A_3. Gammelgaard (1942) was not in agreement with these findings, saying that the amount of A substance secreted in saliva decreased in going to weaker and weaker A subgroups. Secretion was so low in A_4 individuals, he said, that secretor status could not be reliably determined.

McNeil *et al.* (1957a) raised the point that there are some people of blood groups A or B who secrete A or B but no H (as detected by *Lotus tetragonolobus* lectin), while others of group A_1, and especially of group A_2, secrete H but fail to secrete an A which will inhibit the anti-A reaction with A_1 cells. Such persons were termed "aberrant secretors". A substantial number of them were noted among couples in which the women had experienced spontaneous abortions caused by blood group incompatibility (McNeil *et al.*, 1957b). McNeil *et al.* (1960) pointed out that a number of A_2B people do not secrete detectable amounts of A_1 substance in saliva, but do secrete B. Bhatia and Randeria (1970) found the same thing in their study of 194 individuals representing all the blood groups. They used the term "aberrant secretor" to apply to secretors of A and/or B without any H secretion. It was noted that the detection of H in these people depended to a great extent on the anti-H reagent being used. The studies were extended (Randeria and Bhatia, 1971). The lectins from *Psophocarpus tetragenolobus* and *Erythrina subrosa* were very poor anti-H reagents in the inhibition test. The lectins from *Ulex*, *Laburnum* and *Cystisus* were better, but the best reagents were those in human or animal sera. There was more A substance in A_1 than in A_2 secretor saliva, and more in A_2 than in AB saliva. Bhalla and Bhasin (1976) found 13 aberrant secretors among 122 subjects in India, among whom were A people who secreted H but not A in saliva (A−H+), as well as B−H+ and B+H− B persons. AB people were found who were A+B−H+ and A+B−H−. Secretors were categorized as "strong" if they had inhibition titers of $\geqq$ 1:16, "weak" if the titers were $\leqq$ 1:8. It was suggested that a scale be used to score the number of aberrant secretors in populations. Aberrant secretor index (or ASI) was defined as (Number of aberrant secretors/Number of all secretors) X 100. *Ulex* anti-H was used in the study, and it was noted that antisera of the same specificity would have to be used if different populations were to be compared, since results can and do vary with different reagents. Clarke *et al.* (1960) studied the secretor characteristic of a number of sib pairs, and thought that the amount of ABH secreted by secretors was, at least in part, inherited. The distribution of A/H in a series of A secretors was found to behave like a distribution that would be characteristic of a trait controlled by polygenic inheritance. They said that "aberrant secretors" repre-

sented arbitrarily chosen extremes in a continuous distribution of A/H or B/H in populations.

There are differences in the behavior of secretor salivas, depending upon the reagents used to detect the group substances. The point has been made by a number of authors (Wiener, 1943c; Boettcher, 1967b; Randeria and Bhatia, 1971; Bhalla and Bhasin, 1976; Masis, 1964). Many investigators have employed lectins, and the differences in H titer with *Psophocarpus* and *Erythrium* reagents as against those from *Ulex*, *Laburnum* and *Cystisus* (Randeria and Bhatia, 1971) were discussed above. Plato and Gershowitz (1961) found differences in the behavior of various types of secretors toward *Ulex europaeus* and *Cystisus sessilifolius* lectins. The amount of H substance secreted by secretors decreased in the order: $O>A_2>A_1>B>AB$. A_1 saliva showed similar behavior with the two lectins, but A_2 and O salivas were more reactive toward *Ulex* lectin, while B and AB salivas were more reactive toward *Cystisus* lectin. They said that the two lectins were not detecting the same H specificity. Hakim and Bhatia (1965) tested human anti-A serum and lectins from *Phaseolus lunatus* (lima bean) and *Dolichos* with secretor salivas, and found differences in specificity. A_2 secretor saliva showed better inhibition of the lima bean reagent, while A_1 secretor saliva better inhibited human anti-A_1. Boettcher (1967b) found that the mean titer of A_1 saliva with anti-A or with *Dolichos* lectin was significantly greater than that of A_2 saliva. With *Ulex* lectin, the order was $O>A_2>A_1>B$, different from the order for red cell agglutinability. Boettcher said that the rates of production of secreted group substances controlled by the *H/h* and the *ABO* loci were independent, and that aberrant secretors were people in whom the two rates were significantly different. Boettcher (1967a) showed that the inhibition titer of A_1 and A_2 secretor salivas with *Dolichos* lectin was proportional to the amount of precipitate obtained from those salivas with this lectin. Precipitation could, therefore, be used as a quantitative measure of secreted substances. Using *Ulex* lectin, it could be shown that secreted substances from A_1 secretors have a higher ratio of A/H determinants that those from A_2 secretors. Potapow (1970a) said that extracts of the seed hulls from the seeds of *Evonymus sacrosaneta* and *Evonymus alata* make excellent anti-B reagents in inhibition tests, especially if trypsin-treated B detector cells are used.

Other investigators have used precipitin tests as well. Baer *et al.* (1961) tested the H in secretor saliva with a precipitating serum prepared by immunizing chickens with ovarian cyst fluid from O women. Prokop and Geserick (1972) said that A secretors could be detected by a precipitin test with anti-A_{HP} (*Helix pomatia* agglutinin), while B secretor saliva gave precipitin reactions with saturated extracts of *Evonymus europaea* seeds. Agglutinins from animal sources are sometimes called "protectins", as distinct from those of plant origin (lectins). Active anti-A reagents have been obtained from several snail species, including *Helix pomatia*, *Helix aspersa* and *Cepaea memoralis* (Prokop *et al.*, 1965; Grace, 1969; Grace and Uhlenbruck, 1969). An application of the *Helix aspersa* anti-A_1 reagent to the A subtyping of bloodstains is discussed in section 19.10.4. Grundbacher (1973) showed that lectins from *Cystisus sessilifolius* and *Laburnum alpinus* gave a precipitin reaction with the saliva of all secretors. *Ulex* lectin gave this precipitation as well, but comparative immunodiffusion experiments indicated incomplete identity between the *Ulex* precipitin band and those of the other two lectins (the technique is discussed in section 2.2.2). The lectin from *Lotus tetragonolobus* gave two precipitin bands with secretor saliva, and one band with that of nonsecretors. The new antigen being detected in secretor saliva was called "L" (for *Lotus*), and was different from, though related to H and Le^a. A full paper on the L antigen appeared in the following year (Napier *et al.*, 1974). The *Lotus* extract was detecting H as well as L in saliva, and L was not identical to A, B, Le^a, Sd^a or SC_1. It was found by immunoelectrophoresis to be part of a cathodically migrating glycoprotein, and sugar inhibition studies indicated that the terminal sugars of L were the same as those of Le^a and H substances. L is absent from the saliva and red cells of persons of the Bombay phenotype, but all other red cells absorb the lectins from *Lotus tetragonolobus*.

Pereira and Kabat (1974) extensively purified the lectin from *Lotus tetragonolobus*, and the purified preparation had three fractions, all with the same specificity. They determined the specificity of the lectin in terms of the structures of the terminal oligosaccharides of the blood group receptors (see in section 19.9.2). The pure lectin reacted with H, Le^a and A_2, but not with A_1 or B substances in precipitin reactions. It was specific for type 2 chains containing L-fucose on C-2 of the galactosyl residue of β-D-Gal (1→4)-D-GlcNAc, whether a second fucosyl residue was attached to the NAc-D-$GlcNH_2$ residue or not. Similarly substituted type 1 chains did not react.

Holburn and Masters (1974) used ^{125}I-labelled glycoproteins (obtained from Dr. Winifred Watkins) for a radioimmunoassay of A activity in serum and in the saliva of A secretors. A activity was found in all sera examined, there being more in A_1 than in A_2 sera, and the amounts being higher in secretors than in nonsecretors. Sturgeon *et al.* (1973) used an automated system, and could readily discriminate secretors from nonsecretors. Significant differences in ABH concentrations within various ABO types were noted, however.

It has been known for some time that the group substance activity in the secretions of the three salivary glands is not equal. Hartmann (1941) showed that the parotid gland was poor in water soluble group specific substances, while the submaxillary gland was relatively rich in them. Wolf and Taylor (1964) fully confirmed these findings in the saliva of secretors collected from the separate glands. Parotid saliva has the lowest group substance concentration—it can be zero—followed by submaxillary saliva, with sublingual saliva being the highest. Milne and Dawes (1973) found the same results in quantitating A substance in the saliva from the separate glands of A and AB secretors. Sublingual saliva, and the secretions of the minor mucus glands contained

significantly more group substance than whole or submandibular saliva. It was estimated that sublingual and minor mucus glands contribute some 70% of the group substance to saliva, the remainder being contributed by the submaxillary gland. Parotid saliva contained negligible amounts of A substance.

Prodanov (1979) investigated the inhibitive titer of A, B and H substances in adults and in newborns. The inhibiting strength of the A and B antigens in the saliva of newborns was significantly lower than that seen in adults, while the relationship was just the reverse for H substance.

19.8.3.2 Seminal plasma and spermatozoa. Group specific substances in secretor seminal plasma were described by Sirai (1925) and by Yamakami (1926). Landsteiner and Levine (1926a) found group substances on washed spermatozoa. Fernandez-Collazo and Thierer (1972) showed that sperm cells from secretors absorbed anti-A and anti-B to a greater extent than do red cells. Potent, immune anti-A and anti-B did not agglutinate sperm, nor have any cytotoxic effect on the cells in the presence of complement. The absorption of antibody could still be observed following ten washings of the cells in saline. Rangneker and Rao (1970) said, however, that the ABH antigens were readily washed out of secretor sperm cells with saline. The absorption test was negative after two washings. Kerék and Eliasson (1975) studied the amount of group specific substance present in secretor semen using the "split ejaculate" technique. This technique is based on the observation that the accessory glands of the male reproductive tract discharge their secretions in a characteristic sequence and pattern, and by collecting and separately analyzing sequential fractions of the ejaculate, one can sometimes draw inferences about the accessory gland origin of a seminal constituent (Lundquist, 1949). In three A and two O secretors, it was found that the various fractions exhibit very similar hemagglutination inhibition activity toward appropriate antisera or lectin, suggesting that all the fluids which contribute to human seminal plasma possess group specific substances, and in approximately equal quanitities.

Parish *et al.* (1967) have noted that sperm cells will take up soluble A and B substances from seminal plasma. Spermatozoa from A and O nonsecretors could be "coated" with A substance simply by exposure to A secretor seminal plasma. Boettcher (1968) confirmed that sperm cells absorb ABH substances from aqueous solutions. No antigens were detected on the sperm cells of nonsecretors using the inhibition technique. Boettcher found that the inhibition titer of sperm cells correlated with that of the seminal plasma from the same person, consistent with the notion that the cells acquire their antigens from the seminal plasma by absorption. Karsznia *et al.* (1969) detected no group specific inhibition by sperm cells from nonsecretors, and said that they had had no luck in demonstrating the antigens on nonsecretor cells by mixed agglutination technique (see in section 19.10.3.5). However, Ackerman (1969), using cellular microcataphoresis, demonstrated that the mobility of spermatozoa is altered by treatment with antibody, a result consistent with specific antibody binding. This behavior was observed regardless of the secretor status of the sperm donor. Sato and Ottensooer (1967) studied the levels of H substance in O secretor seminal plasma and found them to be from 3 to 70 times greater than in O secretor salivas. There was not a very great variability in seminal H content from the same person on different occasions, but variation was greater than that found in saliva. Secretor semen contained relatively more A and B than did secretor saliva as well. Similar findings were reported by Dorrill and Whitehead (1979). A and B substances were 2 to 3 times higher in semen than saliva, while H substance was about 20 times higher, on the average. There were differences in the A:H and B:H ratios between the two fluids as well.

Karamihova-Tsacheva (1966) examined the inhibition titers of 200 seminal plasma specimens from secretors of groups A, B and AB. Anti-H was unavailable. A sort of bell shaped curve distribution was found in plotting inhibition titer against the number of persons exhibiting the particular value. The curve peaked at around 1:256. The technique employed was that of Wiener and Kosofsky (1941) (see in Section 19.8.4). The curve was broader for A than for B. In AB people, the titers were most often equal for A and B, but there were examples of A>B and of A<B. Interestingly, a prozone effect was seen with two B secretor samples. But for the titration technique, these would have been classified as nonsecretors (see also in section 19.10.5.2 "Elution"). The average inhibition titer for anti-A was 1:256, and for anti-B, 1:64.

Davie *et al.* (1979) presented quantitative studies on the ABH substance content of seminal samples from 225 people. On the average, there was about 4 times more H than A in A secretors, and about 6 times more H than B in B secretors. A:H and B:H ratios were almost always less than one, this in marked contrast to saliva. 65 of the samples were tested for Lewis substances as well. In seven, there were detectable levels of H substance, yet the fluids were Le (a+b−), that is, they typed as "nonsecretors" on the basis of Lewis (see section 19.9.3 and Table 19.8).

Waissbluth and Langman (1971) found that salivary total protein concentration was significantly lower in group O individuals than in members of other blood groups. Serum protein concentration did not show this variation. Salivary protein concentration was higher in males than in females as well, and tended to be high in group A people. There were no clear differences in the concentrations of immunoglobulin classes in saliva, though IgA tended to be higher in secretors than in nonsecretors.

In 1972, Herrmann and Uhlenbruck showed that the A activity in group A secretor semen was due to a heat stable glycoprotein, specifically precipitable with snail agglutinins anti-A_{HP} and anti-A_{HH}. Double diffusion tests indicated the identity of the antigen and human ovarian cyst A substance. These results indicate that the seminal protein has a terminal N-Ac-D-galactosamine residue (see section 19.9.2). For the background on the snail anti-A agglutinins, see Prokop *et al.* (1965), Grace (1969) and Grace and Uhlenbruck

(1969). The A specific glycoprotein was partially purified by Uhlenbruck *et al.* (1973). These workers found too that rabbit antiserum against pooled human seminal plasma (regardless of group) did not precipitate the A glycoprotein from semen. The antiserum did behave, however, as a kind of "incomplete" agglutinin for A red cells, in that pronase treatment of the cells was required to give a significant agglutination titer.

19.8.4 Inhibition tests for group substances in body fluids

It is common to use saliva for the determination of secretor status. Many authors have commented on the technique for carrying out the inhibition test. Even though other methods are used, as noted in the above section, the inhibition technique is still in very wide use. There are a number of variations of the procedure which deserve brief comment, and the considerations apply, at least in a general way, to all body fluids as well as to saliva. Hartmann (1941) titrated the body fluids against a constant concentration of antiserum, and the $-\log_2$ of the highest dilution giving negative agglutination was taken as a measure of the antigen content of the fluid being tested. Thus, if a series of doubling dilutions of A secretor saliva were made, the first tube would be $½^{\circ} = 1$, the second $½^{1} = ½ = 2^{-1}$, the third $½^{2} = ¼ = 2^{-2}$, and so on. If agglutination were observed in the 1:32 tube, but not in the higher saliva concentrations, after inhibition and addition of test cells, the strength of the sample's group substance would be called 4, i.e., $-\log_2 2^{-4} = 4$. The constant concentration of antiserum was selected by titrating it with test cells, and choosing the highest dilution which still gave 3+ agglutination. The next tube in the series gave 2+ agglutination.

Wiener and Kosofsky (1941a and 1941b) discussed the inhibition technique as well, and carried out many control tests to carefully standardize the procedure. If doubling dilutions of antiserum were tested in series with doubling dilutions of secretor saliva, the "inhibitive titer" increased as the serum dilution increased, as expected. The "inhibitive titer" was the reciprocal of the highest dilution that showed negative aggutination, i.e., completely neutralized the particular concentration of antiserum. An illustration of the type of results they obtained is shown in Figure 19.1. The experiment was actually carried out with B secretor saliva and a particular group A serum, but the behavior illustrated is applicable generally. The table has been modified for presentation here, but still illustrates the point. It was found that the change in inhibitive titer as a function of serum dilution was different for different antisera with the same blood group specificity. In some cases the differences were of large magnitude. Two points are noteworthy based upon these studies. First, if the reciprocal of the highest dilution of body fluid which completely inhibits agglutination at a given serum concentration is taken as a measure of the quantity of group substance present, the results obtained will depend very much on the antiserum chosen, and on the dilution of antiserum employed. Second, it is important to select conditions for carrying out the test which maximize the inhibition sensitivity. One does not want an anti-serum whose inhibitive titer changes drastically as a function of serum dilution, for in such a case, small dilution errors in the test could give rise to large errors in estimating the inhibitive titer. Further, it is better to use sera at dilutions where they contain relatively more agglutinin per unit volume to maximize sensitivity. Similar studies were conducted on a number of anti-A sera. Inhibition titers for group substances from either A_1 or A_2 secretor salivas were higher with A_2 than with A_1 test cells, as expected. But the differences in behavior between the subgroups were not great enough to

Figure 19.1 Determination of Inhibitive Titer for an Antiserum with Secretor Saliva Containing the Corresponding Group Substance

Dilution of Antiserum	Dilution of Saliva														Saline Control	Inhibitive Titer
	1:2¹	1:2²	1:2³	1:2⁴	1:2⁵	1:2⁶	1:2⁷	1:2⁸	1:2⁹	1:2¹⁰	1:2¹¹	1:2¹²	1:2¹³	1:2¹⁴		
neat	−	−	−	(+)	+	++	++								++++	2^3
1:2	−	−	−	−	−	(+)	+	+	++						++++	2^5
1:4	−	−	−	−	−	−	−	(+)	+	+	++				++++	2^7
1:8	−	−	−	−	−	−	−	−	−	−	(+)	+	++	++	++++	2^{10}
1:16	−	−	−	−	−	−	−	−	−	−	−	(+)	++	++	++++	2^{11}

make the authors think it should be considered an issue. Wiener (1943c) discussed this whole matter in his book as well.

Prokop and Uhlenbruck (1969) gave an inhibition technique in which a series of doubling dilutions of antiserum is prepared in four rows. The first row contains only saline. The second, third and fourth rows contain saliva in dilutions of 1:1000, 1:100 and 1:10, respectively. After suitable incubation, test cells are added and the tubes read after an appropriate waiting period. Wiener (1943c) noted that the 10-fold dilutions might be too far apart to insure maximal sensitivity.

Boorman and Dodd (1970) recommended a routine technique in which antisera or anti-H lectin is employed at a titer of about 1:64. With one volume of serum is incubated a volume of saliva for about 30 minutes at room temperature. During the waiting period, the anti-serum is titrated by doubling dilution technique. At the end of the incubation period, the antiserum-saliva mixture is titrated by doubling dilution technique as well, red cells of appropriate group being added, and the test being read after 2 hours. A typical secretor saliva would null the antiserum at all dilutions. If a comparison of different secretors is wanted, the saliva may be diluted out in tubes, and incubated with a constant concentration of antiserum at a titer of about 1:32 to 1:64. Saliva dilutions in this case were made with a 1 mℓ pipette, resulting in dilutions of 1:10, 1:50, 1:100, 1:150, etc., out to 1:500. Equal volumes of diluted saliva and antiserum are incubated for 30 min, after which test cells are added, and the tubes read after 1½ to 2 hours. Secretors generally show inhibition up to a point in the 1:100 to 1:500 range, while nonsecretors do not show inhibition above 1:10.

Issitt (1970) gives a one-step method in which antiserum is used at a dilution one tube stronger than that which gives good macroscopic agglutination with appropriate cells. One volume of antiserum is mixed with one volume of saliva (1:2 dilute), and the tubes incubated for 20 minutes. Appropriate test cells (5% suspension) are then added and the tubes read after 15 minutes following a brief, low speed centrifugation.

Race and Sanger (1975) recommended a technique very like Issitt's except that 2% cell suspensions were employed. They say that the antisera should be from donors who have not been "boosted" by frank immunization, and they said that they thought that most routine American antisera came from boosted donors. In cases where a closer look at the secretor property was wanted, the saliva is titrated.

A few other points about technique based upon important principles should be made. It has been known for quite some time that there is an enzyme present in saliva (and in feces) which can destroy the activity of blood group substances (Schiff and Akune, 1931; Schiff and Weiler, 1931; Schiff and Buron, 1935). The principle is heat-labile, however, while the group substances are not. Saliva is heated, therefore, immediately after collection, usually in a boiling water bath for a few minutes, to destroy this activity. It is usually centrifuged hard after heating to remove debris, and the supernatant fluid used for the secretor status tests. This supernatant can be stored frozen for a long time if necessary, and tested at a convenient time. Some authors dilute the saliva with saline before testing, usually 1:1, particularly if a test is being done which does not involve any titration of the saliva.

In testing for the A substance, most authorities have preferred to use A_2 test cells. As Wiener and Kosofsky (1941b) showed, the change in inhibition titer with serum dilution was more suitable for the inhibition test when A_2 test cells were used, giving a better sensitivity. Issitt (1970) and Race and Sanger (1975) both recommended that A_2 test cells be used in the inhibition test for the A group specific substance in secretor saliva.

19.9 Biochemical Studies on the ABO System

19.9.1 Early studies

The older studies on the chemical nature of the group substances are primarily of historical interest. Until the 1950's, biochemical methods were not sufficiently developed to make possible the elucidation of the structures. As will be appreciated by reading through this section, the rather complete basic knowledge that we now have on the chemical nature and biosynthesis of the ABO group substances emerges in large part from the studies of W. T. J. Morgan, Winifred Watkins, Elvin A. Kabat and Victor Ginsberg and their collaborators.

Landsteiner and van der Scheer (1925a and 1925b) and Landsteiner *et al.* (1925) found that partially purified alcoholic extracts of both horse and human red cells would give precipitin reactions with appropriate antisera. The extracts were antigenic, but their antigenicity was enhanced by the addition of foreign serum, and the antisera obtained were different as well. They did not think the active material in the alcoholic extract was protein in nature. Brahn *et al.* (1932) partially purified the blood group A-like substance from commercial pepsin. This pepsin was prepared from pig gastric juice, and pig gastric mucin is a rich source of a group A-like substance. Landsteiner and Chase (1936) obtained a preparation from commercial pepsin that was highly active serologically. Morgan and King (1943) obtained a fairly pure preparation of this material, which Morgan (1943) noted was polysaccharide-peptide in nature. It was not itself antigenic but, when complexed with the conjugated protein component of the O somatic antigen of *B. shiga,* and injected into rabbits, yielded an extremely potent anti-A serum. Group substances have been obtained from pseudo-mucinous ovarian cyst fluid (King and Morgan, 1944), human urine (Jorpes, 1934; Jorpes and Norlin, 1933 and 1934), human gastric juice (Witebsky and Klendshoj, 1941), horse saliva (Landsteiner, 1936), human saliva (Landsteiner and Harte, 1941) and several other sources (Bray *et al.,* 1946). A number of investigators noted the polysaccharide content of the preparations, and D-galactose

(D-Gal), D-mannose, D-glucosamine (D-GlcNH$_2$) and L-fucose (L-Fuc) were found in hydrolysates in some of the investigations. Reviews of this work may be found in Stacey (1946) and Kabat (1949 and 1956). Kabat's (1949) review gives a good overview of the state of knowledge at the time. As noted, any number of relatively pure preparations had been obtained, and Kabat and collaborators had shown by quantitative immunochemical studies, using precipitation as an assay technique rather than hemagglutination inhibition, that a substantial fraction of the preparations consisted of the activity of interest. Morgan and King's introduction of the phenol method for purifying the group substances (1943) had made possible better preparations than had been available previously. Kabat (1956) gave a detailed review of the procedures used in the preparation of bood group substances from erythrocytes as well as from body fluids. By 1956, it was clear that the composition of the H, A, B and Le^a substances showed striking similarities, and that the best preparations contained D-Gal, L-Fuc and D-GlcNH$_2$ and D-galactosamine (D-GalNH$_2$), the latter two most probably as the N-acetyl derivatives.

19.9.2 Chemical nature of the blood group substances

The majority of the detailed studies that eventually yielded the structures was carried out between about 1955 and 1970. The literature on this material is quite extensive, and a complete review would be repetitious and unnecessarily complicated. The details of the investigations may be found in Morgan and Watkins (1969), Marcus (1969), Grollman *et al.* (1970), Morgan (1970), Watkins (1972), Ginsburg (1972) and Hakomori and Kobata (1974). The chemistry of the Lewis substances is discussed in this section because it is closely related to the discussion of the A, B and H structures. The serology of the Lewis system will be dealt with in section 20, though there is necessarily overlap.

A number of approaches, both direct and indirect, were used in these studies. Direct analysis is possible only with highly purified preparations. Indirect approaches have been extremely useful. Watkins and Morgan made use of plant and eel agglutinins, specific for O and H substances. Specific agglutination of A or O cells by these reagents was found to be inhibitable by certain simple sugars and oligosaccharides, from which it could be deduced that the structures capable of giving inhibition were similar to, or identical with the structural parts of the antigens. Thus it could be shown that agglutination by eel serum anti-H was inhibited by L-fucose (Fig. 19.2) and better by methyl-α-L-fucoside, and that agglutination by anti-A from *Vicia Gracca* or lima bean was inhibited by N-acetyl-D-galactosamine (Fig. 19.3) or by methyl-α-N-acetyl-galactosaminide. In this way, an α-N-acetyl-D-galactosaminyl residue was implicated in the A structure and an α-L-fucosyl residue in the H structure. A useful plant or animal source for anti-B was not available when these experiments were done (1952–1953). As oligosaccharides were isolated by hydrolysis of the blood group substances, these could be tested for their ability to inhibit hemagglutination in these systems. Le^a hemagglutination

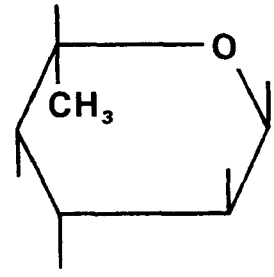


Figure 19.2 α-L-Fucose

was inhibited by a compound called "lacto-N-fucopentaose II" (Fig. 19.4), and that of Le^b by another compound called "lacto-N-difucohexaose I" (Fig. 19.5). Kabat and his collaborators looked at the inhibition of specific precipitin reactions between blood group substances and their immune sera (the natural isoagglutinins are not good precipitins). Inhibition of the B specific precipitation by melibiose (6-O-α-D-galactopyranosyl-O-glucose; Fig. 19.6) implicated the α-D-galactopyranosyl residue in B-specific sites. Group A specific precipitation was inhibited by an oligosaccharide containing N-acetyl-D-GalNH$_2$ released by weak acid hydrolysis. Still another approach was the enzymatic degradation of blood group substances (see, for example, Watkins, 1962), and inhibition of the enzymatic reaction by various carbohydrates ("substrate inhibition"). An enzyme from *Trichomonas fetus* which destroyed H activity was inhibited by L-fucose and D-galactosamine. Enzymatic hydrolysis of A substance by crude extracts of *T. fetus* or *Clostridium welchii* (type B) was inhibited by N-Ac-GalNH$_2$, while that of the B substance was inhibited by D-Gal or its α- or β-D methyl pyranosides. The enzymes which bring about the losses of A, B and H activity are, respectively, an N-acetylgalactosaminidase, a galactosidase and a fucosidase. Watkins (1972) said that no explanation had yet been found for the inhibition of the *T. fetus* H enzyme by D-Gal-NH$_2$. Enzymes from a number of other bacterial sources have been found which destroy the blood group specificities. Enzymatic

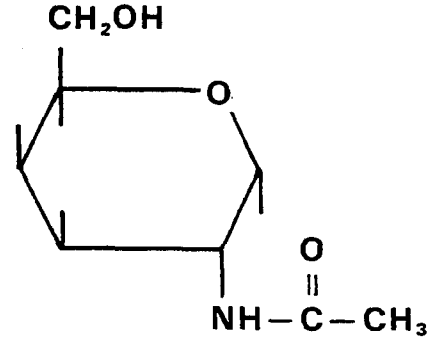


Figure 19.3 N-Ac-Galactosamine

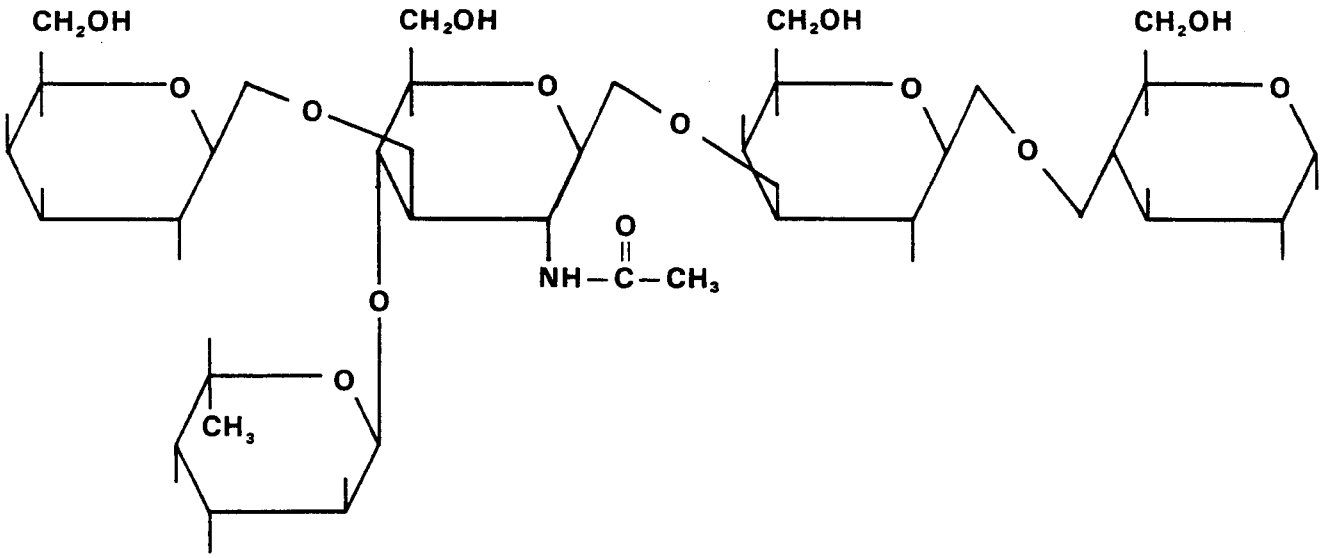


Figure 19.4 Lacto-N-Fucopentaose II

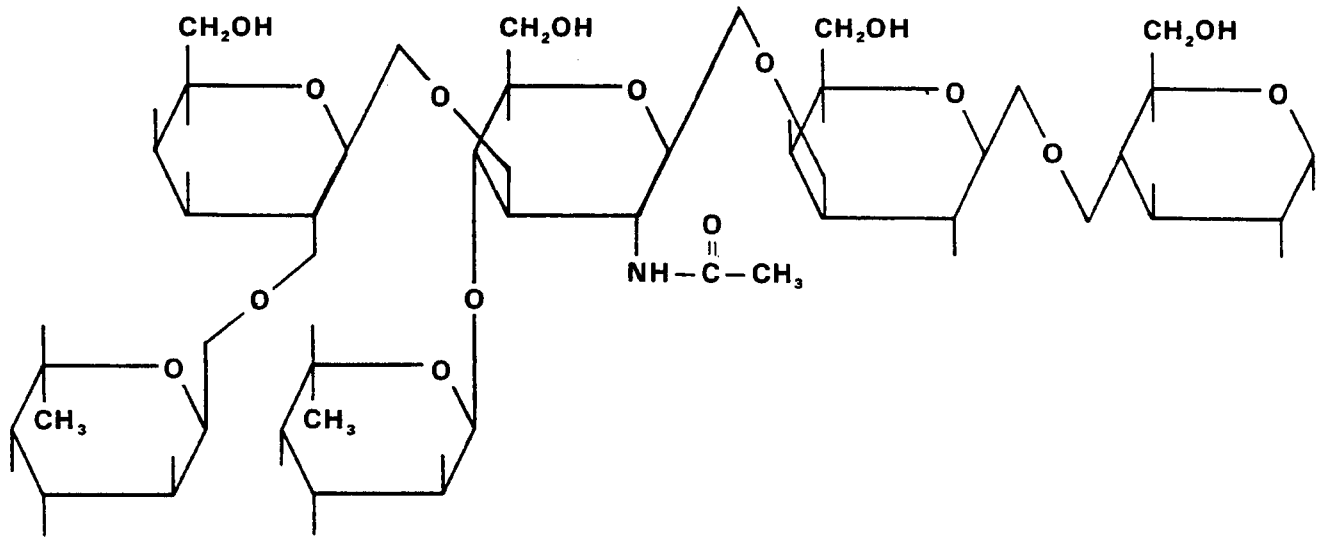


Figure 19.5 Lacto-N-fucohexaose I

degradation of the blood group substances was studied directly as well. Enzymes which specifically inactivated A or B specificities gave rise to increases in H specificity in the process. An A enzyme from *T. fetus* which causes conversion of A→H specificity simultaneously releases N-Ac-GalNH_2. Similarly, a B destroying enzyme released reducing sugar consisting of a major Gal component. A partially purified H enzyme from *T. fetus* liberates fucose in the course of destroying H specificity, and leaves as a product an antigen that reacts with horse anti-type XIV pneumococcal serum. Enzymes have been found that destroy Le^a activity, releasing L-fucose which was in α-1→4 linkage to N-acetyl-GalNH_2 in lacto-N-fucopentaose II, but these enzymes do not release the fucose joined in α-1→2 linkage to galactose in lacto-N-fucopyranose I. Direct analysis of the structures consisted of limited hydrolysis of the blood group substances (enzymatic and chemical), characterization of the products, and inferences about the original structure from the fragments obtained.

The sum and synthesis of many hundreds of studies has resulted in a fairly complete understanding of the chemical structures of the blood group determinants. The immunospecificity without doubt resides in the carbohydrate moieties of the glycoproteins which constitute the soluble group sub-

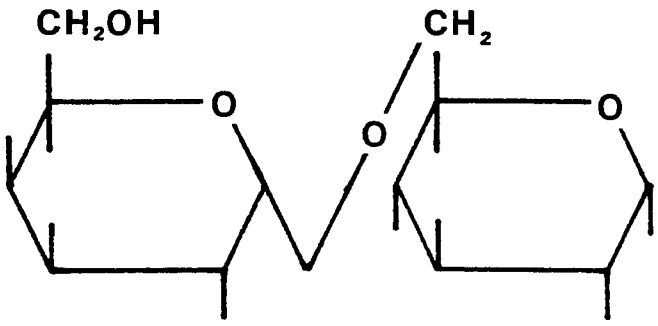


Figure 19.6 Melibiose

stances, and of the glycolipids which constitute the determinants of the red cell. There are two kinds of serologically active carbohydrate chain endings:

Type 1 β-Gal-(1→3)-GlcNAc

Type 2 β-Gal-(1→4)-GlcNAc

A, B and H determinants can be based on either type while Le^a and Le^b structures, in which L-fucose is substituted at the 4-position of NAc-GlcNH_2 are based on type 1 chains. The oligosaccharides characterizing the A, B and H substances are indicated in Fig. 19.7. The structures shown in Fig. 19.7(a), (b) and (c) have type 2 chains. Structures with type 1 chains, i.e., in which the linkage between the terminal N-Ac-GlcNH_2 and the subterminal β-D-Gal is 1→3, occur as well. The oligosaccharides characterizing the Le^a and Le^b substances are shown in Fig. 19.8. Obviously, structures with 1→4 linkages between the galactopyranosyl and N-acetylglucosamine residues cannot give rise to Lewis specificities since the C-4 of the N-Ac-GlcNH_2 is occupied by the glycosidic linkage and no substitution by a fucosyl residue can occur.

The details of the structural work are considerably more complicated than has been indicated. The issues of how the oligosaccharides are put together in the native glycoprotein group substances and the nature of the linkages of the sugar moieties to the amino acids of the peptide chain must await clarification. Composite structures, branched oligosaccharide chains, which include the known structures of several of the group determinants, have been proposed. These have been put forth, at least in part, to account for the many oligosaccharides isolated when the blood group substances are subjected to partial hydrolysis. It has been suggested that the linkage to the peptide chain may be through the N-Ac-galactosaminyl residue to serine or threonine. Goodwin and Watkins (1974) have isolated a number of oligopeptides from the A specific glycoprotein and determined their sequences. Their results were fully consistent with hydroxyamino acid-N-Ac-GalNH_2 linkages in the intact molecule. From the structures of the immunodeterminants that have been determined, it has been possible to characterize the specificities of the so-called "blood group enzymes" that were mentioned above. Some of these are indicated in Table 19.7.

A number of the A-like, B-like and H-like substances from animal sources have been purified and subjected to structural studies. Springer (1970) gave the structure of the B-active receptor from *E. coli* O_{86}. H substance from hog gastric mucosa has been characterized by Kochetkov *et al.* (1976). Newman and Kabat (1976) did extensive studies on the B-active substances from horse gastric mucosa. Pereira and Kabat (1976) showed that the glycoproteins from hog gastric mucosa or human ovarian cyst fluid can be purified by an immunoadsorbent technique in which purified lectins from *Dolichos* or *Lotus* are coupled to Sepahrose 2B support media. Etzler and Kabat (1970) purified and characterized the specificity of the *Dolichos* lectin, and Pereira and Kabat (1974) carried out similar studies on the *Lotus* lectin. It may be noted too that a carcinoembryonic antigen (CEA-M) from human hepatic metastasis is closely related structurally to H substance, and can be modified to possess A and B activities with appropriate enzymes (Bali *et al.*, 1976).

The structual studies on the receptors of the red cell membrane have developed more recently than those on the water soluble materials. The glycolipid structures are more difficult to isolate and purify than the glycoproteins, but considerable progress has been made. The studies are well reviewed in Hakomori and Kobata (1974). Glycolipids with H, A, B, Le^a and Le^b activity have been isolated and the oligosaccharide structures that have been determined thus far are in complete conformity with what is known of the glycoprotein materials of similar serological specificities. The sugar-lipid

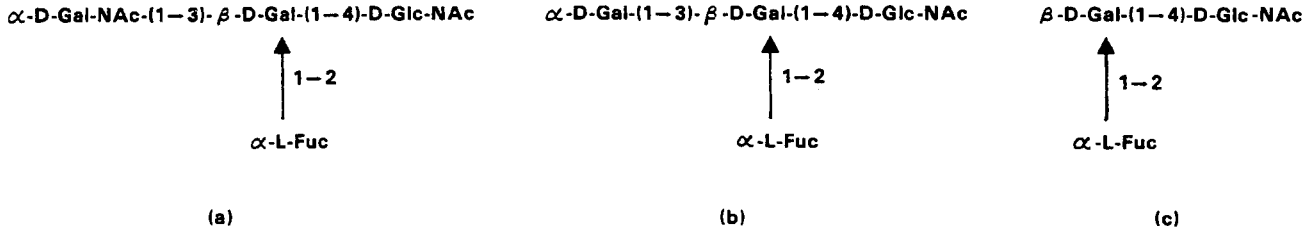


Figure 19.7 Composition of the Carbohydrate Fragments Responsible for Blood Group Activities A (a), B (b) and H (c).

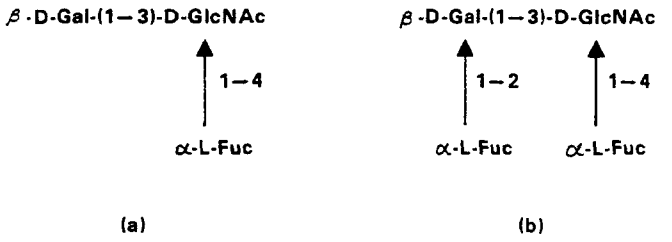


Figure 19.8 Composition of the Carbohydrate Fragments Responsible for Le^a Specificity (a) and Le^b Specificity (b)

linkage is thought to be through a terminal glucosyl residue to a ceramide. Kent *et al.* (1977) have studied the relationship between the glycolipid and glycoprotein blood group active substances in the red blood cell membrane. A procedure for the isolation of ABH blood group active glycolipids from red cell membranes is given by Hakomori (1978). Gardas (1978) purified an A-active glycolipid from red cells, and carried out extensive structural studies which enabled him to propose a structure for the nonreducing end of the chain. It is of interest that the A antigenic receptor sites for anti-A appear to be restricted to the "outside" of the red cell membrane. They are not detected in studies with radio-labelled anti-A on "inside-out" red cell membranes (Schenkel-Brunner *et al.*, 1979).

19.9.3 Biochemical genetics of the ABO, Secretor and Lewis Systems

The biosynthesis of ABH and of Lewis substances are intimately related, and involve the Secretor locus as well. The serological characteristics of the Lewis system will be discussed in section 20, but the biochemical genetics will be included here. The inheritance of the ABO characteristics seemed straightforward for a number of years. There was no particular reason not to suppose a direct relationship between the genes and the blood group determinants. The recognition that the genetic material carries coding information only for the synthesis of proteins (section 1.2.2), and the discovery that the immunospecific structures in the determinants were polysaccharides, required a closer examination of the system. The role of the secretor and Lewis loci had to be accounted for as well, insofar as the presence of Lewis and ABH substances in body fluids are concerned. The basic outlines of the scheme were put forth in the late 1950's, based on the structural work that had been carried out on the ABH substances, and on genetic considerations. These pathways, which have since been shown to be largely accurate, were proposed by Watkins (1958 and 1959), Watkins and Morgan (1959) and by Ceppellini (1959). Four loci are involved in the control of the biosynthesis of ABH and Le^a substances: the Lewis (*Le/le*), the *H/h*. the secretor (*Se/se*) and the ABO loci. Figure 19.9 summarizes the relationships. The genes *O, h* and *le* are regarded as inactive in terms of conversion of precursor substances. The precursor substance is a macromolecular glycoprotein with the peptides fully synthesized and carbohydrate chains incorporated, but not fully completed. There are a few people whose secretions are ABH—, Le(a—b—), but they have in their secretions a glycoprotein which is similar in many ways to the blood group active molecules. It is low in fucose, and cross reacts with horse anti-type XIV pneumococcal serum. This molecule is believed to be the precursor substance for the group substances. The *Le* gene gives rise to conversion of the precursor into Le^a. *H* gene acts to convert the precursor substance or Le^a into H. Both the *Le* and *H* genes cause α-L-fucosyl residues to be added to different sugars in the precursor substance. Le^b activity results from an interaction of *H* and *Le* genes. Gene *Se* is thought to activate gene *H* in tissues producing secreted glycoproteins, so that homozygous recessive *sese* people do not form any soluble H substance. H substance is considered the substrate for the reactions controlled by the ABO genes. The *A* gene controls the addition of α-N-Ac-D-galactosaminyl units to H, while *B* gene controls the addition of α-D-galactosyl units to it.

All the experimental evidence heavily supports the scheme outlined in Fig. 19.9. The *H* gene product has been identified in a number of sources as a fucosyl transferase. The enzyme is not present in the secretory organs of *sese* people, but the synthesis is carried out in the red blood cells regardless of secretor locus genotype. The product of the *Le* gene is likewise a fucosyl transferase but with a different specificity than that of the *H* gene. *A* gene product is an N-acetylgalactosaminyl transferase and *B* gene product is a galactosyltransferase. Type 1 and type 2 chains conversions to the various blood group substances are indicated schematically in Figures 19.10 and 19.11 respectively. It may be noted that the enzyme giving rise to the substances called "X" and "Y" in Fig. 19.11 occurs in human milk, submaxillary glands and gastric mucosa from secretors as well as nonsecretors. The gene giving rise to the enzyme is designated "X" by Hakomori and Kobata (1974) and "3-F" by Watkins (1972). Watkins does not give the substances names. Hakomori and Kobata (1974) designate the positional isomer of Le^a as X, that of Le^b as Y, as has been done in the Figure. No serological specificities were associated with X or Y for a long time, and it was thought that the gene locus controlling the transferase is not polymorphic, and the enzyme is therefore present in all individuals (Watkins, 1972). There is recent evidence that X and Y may have Lewis substance specificity,

Table 19.7 Some Enzymes Acting on Blood Group Specific Structures

Enzyme Source	Blood Group Specificity Affected	Probable Specificity	Effect
Trichomonas fetus	A	N-Ac-Galactosaminidase	Destruction of A; Enhancement of H
Clostridium tertium	A	N-Deacetylase	Destruction of A; No enhancement of H
Clostridium tertium	A	N-Ac-Galactosaminidase	Destruction of A; Enhancement of H
Helix pomatia	A	N-Ac-Galactosaminidase	Destruction of A; Enhancement of H
Trichomonas fetus	B	D-Galactosidase	Destruction of B; Enhancement of H
Clostridium maebashi	B	D-Galactosidase	Destruction of B; Enhancement of H
Coffee Bean	B	D-Galactosidase	Destruction of B; Enhancement of H
Trichomonas fetus	H	1,2-α-L-fucosidase	Destruction of H; Enhancement of Reactivity with Anti-type XIV Pneumococcal Serum
Bacillus fulminans	H	Fucosidase	Destruction of H; Liberation of L-fucose
Clostridium perfringens	H	1,2-α-L-fucosidase	Destruction of H
Aspergillus niger	H	α-L-fucosidase	Destruction of H
Trichomonas fetus	Le^a	1,4-α-L-fucosidase	Destruction of Le^a

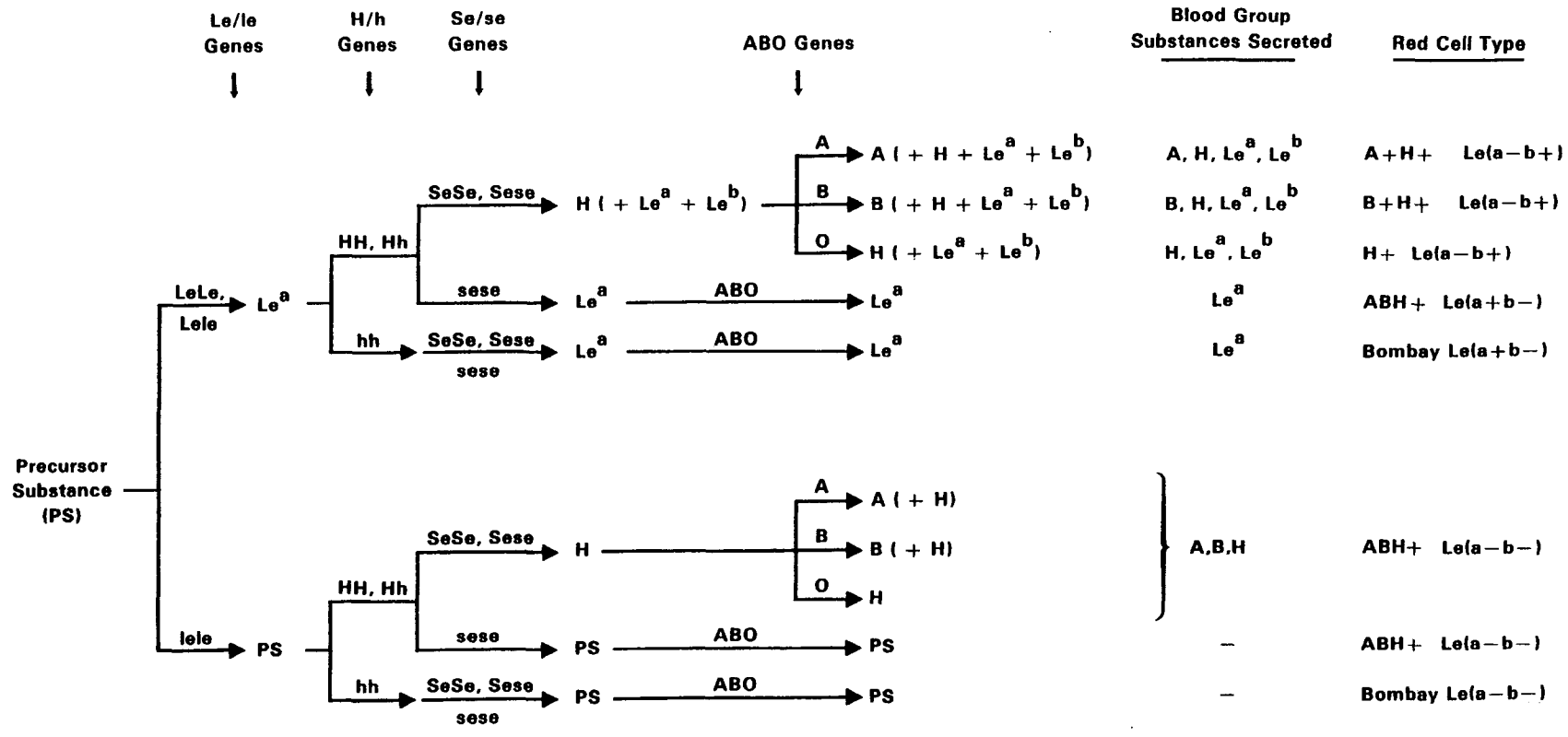


Figure 19.9 Overall Scheme for the Genetically Controlled Synthesis of A, B, H and Le^a

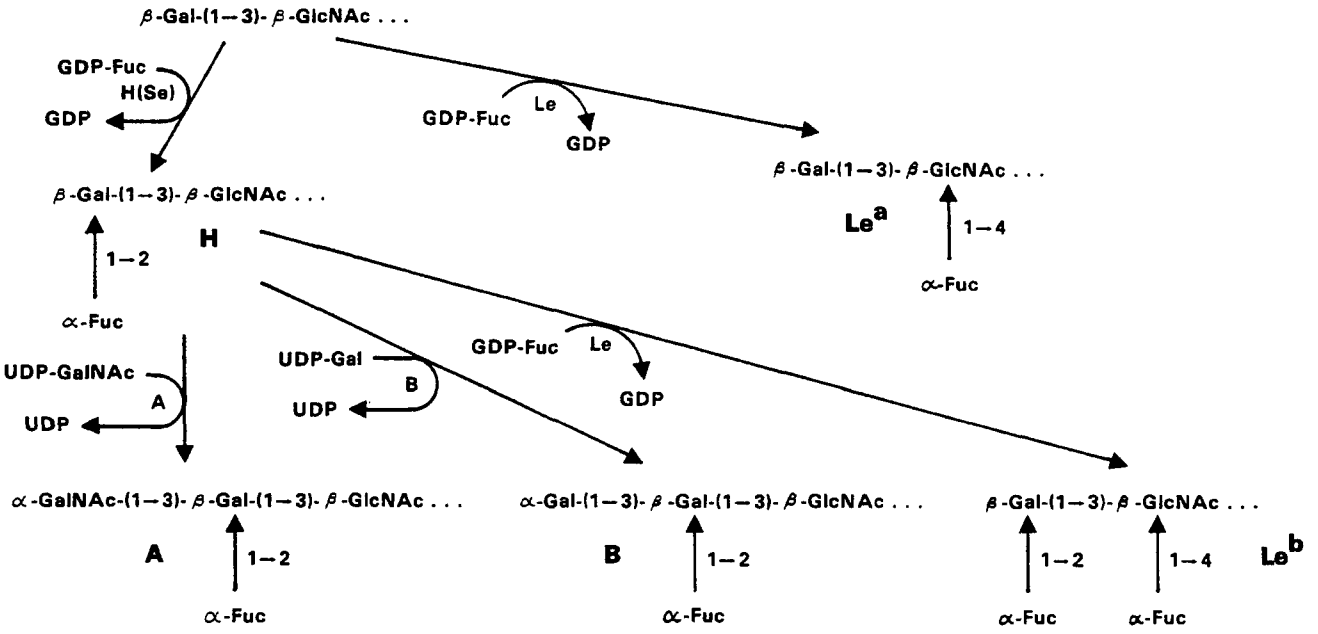


Figure 19.10 Conversion of Type 1 Chain to Group Substances

corresponding, respectively, to Le^c and Le^d (see discussion in section 20.5.1). Carne and Watkins (1977) reported on the purification of *B*-gene-specified α-3-galactosyltransferase. Badet *et al.* (1976) studied the correlation between agglutinability with anti-B sera and the serum concentration of the galactosyltransferase activity in B individuals in several populations. Different correlations were found in African and Caucasian populations. Koscielak *et al.* (1976) found that galactosyltransferase activity in the serum of persons of group B_m (see in Table 19.4) was normal, but that the red cell concentration was very much lower than in ordinary type B cells, perhaps accounting for the weakness of the B antigen in B_m on the basis of the number of H sites converted.

Tilley *et al.* (1978) noted that the α-3′-NAc-D-galactosaminyl transferase specified by the A_1 gene, is appreciably lower in recently delivered mothers than in ordinary adult or newborn serum. A similar, but less striking decrease was noted for H-specified α-2′-L-fucosyl transferase. Although newborns have fewer A and H antigens than adults, the levels of these enzymes in serum were found to be the same. Romano *et al.* (1978) found that O cells from adults, incubated with UDP-galactose and B serum, contained about 200,000 "generated" B sites per cell. The same experiment with group O cord blood cells showed 40,000–70,000 B sites generated per cell, indicating that infant blood cells were lower in the amount of substrate available to the transferase enzyme.

In section 19.6, a number of variant types of ABO expression were discussed. Mulet *et al.* (1979) have studied the biosynthesis of the B antigen in B_h people. The evidence indicates that the B in B_h people is made from H substances, and that the *H* gene is, therefore, not completely silent in these people.

Yoshida *et al.* (1979) have carried out experiments using an antibody to N-acetylgalactosaminyltransferase (anti-A enzyme antibody) with A, B and O sera, and have shown that the antibody cross reacts with B enzyme as well as with a protein found in group O persons, called "O-CRM". This enzymatically inactive, but immunologically cross reactive protein appears to be the *O* gene product. These experiments show that the genes responsible for A enzyme, B enzyme and O-CRM are allelic. Yoshida (1980) indicated that the genotypes of A and B blood could be determined using the anti-A enzyme antibody if the serum was tested for the O-CRM.

The relationships between genotypic combinations and the red blood cell and secretion phenotypes, as modified from Morgan and Watkins (1969), are indicated in Table 19.8. These relationships hold generally for European populations, but there are exceptions (see section 20.2).

19.10 Medico-Legal Applications

19.10.1 Introduction and disputed paternity testing

19.10.1.1 General introduction. The general principles and considerations concerning the medicolegal application of genetic markers in blood and body fluids were discussed in section 18.

In this section, the principal techniques for antigen grouping in bloodstains are introduced, and discussed in some

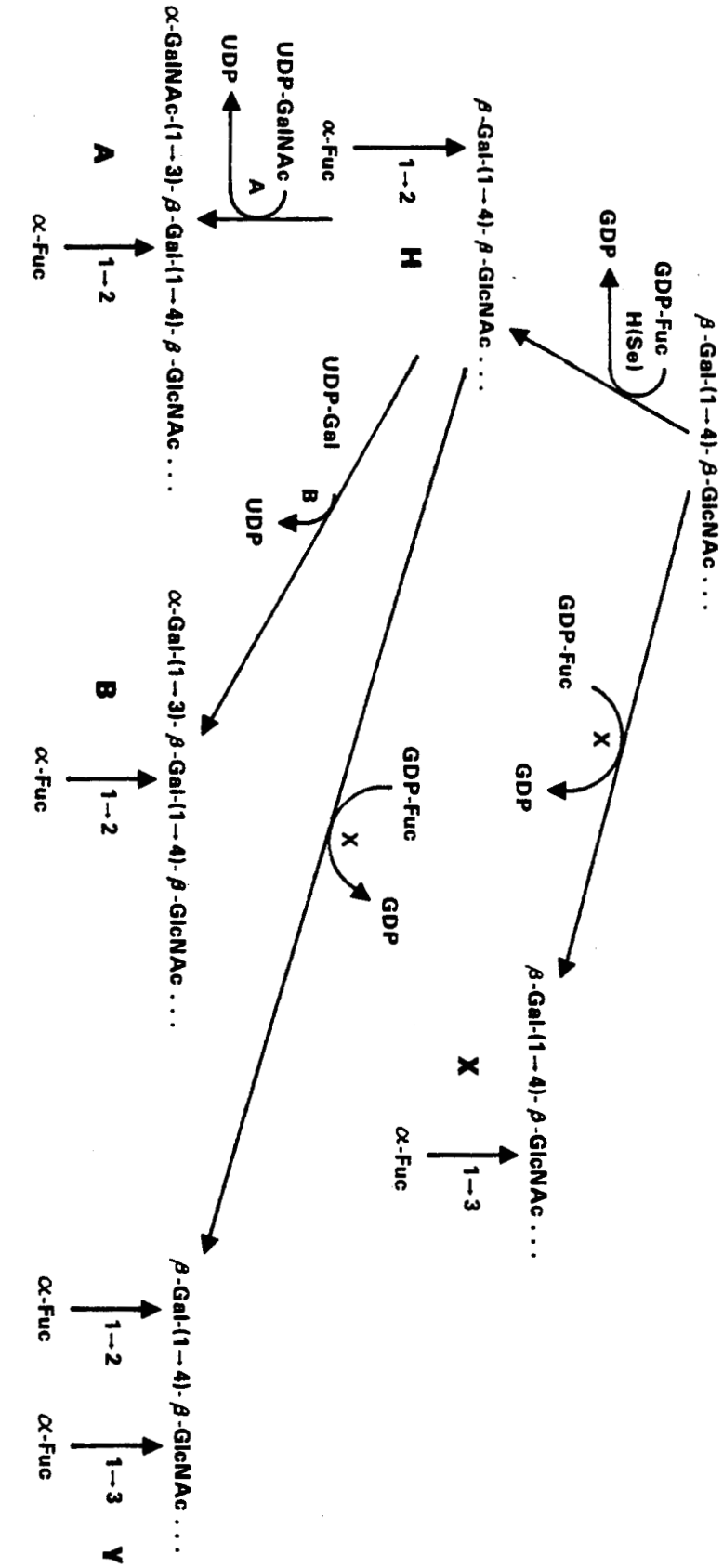


Figure 19.11 Conversion of Type 2 Chain to Group Substances

Table 19.8 Relationship of Genotype to Red Cell and Secretor Phenotype

Genotype Combination	Red Cell Antigens			Substances in Secretions		
	ABH	Le^a	Le^b	ABH	Le^a	Le^b
ABO, H_, Se_, Le_	+	−	+	+	+	+
ABO, H_, sese, Le_	+	+	−	−	+	−
ABO, H_, Se_, lele	+	−	−	+	−	−
ABO, H_, sese, lele	+	−	−	−	−	−
†ABO, hh, Se_ or sese, Le_	−	+	−	−	+	−
†ABO, hh, Se_ or sese, lele	−	−	−	−	−	−

† Bombay phenotypes

detail, since the time they were first employed with the ABO system. The techniques are not very different in principle for the other blood group systems.

19.10.1.2 Some early developments in the medicolegal application of blood groups. The development of medicolegal applications of the ABO blood groups (and later, other group systems as they were discovered) has taken place over the past 60 or 70 years since ABO was discovered. The acceptance of blood grouping as a means of connecting bloodstained materials to individuals and excluding other individuals, and as a means of excluding parentage, occurred at different rates in different countries. Lattes (1932) gives an excellent review of developments in disputed parentage cases. It appears that Germany was one of the earliest countries whose legal system accepted blood tests in disputed affiliation cases as a routine matter. According to Schiff and Boyd (1942), the first case went to Court in Berlin in 1924. Lattes (1932) said that there were 3000 cases tested in Berlin alone in 1924, doubtless because Schiff was there. Popoff (1927) mentioned that Kolzoff first applied grouping in a medicolegal case in Russia in 1921.

In this country, Ottenberg (1921a and 1921b) published a set of exclusionary tables for parentage cases, but the concept was not accepted by everyone. Buchanan (1922a) denied that blood groups could be used as a basis for excluding parentage, and criticized (1922b) Ottenberg's tables. Dyke (1922a) in England agreed with Ottenberg, and published a set of tables as well. In 1930, Dyke said, however, that an affiliation case had yet to be decided by an English court based upon blood grouping results. Wiener (1935) reported on an affiliation case in this country, tried January 18, 1933, in which he had excluded a defendant from paternity. The plaintiff had withdrawn her charges. A baby mixup case in Chicago in 1930 is also reported, in which the children were sorted out by blood grouping tests. As usual, the higher courts were slower to accept the results of the tests. In a New York case in January, 1934, Kings County (Brooklyn) Supreme Court Justice Meier Steinbrink ordered blood tests in a disputed paternity case entitled Beuschel v. Manowitz [271 NYS 277; 272 NYS 165]. The Court apparently used as a precedent the decision of the Supreme Italian Court of Cassation of February 13, 1931, in which it recognized blood grouping as affording a potential means of certain exclusion of parentage (Lattes, 1932). Justice Steinbrink's decision was, according to Wiener (1935), reported in the January 2, 1934, issue of the *N.Y. Law Journal.* Unfortunately, the plaintiff appealed the order, and the Appellate Division of New York Supreme Court reversed Justice Steinbrink's order (see *J. Am. Med. Assoc.* **104**: 344 for 1935). Soon afterward, laws were passed in a number of the States providing the courts with statutory authority to order blood tests in these kinds of cases. Both Wiener and Levine were instrumental in bringing passage of these laws about. The Committee of Medico-legal Problems of the A.M.A. (1952 and 1957) periodically issued recommendations on various aspects of the medicolegal use of blood groups, primarily disputed parentage. The recommendations included the systems that ought to be used, criteria for qualifying experts, model state laws, and summaries of court decisions. They also contained nomenclature recommendations which, in the case of Rh, were not always amicably accepted, as will be discussed in section 22. The most recent guidelines and recommendations were issued in 1976 (Joint AMA-ABA Guidelines, 1976). They recommended the use of 7 systems, ABO, Rh, MNSs, Kell, Duffy, Kidd and HLA in routine parentage investigations. These seven have a cumulative probability of exclusion of 91–93% (see in section 18).

The examination of bloodstains for ABO groups in criminal cases was first reported by Lattes (1916a). In this country, acceptance of this sort of determination appears to have come about in the 1930's. Gettler and Kramer (1936) reported a number of attempts to do blood grouping on case stains. Their results, in trying to follow Lattes' methods, were poor, and they said that the procedures were not very good, and probably should not be used. Landé (1938) at the N.Y. Medical Examiner's Office, reported that the had correctly diagnosed more than 90% of 109 dried blood and 93 seminal stains in a blind trial, and that some of the errors were corrected upon doing the tests a second time. Boyd and Boyd (1937) in Boston took exception to Gettler and Kramer's position. They presented their procedures, which were slight modifications of the ones in use in Europe. They used both human and immune antisera in the tests (inhibition procedure), and could correctly assign the groups of 120 two month old human bloodstains on cotton in a blind trial. They asked Dr. Wiener to send them 28 more stains, half on cloth and half on filter paper, as a further blind trial. All were diagnosed correctly, even a few A_2 stains, and Wiener agreed in a letter to Boyd and Boyd that stain grouping was reliable in competent hands. Wiener (1935) gave a thorough discussion of stain grouping methods, but did not report any criminal cases of his own, or of anyone else in this country. In the 2nd edition of *Blood Groups and Transfusion,* however, Wiener (1939) does mention a case in which he had testified. He had not examined the stains themselves because they were too small, but he had testified that another expert's diagnosis of group AB based on a negative Lattes test alone was completely unwarranted.

19.10.1.3 Disputed Parentage As can be appreciated from the foregoing section, the ABO (and secretor) systems have been applied to disputed parentage cases for many years. All laboratories which do paternity testing at the present time include the ABO system in their tests. A few may include the secretor system, although it is not very useful.

The probability of excluding a falsely accused White father with the ABO system is about 13.4%, while for a falsely accused Black father, it is about 17.4% (Chakraborty *et al.,* 1974; AABB, 1978) The ABO system has its peculiarities, many of which have been discussed in the foregoing sections. These can led to problems of interpretation if they should be encountered in a disputed parentage case (Tippett, in AABB, 1978). There is some indication that problems may be encountered with A_1 and A_2 subtypes in Black people

(Perkins and Morel, 1980). Some people who are genotypically A_1B may type as A_2B. Perkins and Morel (1980) indicated that their analysis of the population data for Black people suggested that as many as 1 in 5 "A_2B" individuals may have the A_1B genotype. Their analysis was prompted by a parentage case in which an apparent A_2B mother (and an O putative father) had an A_1B child.

The only exclusion that can be obtained in the secretor system is in a case where nonsecretor parents have a secretor child. The probability of excluding a falsely accused father with this system is thus quite low, about 3% for White or Black people.

19.10.2 Early studies on grouping bloodstains

Determination of the ABO groups of bloodstains may be said, in a sense, to be as old as the system itself. In Landsteiner's laureate account (1901) of the blood groups, he noted that the agglutinins in a blood sample dried on linen for 14 days would, upon being redissolved, still give group specific agglutination. Based upon this observation, he wrote: "Thus the reaction may possibly be used in some cases for the identification, or better, for the recognition of unidentified blood samples, e.g., for forensic purposes unless rapid variations in the agglutinating ability should be found, which would prevent this application". In 1903, Landsteiner and Richter published a full paper on the activity of agglutinins in blood dried out on glass slides and on linen. In all the tests (blood samples from six different people were used), the reconstituted stain showed the same agglutinating behavior as the original serum, except that in some cases the agglutinins had apparently become inactive. Thus, some samples which were distinguishable in fresh specimens were no longer so in the dried state, but no dried specimen gave a reaction different from what it had given in the fresh state. They noted that it might be possible in certain cases to observe an identity in agglutination behavior between a stain extract and the serum of a person, but not always. Since a person's serum does not agglutinate his own cells, they said that the agglutination of a person's cells by a stain extract excluded that person as the source of the stain. Failure of agglutination, however, could not be interpreted in terms of whose blood might have been responsible for the stain. Checking for agglutination of a suspect's blood cells with a stain extract became known in the literature as the Landsteiner-Richter test. Because of the known fact that many animal sera will agglutinate human red cells, it had to be established that the bloodstain was of human origin before the test was carried out. Although this was not a blood grouping test as such, it was most certainly based on the ABO groups.

Attention continued to be focused on the isoagglutinins for some years. Biffi in 1903 noted that a suspect could be excluded from consideration as the source of a bloodstain if his cells were agglutinated by the bloodstain extract (Landsteiner-Richter). He apparently thought too that agglutinable cells could be extracted from the stain and tested with suspect's serum with the same idea in mind. The testing of the agglutinins in the stain with test cells was carried a bit further though. The isoagglutinins extracted from the stain would behave somewhat differently in the isoagglutination reaction with examples of cells from a number of individuals. If only a few individuals were under consideration in the case as potential sources of the bloodstain, Biffi thought that an "individualization" might be possible by such a comparison. This idea was pursued by Baecchi for a number of years, as discussed below. Florence, writing in 1904, did not accept the idea that results obtained with cells gotten from stains should be treated like those obtained with fresh cells. He seemed to deride the idea of individualization of bloodstains by isoagglutination tests in a statement which, even today, is not without a certain irony: "Il ne s'agit plus de distinguer le sang de lièvre du sang d'homme, mais bien de dire que cette tache a été faite par le sang de Pierre, et non pas par celui de Paul ou de Francois". Florence accepted the idea that a positive Landsteiner-Richter test (agglutination) gave an unequivocal exclusion, as did Galli-Valerio (1905). Verdier (1906), who made the test the subject of his thesis, agreed as well.

In 1910, Baecchi took up studies on agglutinins in bloodstains. Part of his study was concerned with heteroagglutinins in stains, i.e., agglutinins in animal bloodstains that would agglutinate human red cells. This property had been proposed as the basis for a species test by Marx and Ehrnrooth, and is discussed in section 16.6.4. The studies that are of interest here had to do with isoagglutinins in bloodstains. Baecchi thought that the isoagglutinins were fairly stable, and that they retained their activity and group specificity in dried stains. The antibodies resisted heat inactivation and putrefactive change. Like Biffi, he tried to carry the investigations further than the Landsteiner-Richter test. If a stain extract agglutinated a person's cells, the stain could not have originated from that person's blood, since autoagglutination is a rare, pathological phenomenon. But if there were no agglutination, the results could not be interpreted in terms of individuality. It meant simply that the person could not be excluded. This point was made by Lattes (1913) and by Lecha-Marzo and Piga (1914) as well. Baecchi found what he regarded as individualizing differences in the agglutination behavior of a person's serum toward a series of test cells of the same type from different people, e.g., an A serum (β) tested against a series of different B cells. These differences, he thought, would still show up in certain stain extracts. Thus under particularly favorable sample conditions, and if a limited number of persons were involved, he thought that a set of "individual" reactions could be obtained. In a large percentage of cases, he said, an exclusion could still be obtained by these kinds of studies (Baecchi, 1910, 1912, 1913 and 1920).

In 1913, Lattes began his studies on isoagglutinins in bloodstains. He thought that one should proceed to devise the practical tests for medico-legal applications on the basis of fundamental principles. Apparently, not everyone in medico-legal circles was familiar with the blood group literature. Bohne (1913), in a paper he read at the 8th meeting of

the German Society for Legal Medicine, was still making reference to Landsteiner's original groups A, B and C. Lattes' 1913 paper dealt with two issues: the Marx-Ehrnrooth test for species of origin, and the detection of isoagglutinins in bloodstains. The former is discussed in section 16.6.4. Lattes did not agree with Baecchi on the "individual" reactions of bloodstain extracts. He admitted that there were differences in the agglutination behavior of a particular serum towards a series of different cells, but he did not think that this fact formed the basis for individual differences that could be used in legal medicine. He noted too that AB bloods have no isoagglutinins, and will give no agglutination test with A or B cells. Such a negative reaction is indistinguishable from one in which isoagglutinins had been present, but had been denatured or destroyed. Negative results in the test could, therefore, not be interpreted. Positive results with A or B cells or both, however, could be interpreted, and Lattes gave in the paper an early version of his procedure for the direction of isoagglutinins in bloodstains, a procedure which is still in use in some places, and is generally called the "Lattes crust test" in this country. Bloodstains belonging to groups O, A or B could be assigned to their proper group using this technique, and this he thought would be of considerable importance in some cases. Lattes carried out systematic experiments on the technique and, in 1915, published extensive reports on the results in both the Italian (1915a) and French (1915b) literature. The studies had primarily to do with standardization of the procedure. A major point was that the stain extract could be neither too dilute nor too concentrated. In the former case, activity would be too low and agglutinins missed; in the latter case, pseudoagglutination (rouleaux formation) could result giving a false positive result. Lattes reported on the application of the test in two cases in 1916. One was a private matter in which a husband was suspected by his wife of infidelity (the investigation exonerated him), but the other was a homicide case, in which it was a matter of deciding whether bloodstains on the suspect's clothing were his own, as he contended, or could have belonged to the victim, as the police thought. In this case, the suspect was O, and the victim A. The bloodstains grouped as O, and the suspect's version of the story was supported in what was probably the first case in the published literature in which bloodstain grouping played a role in a criminal inquiry (Lattes, 1916a). In the same year, Lattes (1916b) published further experiments on his technique, giving further refinements. Among other things, he noted that subjecting the agglutinated cells to dilution would usually distinguish between true agglutination and rouleaux, since the latter would disappear with dilution while the former would be unaffected.

19.10.3 Further developments — bloodstain grouping methods

19.10.3.1 Detection of isoagglutinins — Lattes Test. As is clear from the previous section, all the early efforts were focused on the determination of agglutinins in stains to determine the group. The procedure has undergone many modifications since its introduction by Lattes. It is now clear that the agglutinogens are considerably more stable than the agglutinins, and the test for agglutinins is used primarily to confirm the results obtained with methods which detect the blood group substances. AB bloods always give a negative Lattes test (except see below). O cells may be used as a control for nonspecific agglutinins. The possibility of naturally occurring antibodies in serum other than ABO isoagglutinins cannot be overlooked either.

Sensitivity has been an issue with the procedure as efforts have been made to determine the group characteristics in ever smaller amounts of stained material. It has been the experience of most investigators that the test is more successful on blood crusts than it is on stains in which the blood has permeated the substratum. This result may be a reflection of the concentration of isoagglutinins in the material actually taken for the test. Extraction procedures have been proposed to try and overcome these difficulties, but some have been cumbersome and not very practical. Lattes and Canuto (1926) said that stains impregnated in fabric can be extracted in a minimal amount of distilled water, and the extract dried down on a slide, dropwise, in a current of air in order to form a crust. Lattes (1932) indicated that this procedure required relatively large amounts of bloodstained material and was not very suitable for smaller stains. In 1927, Müller proposed a technique for extracting, and then concentrating the extract by means of evaportion under vacuum. He called the method "Agglutinin-Anreichungsverfahren" (agglutinin enrichment or concentration technique). About 70% of the stains tested could be grouped correctly in this way. 24 hour extraction times were used. Brunner (1927) carried out a number of studies on this procedure. Colloidal solutions, such as gelatin, albumin and gum arabic were added to prevent rouleaux formation, and stains up to 14 months old could be correctly grouped in some cases. Holzer (1931) reported, however, that he had been unable to get good grouping results with this technique, following Müller's prescriptions closely. Lattes (1932) regarded the procedure as unreliable as well. Serebryanikov (1927) in Odessa proposed a somewhat similar procedure, which was discussed by Popoff (1929). Gettler and Kramer (1936) said that they had obtained reasonably good results with the classical technique in blood crusts, but not in bloodstains on fabrics. Faraone (1942) recommended extraction of bloodstains at 45–50°, and said that the procedure improved the results, especially with older stains in which insolubility was a problem. Harley (1943) used an extraction technique as well. Saline was the extraction medium, and in cases where the bloodstain had permeated the fabric, about 0.5 cm^2 of material, finely shredded, was extracted for 24 hrs in the cold. The extract was collected in a capillary tube, centrifuged, and the supernatant tested with A, B and O cells. The reactions were read over a period of hours, up to 24 hours if necessary. Merkeley (1953) used this procedure in his studies. Nickolls (1956) mentioned that the test could be done with trypsin-treated test cells. Marcinkowski (1959) tested a concentrated extract from a stain, obtained by the

paper chromatographic procedure described in section 7.2 for separating bloodstain components from contaminating material, and concentrating them in the tip of the V-shaped filter paper strip. He said that the agglutinins could be detected. Fiori *et al.* (1963), however, said that they had had no success with this procedure. Ducos (1960) suggested that the sensitivity of the test could be increased by using papain treated test cells. Results could be obtained on three year old stains in this way, but some stains, even recent ones, failed to react. Funk and Tostiak (1965) recommended doing the test in 11% bovine serum albumin. Agglutination could occur as soon as 15 minutes after adding cells, or require as long as 24 hrs depending on the condition of the stain. Wiener (1963) recommended the addition of a solution of gum acacia to the test to increase sensitivity. The stain was extracted with saline and the extract placed separately into three tubes with A, B and O cells. These were kept at 4° for a half hour, then centrifuged. The supernatant fluid was removed, replaced with cold saline, and the tubes then read. Two drops of gum acacia solution (10 g in 90 mℓ water containing 1 g monobasic sodium phosphate, and sterilized) were added, and the tubes re-read after 2 hr at room temperature. A third reading after centrifugation was taken as well. Stains up to two years old could be grouped in this way, the agglutination coming up more strongly with successive readings.

The sensitivity of an agglutination test is a sensitive function of red cell concentration at a given antiserum concentration. While this fact is sort of intuitively clear, a good many workers appear to have overlooked it on connection with the Lattes test. Lund (1941) demonstrated experimentally the importance of red cell concentration to the sensitivity of an agglutination test. Many investigators used cell suspensions ranging from 0.5% to as much as 5% in concentration. Lund found that an agglutination test was some eight times more sensitive at cell concentrations of 0.0625% and about 32 times more sensitive at cell concentrations of less than 0.007% than with the usual 0.5 to 5% suspensions. Outteridge (1965a) made a point of these findings in his review. Kind (1955) recommended a simple tube technique in which 0.5% cell suspensions were put in contact with portions of stained material for 2 hours, then removed and read for microscopic agglutination. Outteridge (1965b) recommended a very similar technique, except that the test was performed in well slides, and 0.01% indicator cell suspensions were employed. Kissling and Neumann (1972) proposed a curious technique for the agglutinin test. They employed suspensions of test cells which were sensitized with the homologous antibody. Sensitization was accomplished by treating the cells with dilutions of antisera eight times more dilute than the titer value (3 tubes more dilute than the last tube giving agglutination). The procedure, they said, gave greater sensitivity. The idea for this approach was based on results obtained by Schulz (1970) in which he had found that such sensitized cells gave greater sensitivity in the mixed agglutination test (see in subsequent section).

Outteridge (1965a) made the important point that the extraction of stains for the agglutinin test with media that do not contain the test cells are not to be remommended, especially where extraction times are relatively long. Many substrata contain substances that absorb α and β agglutinins. Wool can be a particular problem in this way. For this reason, cloth controls are always done in carrying out the absorption tests (see in next section). In the course of a long extraction, the substratum material could absorb a substantial portion of the solubilized antibody, leaving little to react with test cells. If cells are present, they are at least able to compete with substratum receptors, should the latter be present, for liberated antibody. Another point, made by Moureau (1963) in his review, is that test cells should be carefully and thoroughly washed. The reason is that it has been known since the experiments of Schiff (1924b) (see in section 19.8.1), that serum can contain soluble blood group substances corresponding to the red cell type, and these could compete with the test cells for available agglutinin, thus decreasing the sensitivity of the test. The issue of non-ABO isoantibodies being present must be considered. Moureau (1963) recommended carrying out the test in duplicate with both Rh+ and Rh− test cells. This procedure would detect any saline agglutinating anti-D (anti-$\mathbf{Rh_o}$) in the bloodstain. The incidence of natural antibodies to Rh, MNSs, Kell Kidd and Duffy antigens is not very high, and most of them are not saline agglutinating. Giblett (1977) found that less than 1% of a very large series of blood donors in the Seattle area had such antibodies, but the possibility should not be overlooked if the Lattes test results are not in agreement with the results of the tests for ABO antgens. Most investigators have recommended the use of A_1 test cells for α in the Lattes test. In this regard, the occurrence of the so-called "irregular" α_1 agglutinins in A_2 and A_2B sera, which is not all that rare in some populations (see section 19.3.1.1), should not be overlooked (see the case discussed in section 19.10.7).

There have been many conflicting reports on the persistence of the agglutinins in bloodstains. Under favorable conditions, the agglutinins can apparently persist for considerable lengths of time. Detectability is bound to be dependent upon the techniques employed; and solubilization as well as optimization of the concentrations of agglutinins and cell suspensions are undoubtedly important considerations. Levine (1932) said that the agglutinins in a four year old O stain had been detected. Matta (1937) found that, in many cases, the agglutinins could no longer be detected in stains 3 months old, although in some samples, they were detectable in 6 month old stains. Balgairies and Christiaens (1937) found that about three-fourths of 3 month old group O bloodstains retained both agglutinins, while some had lost one or the other. About three-fourths of the A stains still had β agglutinin, and the B stains all contained α. Stains aged in sunlight showed somewhat greater losses of agglutinin in all groups. Kayssi and Millar (1937) indicated that the strength of the agglutinins was not attenuated by exposure of 10 day old stains to a dry 64° heat for an hour, nor by exposure to

ultraviolet radiation for the same length of time. A one hour exposure to 100° dry heat, however, resulted in considerable diminution of activity. Faraone (1942) could always detect agglutinins in stains 7-11 months old with his warm extraction procedure, and occasionally in older stains as well. Merkeley (1953), using Harley's (1943) procedure involving long extraction times and long incubation times with test cells, examined 100 stains 12 to 18 months old for agglutinins. Anti-A could be identified in 34 of 43, and anti-B in 17 of 43 group O stains. Anti-A could be found in 12 of 15 group B stains, and anti-B in 13 of 36 A stains. These results indicated that the β agglutinin in O stains is somewhat more labile than the α agglutinin, in accord with what Balgairies and Christiaens (1937) had reported in a smaller sample of more recent stains. Marsters and Schlein (1958) found that, in some cases, agglutinins could not be detected after 5 days, while in others, they were detectable in stains up to three weeks old (when the experiment was terminated). Wiener (1963) got the correct result in 3 A and 3 O stains, which had been kept cold for two years, using the gum acacia technique. Haseeb (1972) said that the agglutinins could still be detected in a B, 2 O and 2 A stains after 12 years at room temperature on the lab bench. Outteridge (1965a) studied the effect of humidity and heat on the disappearance of agglutinins in stains prepared on filter paper. Humidity was the most damaging condition. The agglutinins were more stable cold than either warm or hot, those stains kept at 50° being inactive after 2 weeks in a humid chamber, and after 2 months if kept dry. Stains kept at 4° in a dry container retained activity the longest. At 2 months, and at 9 months, the A and B stains could still be grouped correctly, but the β agglutinin had disappeared in the O stain. The β agglutinin had been weaker than the α in the O stain to begin with.

Many years ago, there was some interest on the part of clinical serologists in storing grouping antisera in the dried state, i.e., simply dried out on slides. A number of experiments were done to see how long such sera, dried on glass slides or cover slips, retained its agglutinating activity. Sanford (1918) said that dried serum remained active for two months kept at room temperature. Kolmer (1919a, 1919b and 1920) found that isoagglutinin activity had already begun to decrease in dried serum within the first to fourth day. If it were kept cold, it could be used up to two weeks. Karsner and Koeckert (1919) said that the isoagglutinins deteriorated within 2-3 weeks in the dried serum, and after 3-5 weeks lost their group specificity. They said that in 7-10 week old dried serum, they always observed non-specific agglutination. These experiments with dried out serum are, of course, only peripherally related to the issue of agglutinin survival in dried out whole blood, but if the results are correct, they would seem to show that the agglutinins survive considerably better in dried out whole blood than in dried out serum.

19.10.3.2 Detection of agglutinogens in bloodstains by absorption technique—absorption inhibition or agglutinin binding.

• Development of the Technique

This procedure derives directly from the absorption techniques that have been used with red cells. It was a number of years after the discovery of the ABO groups before the technique was applied to dried bloodstains. Medico-legal procedures had been focused on the detection of the agglutinins, as noted above.

The absorption precedure has many variations, but in principle is quite simple: A bloodstain containing an agglutinogen placed in contact with an antiserum containing the corresponding agglutinin will reduce the titer of the agglutinin in the antiserum. This technique is often called "absorption-inhibition" in this country, and sometimes too in England. The Germans have usually called it "Agglutininbindung" technique.

It had been known since the early work of Landsteiner and others that red cells containing an agglutinogen would selectively absorb the corresponding agglutinin from an antiserum containing it. There does not appear to be a record in the published literature of anyone having applied this principle to bloodstains prior to 1921. In that year, H. Schütze at the Lister Institute in London recorded a number of relevant experiments. A case had come to his attention in which some blood was alleged to have poured forth supernaturally from a holy picture, and there was interest in determining not only whether this dried blood was of human origin, but also in determining its blood group if possible. Schütze said that the blood was human by the precipitin test, and that the case had prompted him to conduct the experiments reported. Apparently unaware of Lattes' efforts, he indicated that the agglutinins could be detected in dried bloodstains, and the group determined in this way, unless the stain were of group AB, in which case the negative results would be indistinguishable from those from a stain of a different group in which the agglutinins had become inactive. A technique for determining agglutinogens would be quite desirable for this reason, he said. In the experiments, he dried blood in Petri dishes and on cloth substrata, and reconstituted it in saline. Group O serum was added, and, after suitable incubation, tested again to see which agglutinins had been removed. A group B stain removed the anti-B from O serum after the stain had aged 41 days in sunlight, and a 5 month old group A stain removed the anti-A agglutinin.

It is clear that Lattes, then in Messina, and Siracusa in his institute, had carried out a number of experiments on the inhibition technique in the 1922-1923 period, and perhaps earlier (Lattes, 1923; Siracusa, 1923). Outteridge (1965a) mentioned that the inhibition method had been attributed to the Japanese investigator Kwansuke Sera in 1926, but he said that the method probably pre-dated this work, as indeed it did. The reference to Sera's work, which I could not examine, is probably: *Shakai Igaku Zasshi,* number 474, page 375 (1926). Schiff (1926) gave a technique for determination of the agglutinogens by the inhibition method (Der Agglutininbindungsversuch). He did not recommend using O sera, which contains both agglutinins, for the test because of differences in the agglutinin titers. Many other investi-

gators used O sera. He stressed the importance of substratum controls as well, to detect nonspecific agglutinin binding. Siracusa (1923) conducted extensive experiments on the absorption of agglutinins from O sera by bloodstains which had been treated with heat and a variety of chemicals (see in Unit IX, Translations). An O serum was chosen in which the α and β agglutinin titers were about the same. The serum was diluted 1:3 and incubated with appropriate bloodstained material. After a number of hours, the preparation was centrifuged, and an aliquot of the supernatant fluid tested for agglutination with A and B cells. Exposure of dried blood to 100° dry heat did not abolish the ability of the agglutinogens to absorb homologous agglutinin. Exposure of dried blood to a number of denaturing chemicals, including 1N HCl, 0.1N NaOH, ammonia vapors, 5% HgCl, 1% $AgNO_3$, 3 to 6.7% $K_2Cr_2O_7$, 1% OsO_4, $CHCl_3$, acetone and ethyl ether, did not destroy the agglutinogens. Acetic acid and ethanol inhibited the uptake of agglutinins, but did not completely abolish it, while treatment with 2.4% $KMnO_4$ led to nonspecific uptake of agglutinins (or their destruction). Aliyev (1927) described an inhibition technique with which he said there was no problem in grouping bloodstains up to 8 weeks old. Popoff (1929) discussed the use of this and other blood grouping methods for dried stains in Russia. Higuchi (1929) carried out experiments on agglutinin absorption. He recommended the use of A and B sera, rather than O sera, because he said the absorption characteristics were better. Antisera at a titer of about 1:30 were recommended for the test and absorption was carried out for three hours. He said that correct results had been obtained with some stains that were 21 years old.

In 1931, F. J. Holzer in Innsbruck published an important paper on the agglutinin binding method. His procedure, which became the basis for that used in many laboratories for a number of years, was adapted from the one used by Lattes and Siracusa, except that Holzer checked the degree of inhibition by titration of the antiserum after the absorption process. He employed selected O sera with initial titers of between 1:16 and 1:64 (microscopic agglutination after 30 min incubation). A small amount of bloodstained material was incubated with test serum in the cold for 24 hours. The supernatant serum was then titrated against A and B cells in well slides, and the titer compared with that of unabsorbed serum. A number of materials were tested for nonspecific agglutinin binding, and although most were negative, the importance of substratum controls was stressed, In some 387 tests, the group could be correctly diagnosed in 366 cases. Holzer found that A agglutinogen generally gave greater reductions of α titer than did agglutinogen B of β titer. With initial titers of 1:16, the α agglutinin was usually reduced between 3 and 4 dilution steps, while β was reduced between 2 and 3 steps. The reduction was less marked with lower titer serum, and Holzer said that higher titer antiserum should be employed. Note that he was comparing antisera with titers of 16 and 8 in these experiments. One cannot employ antisera at too high a titer for inhibition or no inhibition will be seen. He tested a number of old bloodstained objects in the collection at his Institute, and although there was no way of knowing the correct group in these cases, the combined distribution of groups agreed well with the Innsbruck population distribution for the ABO groups.

From the foregoing, it is clear that there are two general ways of doing the test. One has been called the "all-or-none" method. In it, relatively low titer antisera is incubated with an amount of bloodstained material or extract sufficient to remove all the corresponding agglutinin. From the complete removal of antibody (absence of agglutination upon addition of test cells) is inferred the presence of the corresponding agglutinogen. This method was employed by Siracusa (1923), Higuchi (1929), Schiff (1932), Wiener (1939), Schiff and Boyd (1942) and White (1954). The other alternative is the method of Holzer, in which the antiserum is titrated after absorption to determine the number of doubling dilution steps by which the test serum titer has been reduced by the homologous agglutinogen. Many authors have recommended this technique, or some modification of it (Therkelsen, 1934 and 1936; Boyd and Boyd, 1937; Harley, 1943; Dahr, 1953; and Tan and Wong, 1963). Funk and Towstiak (1965) recommended a technique similar to that of Holzer, except that they added a drop of 11% BSA to the test tubes following the absorption step and incubated an additional 5-10 minutes in a moist chamber before carrying out the titration.

- Sensitivity

One of the problems with the inhibition test is its relative insensitivity. Fairly large amounts of material are needed to carry out the test. Holzer (1931) recommended 10 mg dried blood for each 0.1 mℓ antiserum used. Other authors (e.g. Therkelsen, 1936) have suggested even larger quantities of blood, up to as much as 80 mg. Various modifications have been proposed to increase the sensitivity of the procedure and reduce the amount of material thus required. Ponsold (1934) described a technique in capillaries which could be carried out on 1 mg dried blood. Hausbrandt (1938) gave a modification of the capillary procedure for which only 0.2 mg dried blood was said to be required. Tan and Wong (1963) used a slide technique and titrated in 1½ fold dilution steps. They said the method was applicable to 0.5 to 0.7 mg dried blood. Kishino (1955) used long absorption times, and with incubation at 37°, then room temperature, then cold, and said that the technique was applicable to 0.04 to 0.1 mg dried blood. He also said that in older stains (1-2 years), a 1-2 hour treatment with 0.3-0.5% solutions of trypsin at 37° made the bloodstain much easier to group by the inhibition method. Obviously, the amount of dried blood required for the test is a function of the titer of the test sera. More material is required to inhibit a higher titered serum.

- The Use of O (Anti-A,B) Sera

The issue of using O sera as against separate anti-A and anti-B has been discussed by many workers. The early workers used O serum. Those who have argued against using it have done so principally on two grounds: first, that the α and β agglutinins are often not of equal titer (Schiff, 1926); and

second, that A stains can absorb some cross reacting antibody, capable of reacting with B cells, and B stains can absorb some antibody capable of reacting with A cells, from O sera. On the first point, Schiff (1926), Schiff and Boyd (1942), Dahr (1953) and Moureau (1963) among others have advocated the use of anti-A and anti-B serum. Harley (1943) did not accept difference in agglutination titer as an argument against the use of O serum. Since the titer difference is determined against a control for purposes of interpretation, he did not think it mattered whether the initial titer of anti-A and anti-B were the same or not. The second matter is more serious. This property of some O sera, wherein B cells can appear to absorb α, or A cells β, was discussed in section 19.7.2. Harley (1936) found an O serum which showed this behavior with an A bloodstain. The phenomenon was confirmed with A cells. B stains or cells did not absorb any anti-A from this serum, however. Harley said, therefore, that O sera must be carefully evaluated for this characteristic, and rejected if they have it. But he did not think it was necessary to abandon the use of O serum for the test, and he still recommended it in his book in 1943, provided it had been evaluated. Many workers, though, prefer simply not to use it. The only advantage to it appears to be a saving of one tube, and, of course, twice as much bloodstain is needed if separate anti-A and anti-B absorptions are set up. Some authors have suggested mixing anti-A and anti-B in equal parts (e.g. Tan and Wong, 1963). The principal objection to this practice is that the serum of A and B secretors can contain significant amounts of A and B substances, thus reducing the titers of the anti-A and anti-B unequally (Schiff and Boyd, 1942). Schiff and Boyd recommended that immune anti-A and anti-B be used in the test along with isoantisera. These reagents were mixed together in equal proportions to make an anti-A,B reagent, since the animal serum would not contain any interfering soluble A or B substances.

- Interpretation

Most authorities have indicated that interpretation of these tests be made with caution, especially when only anti-A and anti-B reagents are used. With bloodstains, the test for agglutinins should always be carried out along with the absorption test. The major difficulty is in the diagnosis of weak A receptors and of group O. Negative absorption tests for anti-A and anti-B constitute necessary but insufficient evidence for the diagnosis of a group O stain. A diagnosis can be confirmed only if positive results are obtained for the presence of both the α and β agglutinins, or if a specific anti-H reagent is used (see below). It is more difficult to diagnose A_2 than A_1 in stains because its absorptive power is lower. A_2 in A_2B stains is weaker still. In this connection, Therkelsen (1934 and 1936) recommended that A_2 test cells be used along with A_1 test cells as indicators for A. If an anti-A serum containing both α and α_1 is used with an A_2 stain, the α is absorbed but not the α_1, and the use of A_1 test cells exclusively might cause one to miss an A_2 altogether in an inhibition test. Wiener (1939) and Schiff and Boyd (1942) completely agreed. Schiff and Boyd said that they would not in fact exclude the possibility of a stain which grouped as B being an A_2B when they reported their results. Similarly, stains which gave negative results with absorption tests with anti-A and anti-B, but in which no agglutinin test results were available, would be said to be "probably of group O", but with the possibility left open that they could be of A_2 or a weaker subgroup of A. Benciolini and Cortivo (1977) said that they had not been able to group the A in A_2B stains successfully using the inhibition technique.

- Inhibition with a Doubling Dilution Titration Series of Antisera

In 1932, Hirszfeld and Amzel introduced a somewhat different way of carrying out the inhibition test, which requires more work and needs more material, but which makes the test considerably more sensitive. The procedure was advocated by Kind (1955). Here, a constant amount of antigen (bloodstained material or extract) is incubated with a series of doubling dilutions of antisera, the entire series being tested for remaining agglutinin by the addition of test cells. Kind (1955) discussed the difference between this procedure and the classical (Holzer) technique, as did Outteridge (1965b) in his review. Suppose an antiserum with a titer of 1:256 is incubated with an amount of antigen-containing material sufficient to remove half the agglutinin. If this absorbed serum is titrated, it will show only a "one-tube" reduction, that is, the titer will be reduced to 1:128. Twice that amount of antigen would reduce the titer of the 1:256 antiserum to zero, though. On the other hand, if the same amount of antigenic material (an amount sufficient to remove half the agglutinin from a 1:256 antiserum) were set up by the Hirszfeld and Amzel-Kind technique, the "titer" would be reduced down to "neat" (i.e. 8 steps). This behavior is illustrated in Figure 19.12. In the classical technique, an amount of antigenic material which reduces the agglutinin in the test serum by one-fourth would be missed altogether. Kind (1955) pointed out that the titration procedure in the classical technique does not yield very much more information, for the amount of extra work involved, than does an "all-or-none" test. The all-or-none test is done with relatively low titer antisera (1:8–1:16) to compensate for the antigen content in relatively small amounts of bloodstain. The problem with such a procedure is that if there is significant nonspecific absorption by the substratum, the substratum control will show a reduction along with the bloodstain in the tests, and make the test results impossible to interpret. With higher titer antisera, the nonspecific absorption would generally be much less noticeable. Fiori *et al.* (1963) said that the "all-or-none" procedures should not be used because of this problem. Schleyer (1957) compared the classical technique with the Kind modification, and found that the classical technique was preferable. Alfultis (1965) described a microprocedure for carrying out the Hirszfeld and Amzel-Kind modified procedure.

- The Problem of Non-Specific Absorption and Interference Due to Contamination

The problem of nonspecific absorption by substratum materials has been periodically investigated. Zipp (1931) found

Figure 19.12 Comparison of Results of Inhibition Test by Holzer vs. Hirszfeld and Amzel-Kind Techniques

Doubling Dilution Titration After Absorption (Holzer):

Condition	Reciprocal Dilutions: neat	2	4	8	16	32	64	128	256	Result
No Absorption (Control)	+	+	+	+	+	+	+	+	+	———
¼ Antibody Absorbed	+	+	+	+	+	+	+	+	+	No reduction
½ Antibody Absorbed	+	+	+	+	+	+	+	+	—	One tube reduction
Complete Absorption	—	—	—	—	—	—	—	—	—	Complete reduction

Doubling Dilution Titration Before Absorption (Hirszfeld and Amzel-Kind)

Condition	neat	2	4	8	16	32	64	128	256	Result
No Absorption (Control)	+	+	+	+	+	+	+	+	+	———
¼ Antibody Absorbed	+	+	—	—	—	—	—	—	—	Seven tube reduction
½ Antibody Absorbed	+	—	—	—	—	—	—	—	—	Eight tube reduction
Complete Absorption	—	—	—	—	—	—	—	—	—	Complete reduction

that a number of materials used in textile manufacturing would absorb agglutinins. Werkmeister-Freund (1932) did not encounter problems with nonspecific absorption in bloodstains mixed with clean sand, garden soil or sawdust. Holzer (1931) in the original paper had noted that the mud in a sample of blood-soaked mud showed some absorption of agglutinins, which interfered with the test. Berger (1933) studied 64 dirty bloodstains by the absorption technique and found that 14 of these gave problems due to non-specific absorption of agglutinins. Therkelsen (1936) examined 179 different fabric samples from underwear, regular clothing and outerwear. In 19 cases, absorption was observed, most often of α when tested with A_2 cells. A three tube reduction was seen in some cases. He also examined the absorption properties of papers and the bark and wood of a number of trees. Parchment gave substantial absorption, as did the wood and bark from trees, including chestnut, beech and fir. A number of mineral materials, such as zinc and aluminum powders, and finely pulverized marble, feldspar, kaolin, silica gel and talcum gave no absorption. Guareschi (1937) reported nonspecific absorption by silk, and Granata (1955) observed it with cotton but not with wool. DeRen *et al.* (1970) found that stains on nylon created serious problems in attempting to get an ABO group by the inhibition procedure.

A related problem is that of contamination of the bloodstain with various materials or laundering agents. Siracusa's experiments (1923) on the effects of a variety of chemicals were discussed above. Eisele (1973) said that blood treated with 10% NaOH, 1% acetic acid or 1% $FeCl_3$, and then used to make stains could yield false positive inhibition results upon grouping the stains by the Holzer method. 1% eosin and 1% dextrose treatment had no deleterious effect. Depending upon how the test is carried out, the contaminants may have an effect on the antiserum or on the test cells. Kirk *et al.* (1954) raised this problem in connection with the presence of laundering agents in fabrics. With the detergents that were (and are) available, they noted that much less rinsing was required than was formerly the case with soap, and that there was a stronger possibility of the presence of residual detergent in a fabric. It could be shown that these agents caused lysis of the red cells when present in higher concentrations, and when present in lower concentrations, gave inhibition of antisersa in the agglutinin binding test indistinguishable from that of group specific substances (false positive). They had looked into a procedure for alcohol precipitation fractionation of the agglutinogens in such cases, but said that they had had limited success. Kind (1955) found that the hemolysis observed in the presence of soap or detergent residues in cloth was proportional to the soap solution:AB serum content of the antiserum used. The AB serum apparently served as a protein medium in which to dilute antisera, and protected the cells from hemolysis. However, AB serum can contain A and B substances, which can reduce the titer of the antiserum for which it serves as diluent (Kind, 1956) and it was found that 3% BSA worked just as well for the purpose as the AB serum. Marsters and Schlein (1958) tested the effect of a number of potential contaminants on the inhibition test, and did not find interference, except that the presence of soap or detergent could cause hemolysis of the cells. Simon (1964) tested the effect of two detergents on linen and wool for its effect on grouping by the Holzer technique. The experiment ran for 43 days. Bloodstains alone and detergent residue alone gave the same results with the inhibition test in terms of titer reduction during the entire course of the experiment. Bloodstains on detergent-residue containing cloth, however, were progressively less active with time, i.e., less and less group specific inhibition was observed, and after a few weeks, the group could no longer be determined. Tomita (1967) presented a method for extracting interfering detergent and surfactant materials from bloodstains by chloroform and other solvent extraction procedures. Gramer (1975) investigated the effect of several detergents, a fabric softener and an enzyme-containing pre-treatment product on the determination of the group in bloodstains by the Holzer technique. The enzyme product contained a protease derived from *B. subtilis*. A variety of fabrics were washed or dipped in these materials, and then artificial bloodstains were made on them for the experiments. A number of the fabrics themselves showed nonspecific absorption of agglutinins, and treatment with the cleaning agents in general had a more or less deleterious effect on the test. In a few cases, hemolysis was noted. The importance of comparing a bloodstain test to a proper substratum control test was stressed. While in a number of cases, no results could be obtained, there were no false positives with A_1, A_2, B or O stains. AB stains could be misdiagnosed. Kijewski and Mülmann (1975) found that dried blood mixed with various soils could not be grouped reliably because the concentration of agglutinogen was apparently too low. Clean sand was an exception. With some concentrating procedure, e.g., the filter paper chromatographic technique (section 7.2), the group could be determined.

- Lectins

The unequivocal determination of group O stains was a serious problem with the inhibition method until the development of anti-H reagents. Many authorities felt that a diagnosis of group O could not be made on the basis of negative absorption tests with anti-A and anti-B, unless the presence of both α and β agglutinins could be demonstrated. Before the discovery of useful anti-H lectins, the so-called anti-O reagents—usually animal serum of some kind absorbed with AB cells—were not easy to obtain. Ox serum was a common source of the reagent. Wiener (1939) pointed out the value of such reagents in the inhibition test, and showed that a reagent he had prepared was inhibited by O and by A_2 bloodstains, but not by B or A_1 stains. He said, however, that the reactions were not always clear cut. In 1958, Wiener *et al.* introduced the use of anti-H lectin from *Ulex europaeus* for the test. The reagent used had a titer of about 1:8 to 1:16 against O or A_2 cells. Many investigators began to use anti-H reagents in the test as more sources were discovered and became available (e.g. Funk and Towstiak,

1965). Kissling and Neumann (1972) used an anti-H from *Laburnum alpinum,* and did not get good inhibition results with it at higher titers (1:64 to 1:128). At lower titer values, it worked well. The higher titer material could be used, however, and sensitivity retained, if O test cells, previously sensitized with subagglutinating concentrations of lectin, were used. Potapow (1970a) recommended the use of an anti-B lectin from *Evonymus alata* for the inhibition test. Tryspin treated B test cells were used.

• Testing of the Immunoglobulins in Antisera

It may be noted that Misawa (1968) fractionated the immunoglobulins of anti-A in order to separately test them in the inhibition procedure. IgM was found to be the most easily absorbed antibody (among IgM, IgG and IgA), and test sera containing high concentrations of IgM were recommended for this test.

• Gamma Globulin Deviation Procedure

A rather complicated variation of the inhibition test may be mentioned for the sake of completeness. In 1957, Dell'Erba and Ambrosi proposed to quantitatively assess the amount of antiserum remaining after absorption by means of paper electrophoresis, followed by densitometric quantitation of the gamma globulin fraction. This procedure is the same one as was proposed by Ambrosi for species differentiation (section 16.6.7). Adamo (1957) believed the technique had promise, and recommended it. Laudanna and Segre (1959) criticized the procedure. They said that the extraction of serum protein from the stain and hemoglobin contamination due to hemolysis in the stain interfered with the determination. Dell'Erba and Ambrosi (1960) said that these points were not of practical importance in the method, and did not accept the criticism.

19.10.3.3 Detection of agglutinogen A by inhibition of hemolysis. This technique is mentioned as a matter of historical interest. It was never widely employed in practice, probably because it was never really applicable to group B. Rabbit antisera to group A cells, or alcoholic extracts of them, are also potent sheep red cell hemolysins. Similar sera can be obtained by immunization with sheep red cells. A bloodstain can be tested for its ability to inhibit the subsequent hemolysis of sheep red cells in the presence of complement (e.g. guinea pig serum). From the inhibition of hemolysis is inferred the presence of the A antigen in the bloodstain. The assay is very sensitive. It was put forward by Witebsky in 1927. Hirszfeld and Amzel (1932) confirmed the value of the test for group A stains, and Jadin (1934) thought that the test ought to be used in conjunction with the Lattes test and the agglutinin binding test.

19.10.3.4 Detection of agglutinogens in bloodstains by absorption-elution technique

• Development of the Technique

Absorption-elution is probably the most widely used technique for grouping bloodstains at the present time. The technique is based on the principle that the agglutinogens in dried blood will bind their corresponding agglutinins, the antigen-antibody complex can be dissociated subsequently, usually by raising the temperature, and the agglutinins recovered and detected by agglutination of appropriate, exogenously added test cells. The technique is very sensitive.

The development of the technique grew out of the early studies on agglutinin-red cell reactions, which showed that the antigen-antibody reaction was reversible and that binding was temperature dependent. In 1902, Landsteiner showed that serum agglutinins, absorbed onto red cell receptors at lower temperatures, could be recovered from the cells by raising the temperature to 40–50°. The temperature dependence of agglutinin binding to red cell receptors was studied in detail by Landsteiner and Jagic (1903), and in the same year, Landsteiner indicated that "cold agglutinins" in animal bloods could be recovered from cells by raising the temperature to 20–30° (Landsteiner, 1903). These results were confirmed by Koeckert (1920), Jervell (1921) and many others. Huntoon (1921) eluted bacterial antibodies from their receptors in the same way.

In 1923, Siracusa, working in Lattes' Institute at Modena, first applied the principle to the grouping of bloodstains. He subjected bloodstains to the action of heat, and to a variety of chemicals, and then tested them for their ability to absorb agglutinin (as indicated by the inhibition procedure). He then washed the cellular residue in the cold, added a drop of saline, raised the temperature to 45–50°, centrifuged using a water mantle maintained at 45°, and tested the eluate for agglutination with appropriate test cells. These experiments are discussed in section 19.10.3.2, where the absorption results are given. Absorption was carried out for a number of hours in the cold using O (anti-A,B) serum. In general, those residues which retained their group specific agglutinin binding (inhibition) properties (section 19.10.3.2) would also yield up the bound agglutinins after washing and incubation at 45–50° for 15 min. The paper has been translated, and may be read in Unit IX. In 1927, however, Siracusa called the reliability of the method into question, based on some discordant results that had been obtained with the inhibition and elution procedures. The elution test showed that nonspecific absorption of agglutinins had occurred, which had not been detected by the inhibition test. The problem was not believed to be the result of isoagglutinins present in the stains themselves. Undoubtedly, it had to do with the cross-reacting antibodies in O serum (section 19.7.2), and the fact that the elution test is considerably more sensitive than the inhibition test. Siracusa said that the elution test should be used as a means of confirming the results of the inhibition test. Lattes (1928) mentioned that cold agglutinins in the antiserum might be causing part of the problem of apparent nonspecific absorption in the elution test. Popoff (1929) proposed certain modifications of the elution test, including heating the stained material to 70–80° for a half hour to inactivate the isoagglutinins, to which he attributed the nonspecific reactions. He also recommended washing with, and elution into phosphate buffered saline. Popoff also said that absorption and elution could be carried out a number of times on the same piece of stained material. The elution step could be done several times into the same saline, or else several eluates could be combined

and concentrated, in order to avoid negative results caused by the level of eluted antibodies being too low. Siracusa, in the original work, had tried this device as well. Siracusa (1937) recommended against the use of either O sera, or mixtures of anti-A and anti-B for the elution test. He gave a lengthy procedure, in which the stain was heated to destroy its agglutinins. The inhibition test was then carried out with dilute antisera, followed by reincubation of the stain with strong antisera for the elution test. The stain was then washed with 45° saline, and reheated to 80° to inactivate any agglutinin still present, and the inhibition and elution procedure repeated again with different antisera. In 1941, Faraone working in Siracusa's Institute, investigated the reasons for the nonspecific reactions. He thought that cold agglutinins could be a problem, since absorption was carried out at 4°, and said that cold agglutinin-free serum had to be used for the test. Separate anti-A and anti-B were recommended as well, because of the cross reacting antibodies in some O sera. The phosphate buffered saline recommended by Popoff (1929) was found to inhibit specific agglutination reactions. Schiff (1926) described an absorption-elution technique in his book, calling the procedure "Absprengung des gebundenen Agglutinins". A 2–24 hour absorption in the cold with an equal mixture of anti-A and anti-B were recommended. The problem of false positive results because of nonspecific absorption was discussed, and Schiff regarded the test as confirmatory to the inhibition procedure results. Elution results which did not agree with those of a properly executed and controlled inhibition test which had given good results, he said, should not be taken seriously. Throughout this period, and up until 1960, the technique does not appear to have been widely employed as a means of bloodstain grouping.

Subsequent development of elution techniques took place in England, and the procedure has undergone numerous modifications. It is quite widely used at the moment for the grouping of bloodstains. Kind resurrected the procedure in 1960, and showed that it could be used to group blood crusts on glass slides. Crusts, consisting of 5–10 $\mu\ell$ of blood per cm^2 on slides, were fixed by plunging into 100° McIlvaine's buffer for 30 sec, [a citric-acid-phosphate buffer (McIlvaine, 1921)]. Absorption was for 3 hours at room temperature with occasional agitation and, after rinsing the material with ice cold saline, test cells were added and the temperature raised to 50° for 5 min (Kind, 1960a). Very soon afterward, Kind (1960b) said that the technique, essentially the same one as applied to blood crusts, could be applied to bloodstains on fabrics, a piece about 2 × 5 mm being used for each antiserum being tested. Kind elaborated on the technique in 1962. Among other things, he noted that fabric samples can and do show some nonspecific absorption of agglutinins. The use of thin test cell suspensions was not recommended as a means of increasing the sensitivity, therefore, because one might very well observe agglutination with the cloth control. The fixation in boiling McIlvaine's buffer (pH 7.4) was said to create an impermeable layer of protein on the fabric or surface of bloodstained material, so that nonspecific absorption of agglutinin was inhibited. Evidence that this was so came from showing that a fabric which did absorb anti-A nonspecifically would, after impregnation with group O blood, drying and fixation, fail to do so. Treating the bloodstained material with trypsin solutions inhibited absorption. The fixation process was said to have the additional advantage that it removed soluble soap or detergent material which might hemolyze the test cells, and Kind said that oily contaminants could be washed out of the bloodstain with petroleum ether prior to absorption. Fixation was also said to remove any soluble ABH substances that might be present. Kind said that 68 of 70 samples of dried blood on filter paper, sent by Dr. Dunsford for blind trial, had been correctly grouped. These included 35 A_1, 16 A_2, 2 A_1B, 4 A_2B, 7 B and 6 O stains. Two of the A_2 stains showed no absorption, perhaps because the artificial stains had been made from clotted whole blood specimens, and contained mostly serum. Kind said that a result was not regarded as certain unless the elution results were confirmed by the Lattes test.

Outteridge (1962a) confirmed the value of Kind's procedure, and said that aqueous extracts of stains dried in well slides had worked very well in his hands. A and B were detectable in 10 μg material, and H in 200 μg. Yada (1962) also reported that the technique worked well. He used an alcohol fixation, and did his absorption at 37° for 1 hour. He used 0.1–0.2% cell suspensions, and said that the technique was sensitive down to 0.001 mg dried blood for A and B. In 1961, Schleyer had tried out the technique, but he used tiles rather than cavity slides. He also used an anti-H reagent from *Laburnum*. A_1, A_2 and B stains that were relatively fresh could be correctly grouped, but A_1 and A_2 could not be differentiated (they grouped as A), and problems were encountered with A_1B and O stains. Non-specific anti-B absorption was noted with an A_2 stain, but also with the cloth control, and perhaps could have been accounted for in the bloodstain by insufficient washing. Schleyer recommended that the test be used along with the inhibition test. Outteridge (1962b) noted that Schleyer's difficulties may have arisen from his use of A_2 test cells, the use of *Laburnum* rather than *Ulex* anti-H which perhaps had too low a titer for elution technique, and from the employment of test cell suspensions that were too concentrated. In 1962, Nickolls and Pereira recommended a modified elution technique in which the boiling water fixation step was eliminated, because it was found to reduce the reactivity of the stain considerably. Threads from bloodstained fabrics were employed, rather than larger pieces, because they were easier to wash thoroughly. The test was carried out in cavity slides, with elution at 50° for 10 min into saline containing 1% BSA. Takagi (1968) found the procedure to be completely satisfactory. Fiori *et al.* (1963) proposed an elution procedure carried out in tubes, for which 2 to 3 threads or 0.5–1.0 mg dried blood (pulverized), previously fixed for 15 min in methanol, was used. Absorption was carried out at 4° for 5–6 hours or overnight. The test material was washed 3–4 times with cold saline, appropriate test cells being added

to the last washing, and checked for agglutination to insure that excess antiserum had been washed out. Elution was at 56° for 10 min with gentle shaking into 30–50 $\mu\ell$ saline, which was removed before adding the test cells. Budvari (1963) found this procedure to be completely satisfactory. Göring *et al.* (1969) reported a much higher success rate in grouping stains by Budvari's procedure (actually Fiori *et al.*'s procedure) than with either the Lattes technique or the Holzer inhibition technique. LaCavera and Scafidi (1972) used the technique as well, except that the mixing of the eluate and the test cells could be carried out in a fine bore capillary. After reading, a little methanol could be added, the ends sealed, and the capillary saved. Kind (1963) raised the issue of antiserum selection in connection with the elution test, as well as the inhibition and mixed agglutination (section 19.10.3.5) tests. Goodman (1962) had conducted experiments in which columns of polyurethane support media and formalin treated red cells were prepared, and agglutinin binding studied as a function of temperature. There was significant heterogeneity in the antibody population with respect to the temperature at which the molecules eluted from the column. Kind said in 1963 that these properties of antisera had to be taken carefully into account in the selection and preparation of antisera for the medicolegal tests. Further work on the subject was recommended.

• Further Modifications

A technical modification of the elution test, which is quite useful for routine work, was proposed by Outteridge (1963). Bloodstained threads or fibrils teased out of the fabric were glued into the wells of well slides by one of the ends. Absorption, washing and elution could then be carried out on the same slide. The mountant gave no interference with the test. Outteridge said that single threads could be used, but that he usually used more if there was sufficient material. Howard and Martin (1969) described a very similar technique for single threads on cellulose acetate sheets. Threads from the stained material, about 3 mm long, were affixed to the cellulose acetate using a glue made from cellulose acetate shavings in acetone. Absorption at 4° for 3–24 hours, washing with cold saline or water, and elution at 50° for 15 minutes into added test cells could all be conveniently carried out directly on the threads on the cellulose acetate sheet.

Ueno (1963) proposed a "transfer" technique for carrying out absorption-elution. A thread 6–10 mm long was moistened with distilled water and applied against the bloodstain causing some of the bloodstain material to be transferred to it. The thread could then be cut into three segments, fixed with methanol, and subjected to the elution procedure with anti-A, anti-B and anti-H. With older stains, the transfer could not always be achieved, in which circumstance a thread of the bloodstained material itself could be employed for the test. Ueno said that, in his experience, the fixation step recommended by Kind, in which the fibers were plunged into boiling buffer, did not remove the contaminating soluble group substances present in the stain.

Bashinski and Davis (1973) described a modification of the technique in which the washing step was carried out with the help of a vacuum filtration device. The same sample could be tested repetitively if necessary. Madivale *et al.* (1971) used a Pasteur pipette-shaped "washing tube", drawn so that the threads being tested did not pass through the fine bore of the capillary. Cold saline could then be poured in carefully and allowed to drain out, giving a completely controlled washing step. They determined the optimal washing volumes and flow rates through the washing tubes.

A number of workers have recommended fixation of the stain prior to applying the absorption-elution procedure. Popoff (1929) recommended a 70–80° heat fixation step, which was designed in part to inactivate the isoagglutinins of the stain. Kind (1960a and 1960b) fixed the test material in boiling McIlvane buffer, and Outteridge (1962a) followed this procedure as well. Yada (1962) fixed stains in absolute alcohol before the absorption step. Fiori *et al.* (1963) said that fixation in methanol for 15 min was essential, especially with dried blood clots and fresher stains, before proceeding to the absorption step. Nickolls and Pereira (1962) said that the boiling water (buffer) fixation step decreased the reactivity of the stain.

Extraction techniques have been employed as well. Outteridge (1962a) used an aqueous extraction procedure and carried out the tests on the dried extracts. In 1969, Kind and Cleevely introduced an ammonia extraction procedure. The absorption of agglutinins takes place only at the surface of a bloodstained fiber, they noted, and extraction procedures, followed by fixation of the extract on a glass surface, have the advantage of increasing available antigen receptors. The ammonia was introduced as an extraction medium because of the insolubility of older stains, and the resulting difficulty of extracting them with other aqueous solvents. Bloodstained material about 2 × 2 mm was extracted with 0.2 mℓ 0.880 aqueous ammonia, and the extract divided into six aliquots for duplicate testing with anti-A, anti-B and anti-H. A and B could be detected in about 2 μg dried blood, and H could be detected in 20 μg group O dried blood and 80 μg group A_2 or B dried blood. Absorption was for 90 min at 4°, followed by 30 min of washing with ice cold saline, and elution was carried out at 50–55° for 10–15 min. Chisum (1971) used a modification of this procedure in which very short absorption times (2 min) were employed. Washing was carried out in less than a minute, and the technique was found to be suitable for routine work. Its value, of course, is primarily the amount of time saved. Tröger (1973) found the Chisum procedure to be completely satisfactory, except he said that he used papain-treated O cells for the detection of eluted anti-H. Kind and Lang (1975 and 1976a) reaffirmed the value of the ammonia extraction procedure, in comparison with aqueous extraction, in a series of experiments on various parameters affecting the technique. They noted that they preferred the ammonia extraction to direct determination on threads, as was advocated by Howard and Martin (1969).

• Sensitivity

One of the singular advantages of absorption-elution, in

comparison with absorption-inhibition, is its sensitivity. Most estimates of the minimal amount of dried blood in which the blood group can be determined by inhibition are in the range of 1 to 50 mg, although a few estimates between 0.1 and 1.0 mg have been given. Outteridge (1962a) estimated the sensitivity of the aqueous extraction absorption-elution procedure to be 10 μg dried blood for A and B, and 200 μg for H. Yada (1962) estimated 1 μg dried blood for A and B. Outteridge (1963b) noted that the microtechnique (Outteridge, 1963a) is at least this sensitive, and perhaps more so. Fiori *et al.* (1963) indicated that consistently clear results were obtained with a 10 μg dried blood. Elution is, therefore, at least 100 times more sensitive than inhibition, keeping in mind that any such estimates for either procedure depend very much on the technique employed, the antisera used, and probably on the experience of the person carrying out the test as well. Vitullo (1966) said that the sensitivity of the test could be improved by using bromelin-treated test cells. Seven stains which gave no results with untreated cells were groupable using the enzyme treated ones.

- Interference by Adventitious Substances

The matter of the presence of substances which might interfere with the test has been discussed by a number of authorities. Any discussion of interference with bloodstain grouping tests is related to the fact that ABH-like substances occur in a wide variety of living things, from bacteria to other mammals (see in section 19.7.3). Kind (1962) noted that oily or fatty contaminants could be washed out with petroleum ether prior to carrying out the test, with no deleterious effect on the group substances. Fiori *et al.* (1963) tested bloodstains on wall material, wood and soil, and found that these substrata did not interfere. Sometimes, as with blood in soil, the blood material needs to be reconcentrated for testing, and this may be accomplished by the paper chromatographic method described in section 7.2, and which Fiori *et al.* (1963) also described. The hemoglobin concentrated in the tip of the filter paper strip, and could be recovered for identification and species tests with anti-human Hb antisera. The group substances distributed all along the paper, but were most concentrated in the zone closest to the edge in contact with the solvent (saline). Absorption-elution could be applied for their determination. Fiori *et al.* said that small strips had to be used, so as not to spread out the blood group substances over too large an area of the paper.

It is considered unlikely by some that soluble blood group substances from contaminating body fluids of secretors would present a problem in the absorption-elution procedure. Since they are soluble, the likelihood is that they would be washed out in the washing steps, or in the initial hot water (buffer) fixation step if it is used. Benciolini was said by Fiori *et al.* (1963) to have shown that soluble group substances in stains do not yield up antibodies upon elution. This was attributed to the possible formation of an antigen-antibody bond which was stable at elution temperature, but, as Outteridge (1965a) has noted, it could have been caused by the antigen-antibody complex being washed out in the washing steps. Tha Aye (1978) noted that garments stained with sweat could cause problems in determining the ABO group of a bloodstain on such garments by elution. He tested A and B bloodstains on garments which were contaminated with A and B perspiration. Saneshige *et al.* (1980) recently indicated that determination of the agglutinins (Lattes procedure) could be very helpful in sorting out mixed blood and sweat stains.

Kind and Lang (1976b) conducted a series of experiments on a number of potential contaminants of bloodstains, and their interference with the grouping tests. The studies focused on contaminants which are themselves known to contain ABH receptors. ABH activity has been reported in many animal bloods and tissues, and in numerous plant and bacterial sources. The ammonia extraction technique (Kind and Cleevely, 1969; Kind and Lang, 1976a) was used in the experiments. A number of samples of household dust (from vacuum cleaners), of soil, wood (shavings and sawdust) and microorganisms from the air (allowed to fall on blood-agar and grown) were tested. Twenty-two of 30 dusts reacted, 11 of these with one of the antisera, and another 11 with more than one. Four samples reacted for A, B and H. Both the strength and type of reactivity of the dust samples were greatly affected by the dust concentration. There was some correlation between the group reactivities of the dust samples and the groups of the usual occupants of the locations from which the dust was collected. Three of 24 soil samples, all with higher organic content, gave single or multiple group reactions. Twenty-four of 42 wood samples showed reactivity for B, H or both. No A reactions were seen. Samples were taken from 257 microbial colonies, and 72 showed A, B or H reactions, while 8 others showed AH, BH or AB reactions. Bacterial contamination of a bloodstain, caused mainly by slow drying, can render the stain completely unreliable for ABO grouping. If glassware for the serological tests is washed and re-used, contamination from prior tests could be a problem. The point was raised by Tha Aye (1977) who said that the difficulties are easily overcome by cleaning the glassware with 0.5M periodic acid. Periodic acid, in concentrations of the order of 1 to 5 mM, destroys the blood group receptors A, B, H, M. N, P, Le^a and D (Rh_o) on red cells (Morgan and Watkins, 1951). Fiori *et al.* (1963) noted that, in cases of suspected contamination of bloodstains being tested by the inhibition method by soluble body fluid group substances, a periodic acid treatment of the cloth control might shed some light on the problem, but they did not think this would be necessary when using elution tests. It is of interest to note that Tandon and Naik (1976) found H substance in the saliva of 56 dogs they tested. 92.8% were found to have A subsdtance, and 25% to have B substance in saliva. Contamination of fabrics with dog saliva might thus interfere with grouping of stains on such a fabric.

- Selection and Evaluation of Antisera for Absorption-Elution

Selection of antisera for the elution test was discussed by Kind (1963), who noted that it was important to recognize

that antisera contain heterogeneous populations of antibodies with respect to their thermal dissociation characteristics in antigen-antibody complexes, as shown by Goodman (1962). Everyone is in agreement that relatively high titered antisera are required for absorption-elution. Fiori *et al.* (1963) observed that anti-A and anti-B at 1:16–1:32 give weak, if not negative reactions in the test. Anti-A_1 sera and lectins did not give good results because the titers were too low. *Ulex* anti-H at 1:64 gave good results. Popoff's (1929) "enrichment" method, in which successively absorbed stain material is eluted into the same saline to try and enhance the agglutinin concentration, was tried to see whether it would improve the results with low titer sera or lectins, and it did not. It must be kept in mind that the "titer" of an antiserum is a function of the technique used and the conditions under which it is titrated (see in section 1.3.4.1). Anti-H, for example, will usually give a higher titer with papainized O cells than with untreated ones. Lang (1975a and 1976a) conducted a series of experiments on antisera intended for use in bloodstain grouping. Series of doubling dilutions of antisera were tested using the ammonia extraction elution technique against a series of different amounts of dried blood material. In the original studies on the ammonia extraction technique (Kind and Cleevely, 1969), neat antisera were tested against extracts of dried blood material ranging from 0.002 mg to 1.26 mg. Agglutination with eluted antibody increased up to a point, levelled off, and decreased again in some cases at the highest antigen concentrations (prozone effect). Lang's (1976a) results may be summarized as follows: There was a general positive correlation between antiserum titer and the agglutination score in the elution tests. In some cases, with more dilute antisera, a decrease in agglutination score was seen at high antigen concentrations (prozone). Different antisera with the same group specificity showed different optimal antigen concentrations, as judged by the agglutination score with the eluted antibodies. More A_2 blood solids were required to optimize reactivity with anti-A than A_1 blood solids. For ordinary purposes, it was found to be sufficient to check the antisera in a few dilutions with constant, near optimal concentrations of antigen (one dimensional test). It was noted that the antisera being used had already been quite carefully selected for red cell grouping, and had been tested for spurious irregular antibodies against Rh, MNSs, P, Lu, Le, Kell, Fy and Jk antigens. If such screening had not been done by the supplier, antisera should be tested against a panel of O cells for saline agglutination. Lang also pointed out that the amount of effort to be invested in antiserum screening must, in part, be a function of the size of the stock received at one time. It would not be very productive to invest several days and half the reagent in the screening of a quantity of antisera that was only going to last for a small number of tests.

Among the most carefully controlled quantitative studies on the parameters of the absorption elution procedure are those of Lincoln and Dodd (Lincoln, 1973; Lincoln and Dodd, 1973). The antigen-antibody reaction is reversible, and can be looked at thermodynamically. This matter was presented and discussed in section 1.3.4.1. The uptake of antigen and its elution are affected by the concentrations of antigen and antibody, and by the equilibrium constant for the reaction. The following parameters were studied: Rate of absorption, effect of varying the concentration of reactants during absorption, variations in the type of antibody, effect of varying ionic strength, elution temperature and the effect of cell suspension concentration on the detection of eluted antibodies. The tests were carried out by incubating antisera of known titer with known amounts of bloodstain for specified times, washing, and then titrating the eluates to determine the quantity of antibody recovered. Experiments were conducted with anti-A, anti-D and anti-c. Papain treated cells were used so as to obtain high titer values. The findings may be summarized as follows: (1) Comparison of absorption times of 2 and 16 hours with anti-A showed that 16 times as much antibody was recovered from stains absorbed for 16 hours as from stains absorbed 2 hours at an initial anti-A titer of 1:1000, and 8 times as much antibody at an initial titer of 1:256. Using the 16 hour absorption period, 4 times as much antibody was recovered from the stains incubated with 1:1000 anti-A as from those incubated with 1:256 anti-A. With anti-D, it could be shown not only that the use of *very* high titer antisera (1:1000–1:32,000) for absorption did not increase the quantity recovered from D+ stains, but that D-negative control stains showed nonspecific uptake of antibody. At very low anti-D titer, recovery from D+ stains was low. The nonspecific reactions in the negative control were attributed to failure to remove all the excess antibody in the washing step with the very high titered sera. (2) With anti-A at an initial titer of 1:1000, and varying the quantity of bloodstained material (amounts equivalent to 5, 2.5 and 0.25 $\mu\ell$ packed cells were used), the antibody recovery was 16 times higher in the 2.5 $\mu\ell$ specimen as in the 5 $\mu\ell$ one, and was unchanged by the additional tenfold decrease in going from the 2.5 to the 0.25 $\mu\ell$ specimen. (3) Using cells, it could be shown that the same quantity of red cells can absorb considerably more anti-A than either anti-D or anti-c of similar titer in a 1 hour absorption. This probably results from the very great differences in the number of receptor sites per cell. (4) Using anti-D diluted in 0.145M and 0.03M saline, the eluates from the D+ stain had about twice as much antibody in the lower ionic strength medium, but D-negative control stains showed significant reactivity. (5) Elution temperatures varying from 4° to 70° were tested for both anti-A and anti-D. The optimal temperature for anti-A was 45–65°. 55–65° was effective for anti-D as well, although 70° yielded the greatest amount of this antibody. These differences are attributable in part to heterogeneity in equilibrium constants in the various antibody populations in the test sera. In another experiment done with A cells, the titers of the eluates from cells sensitized with an anti-A were compared in the cases where (a) the eluate was immediately separated from the cells while the temperature was still 56°, and (b) the tube was allowed to sit at room temperature for 5 min before the eluate was separated. Sixteen times as much antibody was

recovered from the eluate which had been separated from the cells immediately (as assessed by both saline and papain titer). In practice, therefore, the eluate should be removed before the samples have a chance to cool. (6) The concentration of cell suspension used to detect the eluted antibody was varied in an experiment using an anti-c serum. Three different dilutions of anti-c were used for absorption. With the more concentrated anti-c, it was clear that detectability of recovered antibody was better with 0.5–0.05% cell suspensions than with 2% ones. At the highest dilution of anti-c, the detectability of eluted antibody increased steadily as cell concentration decreased, 16 times as much antibody being detectable with 0.05% suspensions as with 2% ones. In sum, these experiments indicated the importance of carefully standardizing the test conditions for the antigen being tested. Different antisera behave differently, and must be evaluated. The measurable recovery of anti-A from bloodstains eluted at 22° (saline titer of eluate = 1:4) points up the importance of washing out the excess antibody at 4°. Washing with warmer saline would cause considerable loss of absorbed agglutinin. Additionally, there is an advantage in titrating the eluate, rather than simply adding test cells to it. In those cases where the negative controls showed some reactivity, attributable no doubt to incomplete washing, the titers of the eluates from these were very low in comparison to the antigen-positive material. These differences, however, would not be seen without the titration. The procedures used in these experiments could be productively applied to the evaluation of antisera intended for use in the elution test, and it cannot be assumed that a particular lot of antisera will behave like any other lot. A final point is that the procedures employed must, of necessity, be a function of the nature of the antibodies (IgM, IgG, etc.) in the antiserum being employed. Some antibodies must be detected in colloidal media, others with enzyme treated cells, or by the anti-globulin test (see in section 1.3.4.1).

Lang (1975b and 1976b) showed that considerable enhancement of the reaction of anti-H lectin from *Ulex europaeus* with O and A_2 stains, using the ammonia extraction technique, could be obtained by the addition of BSA. The optimal concentration was found to be 20% BSA with other factors optimized, but this concentration was difficult to work with, and 7.5% BSA concentration was recommended. In some cases, O stains could be detected in this way which were not detected in saline.

- Reliability and Specificity

The age of stains is apparently not a serious drawback to the detection of ABH agglutinogens by elution technique. Yada (1962) said that he had correctly grouped a stain 62 years old, and it is undoubtedly possible to group stains even older, provided that they have not been contaminated. Rees *et al.* (1975) noted that blood preserved for blood alcohol testing could be correctly grouped after more than a year's storage at room temperature in the presence of NaF and oxalate. The elution technique was applied in these studies to stains prepared from the aged blood. Denault *et al.* (1978) recently found that ABH antigens were detectable by elution technique in bloodstains up to 26 weeks old on a number of substrata, including cotton, denim, wool, nylon, and permanent-press fabric, even if the humidity was high.

Most authors have agreed that the results of a properly executed and controlled elution test are entirely specific. With the exceptions noted in foregoing sections (contamination by adventitious substances), the test is ordinarily reliable. It is possible to miss a weaker antigen, however. In an informal quality control test arranged by Fox (1974), one of the laboratories failed to report the A antigen in an AB bloodstain. Benciolini and Cortivo (1977) said that A_2B stains presented problems, only 9 of 11 examples of them being groupable after 5 days aging. Better results were obtained when the stains were methanol fixed than when they were not. False positive results were possible, even in the apparent absence of contaminants. Denault *et al.* (1978) saw occasional false positive A and B reactions in 26 week old bloodstains on denim or on nylon.

- Other Methods of Eluting Antibodies

It may be noted, finally, that antibodies can be eluted from their antigenic receptors in a number of different ways. Heating is probably the only method commonly used in bloodstain grouping. This method of eluting antibodies originated with Landsteiner (1902). Some authors trace the technique of heating to 56° for 5 min to Landsteiner and Miller (1925), and although there is no doubt that they did use this elution technique, it was not the first time that agglutinins had been heat eluted from cells. Of all the different techniques, heating is probably the simplest, and can be applied to the grouping of micro amounts of stain material.

In 1936, Landsteiner and van der Scheer eluted antibodies to haptens on red cell stroma from these "antigens" by brief exposure to 0.1N acetic acid. Kidd (1949) eluted incomplete antibodies from red cells by exposing sensitized cells to 0.1M citric acid-HCl at pH 3.2–3.4. Hughes-Jones *et al.* (1963) gave an acid elution technique for anti-D which yielded up 80% of the bound antibody. Rekvig and Hannestad (1977) applied an acid elution procedure to IgM and IgG antibodies with A, B, D, C, c, E, e, Fy^a and K specificities. Vos and Kelsall (1956) used an ether elution technique, which was modified by Rubin (1963). Weiner (1957) used a cold ethanol procedure to elute immune antibodies to A, B, C, D, E, K and k from cells. Jensen (1959) said that Weiner's method gave better results than heating, but noted that it was quite a bit more involved as well. Chan-Shu and Blair (1979) described still another procedure, using xylene.

19.10.3.5 Detection of agglutinogens in bloodstains by mixed agglutination technique. The use of the term "mixed agglutination" in senses other than the one used here was discussed in section 1.3.4.1. Mixed agglutination, as used in this section, means a technique in which an antigenic receptor is identified in a cell or tissue by using a specific antibody to link the cell or tissue to indicator cells which themselves contain the antigen. The technique was first applied by Coombs and Bedford (1955) to identify A and B receptors on human platelets. In 1956, Coombs *et al.* used the technique to identify A and B on human epidermal cells. It has

been applied to assess the distribution of the A receptor in a variety of human tissue cells (Holborow *et al.*, 1960), the distribution of Forssman antigen in guinea pig tissues (Hawes and Coombs, 1960), and the distribution of ABH antigens in the tissue cells of frogs (Yada *et al.*, 1962). Ogata (1960) reported application of the technique to the grouping of red cells in a thin smear. He called the technique "hemagglutination on a smear" or HOAS, and noted that he had read a paper on it in 1958 at a meeting of the Serological Society of Japan, and published a preliminary report in Japanese in 1959. Medicolegal application of the procedure to the grouping of tissues and secretions is discussed subsequently (section 19.10.5.2). Its application to species determination (mixed antiglobulin technique) has been discussed in a previous section (16.4).

In 1961, Coombs and Dodd applied mixed agglutination to bloodstain grouping. Individual threads about 0.2 mm in length were cut from the stained material, and teased apart. The fibrils were pretreated with dilute acetic acid to fix and clear them. They were then washed in dilute normal rabbit serum (absorbed free of any human A, B or H agglutinins), and incubated with anti-A, anti-B or anti-H for 1-24 hours at room temperature. After washing, indicator cell suspensions were added. A, B and O stains could be grouped reliably. Papain treated O cells were employed for the detection of the bound anti-H lectin. Yada (1961) reported that the procedure was applicable to bloodstains. Fiori (1961) gave a slightly modified technique. He said that some problems were encountered with the preparation of thin microscopic test samples from some substrata, such as wood and paper. Fiori *et al.* (1963) quoted Beniolini as having encountered some false negative results with stains on wood or nylon, and there could be problems with false positive microscopic images with some substrata. Fiori *et al.* thought that the technique was more difficult than elution in its manipulations, but that with most stains it was reliable. Nickolls and Pereira (1962) found that a simplified version of the original Coombs and Dodd technique was quite satisfactory. They omitted the acetic acid step, substituted saline for the normal rabbit serum, and said that the papain treated O cells were not required to obtain satisfactory reactions with *Ulex* anti-H. Good results could not be obtained using *Dolichos* lectin. For most routine work, the elution technique was preferred. Roychowdhury (1963) described a version of the test, which he preferred to call "triple bonded agglutination", or TBA technique. Absorption for 1-2 hrs at room temperature was carried out on 2-2.5 mm long fibrils of stained material, which had been teased apart with a fine needle. After a saline wash, test cells were added and left 1-1½ hours. A gentle wash with saline preceded transfer to a slide, application of a cover slip and reading. Roychowdhury elaborated on this method subsequently (1973, 1974 and 1975), and said that the success rate with it was far better on case samples than with inhibition technique. Mitra and Ganguly (1973) modified the technique, saying that they merely withdrew the excess antiserum by aspiration following incubation with the stained fiber, i.e., the washing step was eliminated. The presence of agglutinates not associated with the fiber in the field due to residual antiserum did not interfere with interpretation in their view. Maresch and Wehrschütz (1963) adopted a technique very similar to that of Nickolls and Pereira (1962) and found it to work quite well. They noted that the positive results could be confirmed by subjecting the mixed agglutinates to a 50° elution step and looking for agglutinates in the field. Certain stained fibers (Nylon) apparently required this confirmation. In 1965, Akaishi described a mixed agglutination procedure in which the dilute acetic acid treatment of the fibers, originally employed by Coombs and Dodd (1961), was used. This step followed methanol fixation of the fibers. The technique gave good results, and it was suggested that it be called the "group specific double combination method" (GSDCM) for reasons mentioned in section 1.3.4.1. Mikami *et al.* (1966) said that catalytic tests for identification of blood (section 6), the fibrin plate method for human species determination (section 17.2) and the GSDCM for the ABO group could be applied consecutively to the same few threads of bloodstained material. In 1967, Halvorsen and Nordhagen described a mixed agglutination technique in tubes. They said that the results did not differ if the stained thread was incubated with antisera at 4° or at 18°, and that the maximal amount of antibody had been absorbed after 12 hours. In 300 samples tested, there was one error in which an A stain was grouped as an O (anti-H was not used). Nordhagen (1967) noted that woolen garments, well worn by A secretors, gave A reactions in mixed agglutination tests even if they had been washed a number of times. Three such A reactions were found in 24 fabric samples tested, and Nordhagen said that this finding emphasized the importance of substratum controls. Göring *et al.* (1969) said that they were considerably more successful in grouping dried bloodstains by the Halvorsen and Nordhagen procedure than with the Lattes test or the Holzer inhibition test. Benciolini and Cortivo (1977) noted that the A reaction in A_2B stains was not detectable by means of mixed agglutination, using the method of Ishiyama and Okada (1975). The latter investigators found that a number of different types of samples could be grouped for ABO using a modified mixed agglutination procedure called the mixed cell agglutination reaction (MCAR). The technique was used by Davidsohn and his collaborators to determine ABO receptors in thin tissue sections (Kovarik *et al.*, 1968). It has developed that this procedure may be applicable to the diagnosis of cancer (Davidsohn, 1972). In effect, the procedure consists of treating the sample with antisera on a microscope slide for 15-30 min at room temperature, washing, and adding test cells. The test cells are allowed to settle onto the specimen for 15 min or so, after which the slide is rapidly inverted and placed on props in a Petri dish filled with buffer or saline, such that the specimen just contacts the liquid. Unagglutinated cells settle out, and the sample can be examined under low power *in situ*. Ishiyama and Okada (1975) modified the procedure slightly for use with samples of medicolegal interest. Ishiyama *et al.* (1977) said that the MCAR mixed

agglutination procedure could be employed to determine the ABO group in latent fingerprints, lifted from surfaces with cellophane tape and transferred to microscope slides. Chatterji (1977) reported good results, equivalent in specificity and sensitivity to absorption-elution, with a mixed agglutination procedure on 2–3 mm long threads affixed to a cellulose acetate sheet. The technique was faster than elution, and saved work.

The mixed agglutination technique is probably equivalent in sensitivity to the elution test. Fiori *et al.* (1963) noted that some limitation is imposed in both methods by the fact that the manipulations can no longer be carried out if the sample is too small. Akaishi (1965) said that stains made from whole blood diluted 1:25,600 could be grouped using mixed agglutination.

19.10.3.6 Detection of ABO agglutinogens in bloodstains by fluorescent and ferritin labelled antibody techniques. In 1962, Hasebe employed fluorescent-labelled anti-A, anti-B and anti-$\mathbf{Rh_0}$ (D) for the detection of the corresponding antigens on red cells (Hasebe, 1962a). Two methods were used, one in tubes and the other on blood smears. In the same year, Hasebe (1962b) extended the application to bloodstains using high titered rabbit immune anti-A and anti-B, and detection of binding with fluorescent anti-rabbit globulin serum prepared in sheep. Both slide and tube methods were used. The latter gave better results, in that there was less non-group specific fluorescent antibody binding. Results with blood crusts were completely acceptable, but Hasebe noted that nonspecific binding of fluorescent antibody to cloth, fiber and wood substrata were still a problem in trying to make the determination on these types of bloodstains. Outteridge (1965a) noted that the method was not as complicated in practice as it seemed upon first looking at it, and that it had promise. Pollet (1969) reported that he had obtained good results with a procedure which he described in full. For ABO and MN antigens, fluorescent-labelled AHG serum was used. Kind and Cleevely (1970) found the procedure to be satisfactory with blood smears, but not with bloodstained fabrics. The fabrics often fluoresced, and an extraction technique was tried to circumvent this problem. Results were not good, and they thought hemoglobin might be absorbing most of the fluoresced light. An agar diffusion procedure was then used to try and separate the blood group active material in the extract from the hemoglobin, but in this case the fluorescent staining became nonspecific. They did not think the procedure looked very promising for bloodstains, but they were able to get good results with secretor saliva stains.

Suzuki (1970) reported that he had been able to group bloodstains by using ferritin-labelled antibodies and then examining the stained material in the electron microscope. A number of artificial bloodstains on fabrics could be correctly determined for ABO in this way.

19.10.3.7 The use of formalin treated red cells. In 1957, Moskowitz and Carb observed that treatment of A red cells with formalin renders them inagglutinable with anti-A reagents. Treatment of the cells with 10% formalin at 25° for 24 hours made the cells completely inagglutinable. Shorter treatment times, or lower formalin concentrations, or a combination of these, reduced the agglutinability of the cells without completely abolishing it. The receptors were apparently not damaged, however, as the formalin-treated cells absorbed the anti-A from an anti-A reagent as effectively as did fresh cells. Gold *et al.* (1958) confirmed the observation, and noted too that formalin treated red cells would, after sensitization with antibody, yield it up at 56° (elution) in the same way as fresh cells. The formalin treated red cells could also be lyophilized, and reconstituted in saline without losing their specific absorptive capability.

Marcinkowski (1970) proposed a procedure for grouping bloodstains based on the use of formalin treated red cells. He did not cite the work discussed above, and apparently thought that the formalin treatment stripped the antigens off the cell. He treated 1 part packed cells (group O preferably, but any group was usable) with 10 parts of 4% formalin in saline (final concentration) for 24 hours at 4°. He then washed the cells, resuspended them in saline and checked them for agglutinability with anti-A and anti-B. He called the inagglutinable cells "E-Li" (from Erythrocytus liber—antigenically free). The cells could be incubated with bloodstain material or its extract, and would take up antigen onto their surfaces. If the cells were washed, and tested with antisera, they agglutinated if they had been in contact with stain material containing the appropriate antigen. Marcinkowski said that 20 stains had been correctly grouped in this way.

19.10.3.8 Reversible agglomeration technique. This procedure is not so much a grouping technique, but a means of getting red cells which are in reasonably good condition from stored blood or cadaveric blood. In 1963, Huggins was looking for simple means to wash the dimethylsulfoxide out of frozen red cell preparations prior to transfusion. These blood units had been cryogenically preserved in glucose solutions containing DMSO. He found that if red cell suspensions were diluted with large volumes (1 vol red cells:10 vols diluent) of isotonic glucose or sucrose, the cells settled out at the bottom of the container quite rapidly (agglomeration). They could be washed with diluent, and allowed to resettle. If an equal volume of plasma were added to the settled cells, they were easily resuspended, and behaved normally (reverse agglomeration). The agglomeration depended on the presence of large quantities of isotonic nonelectrolyte, and on the pH being between 5.2 and 6.1. The mechanism was thought to have to do with the precipitation of γ-globulins which could bind to the red cell surface lipoprotein, and thus carry the cells down as well. In 1977, Michailow said that he had been able to use this procedure to recover red cells in reasonably good condition from putrefying, hemolyzed blood samples for grouping. A substantial number of samples treated in this way could be reliably grouped.

19.10.4 Determination of A subgroups in bloodstains

Efforts to distinguish between A_1 and A_2 in bloodstains are not new. Ponsold (1937) discussed a microprocedure for

carrying out the differentiation by the inhibition technique. Differentiation of A subgroups is mentioned periodically in textbooks prior to the 1960's (e.g. Huber, 1957). A number of authors have said that the inhibition procedure could be used to distinguish between A_1 and A_2 (as well as between A_1B and A_2B) using lectin reagents. Leister and Kirk (1961) tested a commercial *Dolichos* anti-A_1 reagent as well as fractions of *Ulex* extract, separated by continuous electrophoresis, and these fractions were referred to as "anti-A_2" and "anti-O". It was found that A subgroups could be determined in stains. Leister *et al.* (1961) confirmed the usefulness of the lectin reagents in subgrouping stains and red cells, although they appear to have believed that "anti-A_2" and "anti-O" specificities could be separated and used to distinguish between A_2A_2 and A_2O cells. Except for the *Dolichos* reagent, the intensity of the lectin reagent reactions with different cells was shown to be a function of the time of incubation (Sylvia and Kirk, 1961), and the test conditions had to be carefully standardized in order to achieve the desired discrimination. Grünwald and Lackovic (1963) said that anti-H from *Ulex* could not be used to discriminate between genotypes in the ABO system (e.g. AA vs AO). Moureau (1963) reported that there was no difficulty in determining A_1 and A_2 in bloodstains by the inhibition procedure using *Ulex* lectin. DeRen *et al.* (1970) reported, however, that they had been unable to distinguish A_1 from A_2 by an inhibition procedure with H and A_1 lectins.

Poon and Dodd (1964) reported that A_1 and A_2 could be discriminated in bloodstains by mixed agglutination using *Dolichos* anti-A_1 lectin, and testing with papain treated A_1 test cells. Kind's (1960b) studies on the elution procedure indicated that A subgrouping was possible as judged by the *Ulex* anti-H reaction. Outteridge (1962) confirmed Kind's results. Schleyer (1961) had difficulty with A_1–A_2 differentiation by the elution procedure using a *Laburnum* anti-H reagent, but this result could have come about in part because the titer was too low. Morgan and Richards (1967) tested *Dolichos* anti-A_1 in the elution system of Nickolls and Pereira (1962). They found that the intensity of the reaction decreased both with stain age and with the age of the frozen lectin preparation. Six month old reagent did not work, even with 1 day old stains, and 6 month old stains worked only with 1 day old lectin. They tried anti-A_1 serum, obtained by absorption of anti-A with A_2 cells, and did not find it to be very satisfactory. Enzyme treated test cells were used with the serum anti-A_1. Hayward (1969) described an elution procedure for diagnosing A_1 stains with *Dolichos* lectin. The stains were trypsin treated at 37° and washed prior to a 15 hour 4° absorption. Following absorption the preparation was frozen, and washed with ice-cold, partly frozen saline. Elution was at room temperature into trypsin treated A_1 cells. He said, too, that agglutination was optimal at 4°, and ran this part of the test in the cold. A 20 year old A_1 stain was diagnosed by this procedure, while a similarly aged A_2 stain was negative. Hilgermann (1971) described an absorption-elution procedure for differentiating A_1 from A_2 in bloodstains. *Dolichos* lectin and anti-A_1 serum reacted with A_1 and A_1B stains if A_1 test cells were used. A_2 test cells gave negative results. A_2 and A_2B stains reacted with *Ulex* or *Laburnum* lectin if A_2 test cells were used, and A_1 test cells gave negative results. If, however, enzyme treated cells were used (bromelin, papain, ficin, etc.), *Ulex* and *Laburnum* lectins reacted with A_1 and A_1B stains using A_2 test cells, and *Dolichos* lectin reacted with A_2 and A_2B stains using A_1 test cells. Poon and Dodd (1964) had not observed any reaction of *Dolichos* lectin with A_2 stains in mixed agglutination using papain treated A_1 test cells. Tröger (1973) noted that his initial results indicated that *Dolichos* lectin could be used to diagnose A_1 stains by the Chisum (1971) rapid ammonia extraction procedure if papain treated A_1 test cells were used. Pereira (in Culliford, 1971) questioned the reliability of the subgrouping techniques in bloodstains by absorption-elution procedure. Khalap and Divall (1979) reported that bloodstains could be successfully typed for A_1 and A_2 subtypes by elution, using the specific anti-A_1 protectin from the albumin glands of *Helix aspersa*.

19.10.5 ABO grouping in body fluids and tissues

19.10.5.1 Introduction. Medicolegal application of the group substance determination in body fluids could be of two kinds: Determination of secretor status (in saliva) as an additional marker in affiliation cases; and determination of the ABO group from body fluids, stains or human tissues in connection with criminal investigations. Both applications have been practiced for quite some time. There was some hesitation on the part of experts to use the secretor property in affiliation cases for a while, because not everyone was convinced that the distinction between secretor and nonsecretor could always be made with certainty. Holzer (1937) carried out extensive studies, but warned against the introduction of this "new" marker into parentage cases until further experiments had been done. He noted, too, that since most people are secretors, exclusions by the system would be quite rare. In fact, the secretor characteristic can be employed in disputed parentage cases (Boorman *et al.*, 1977). The only type of exclusion that can be obtained, however, is in the case of two nonsecretor parents having a secretor child. Such a result is expected in a very small percentage of cases (see in section 19.10.1.3). Determination of the groups in body fluids, stains or tissues from secretors has long been accepted, provided a clear, unequivocal result was obtained. Fujiwara (1930) reported a case in which he had determined the seminal type as A in a rape-homicide case where the victim was an O. Holzer (1937) mentioned that saliva stains from secretors could be grouped without difficulty.

19.10.5.2 Methods of ABO grouping in body fluids and tissues.

- Inhibition

Up until about 1960, inhibition was the only method used for grouping body fluids. This procedure has been discussed at some length in section 19.8, and most of the considerations that apply to grouping body fluids apply equally to the grouping of body fluid stains. An inhibition technique is often the method of choice with body fluid stains from secretors, because of the high group substance content.

Andersen (1951) conducted an extensive study of the occurrence of H substance in secretor fluids. Using eel serum anti-H, he could always detect H in secretor saliva provided it was concentrated about 4 fold. Nonsecretor saliva had no detectable H. The presence or absence of H in saliva, he said, could be used as a criterion for secretor status, provided the concentration step was carried out. The secretor characteristic, however, should be used as a sole criterion for exclusion of affiliation only with caution, and only when the results are very clear. Seminal stains from secretors could be grouped without difficulty, the distinction between O secretor and nonsecretor being possible using the eel anti-H reagent. The introduction of anti-H reagents simplified and assisted in the interpretation of these tests with regard to O secretors. Jungwirth (1955) used an anti-H serum for this purpose. Wiener *et al.* (1958) introduced the use of anti-H lectin from *Ulex* for grouping blood and saliva. The reagent was equally applicable to seminal typing in rape cases (Helpern and Wiener, 1961). Raszeja and Dziedzic (1971) found that *Ulex* and *Cystisus* lectins could be employed in the differentiation of secretor from nonsecretor saliva in groups A_2, A_2B and O without difficulty. Caution was required in the case of A_1, A_1B and B.

- Mixed Agglutination

In 1961, Yada noted in his paper on the grouping of blood smears by mixed agglutination that the technique was perfectly useful for semen, saliva and vaginal secretion stains as well. Nickolls and Pereira (1962) found that their modification of the original Coombs and Dodd (1961) technique was fully satisfactory in grouping seminal and saliva stains. Dodd and Hunter (1963) compared the mixed agglutination procedure with the classical inhibition method for the grouping of saliva stains. Serial dilutions of saliva in saline were prepared, and used to make stains on cotton fabric. The sensitivity of the mixed agglutination procedure was enormously greater than that of the inhibition technique. In one case, the A antigen could be detected by mixed agglutination in a stain prepared from a 1:256 dilution of saliva, while the corresponding inhibition test detected it only in dilutions out to 1:8. In addition, mixed agglutination required considerably less material. Methanol fixation of the stained threads improved the mixed agglutination results. A, B and H substances could be detected in some saliva stains from nonsecretors as well by mixed agglutination. Maresch and Wehrschütz (1963) carried out mixed agglutination grouping on saliva stains on filter paper. In some laboratories, when saliva needs to be collected for secretor status determination in an affiliation case, it has been found convenient to prepare the sample as a stain on filter paper (see for example Hennig and Rackwitz, 1961). Prokop and his collaborators apparently follow this practice. Maresch and Wehrschütz (1963), noting that groups could sometimes be diagnosed in nonsecretors by mixed agglutination, questioned the ease with which secretor status could be diagnosed, and raised the issue of its validity as a genetic marker. Prokop and Gibb (1965) responded that there was no reason to question the validity of the secretor characteristic as a marker, that inhibition was the method of choice in diagnosing secretor status, and that mixed agglutination on filter paper stains should not be used for the purpose. In 1970, Schulz said that the sensitivity of the mixed agglutination test could be improved by using test cells that had been sensitized with subagglutinating doses of appropriate antisera (the dose used was two doubling dilutions beyond the agglutinating titer). The test was carried out on slides, and found to be applicable to semen, saliva and sweat. Schulz extended his studies in 1974. Secretor and nonsecretor samples (as determined by the inhibition test on saliva) of saliva, semen, vaginal secretions and sweat were tested with anti-A, anti-B and anti-H by mixed agglutination. In vaginal secretion and sweat stains, exceptions were found to the general rule that secretors always secrete H (using the inhibition test). One vaginal secretion stain from an AB secretor failed to react with anti-A, and a sample from another A secretor reacted with anti-A but not with anti-H. Another sample from an A secretor reacted with anti-A but very weakly with anti-H. A sample from a B secretor reacted with anti-H but only very weakly with anti-B (it would have been diagnosed as an O secretor). These were the only four exceptions in 86 samples. By mixed agglutination, the group substances were all diagnosed correctly. Some exceptions were noted in sweat using the inhibition procedure as well. Two A secretor samples did not inhibit anti-H, one O secretor sample did not inhibit anti-H, and one O secretor sample inhibited anti-H and anti-A. Using anti-A and anti-B in the mixed agglutination test, one A secretor sample failed to react with anti-A, but the O secretor which had given the false A positive reaction in the inhibition test did not give an anti-A reaction by mixed agglutination. Palatnik and Carnese (1970) reported good results in grouping saliva stains from secretors by mixed agglutination. Bromelin treated O cells were used to detect the *Ulex* anti-H binding.

- Elution

In 1963, Pereira reported that she had obtained very good grouping results with semen and saliva stains using the absorption-elution method described by Nickolls and Pereira (1962) (Pereira, 1963b). Ueno (1963) said that the elution procedure was not applicable to body fluid grouping, and that the results always came up as group O, regardless of the actual group. Thus, he said that bloodstains could be grouped by elution even in the presence of contaminating soluble group substances. Fiori (1963) rejected both elution and mixed agglutination procedures for body fluid stain grouping. In 1969, Pereira *et al.* conducted a series of experiments on the applicability of absorption-elution and mixed agglutination procedures to group determination in body fluid stains. Both methods were found to be entirely satisfactory. O secretor saliva detection by anti-H with mixed agglutination technique required the use of papain treated O cells. H reactions were occasionally seen in nonsecretor samples as well. With nonsecretors, weak reactions were generally seen with mixed agglutination, while the reactions were generally strong in these samples by absorption-elution. Fixation was not used, but a saline wash step

preceded the addition of antiserum to the threads in order to get some of the soluble group substance out which would dissolve in the antiserum and attenuate its strength. Pereira *et al.* said that, if the threads were not washed, and the fibers teased apart in the antiserum (at the start of the absorption), stronger reactions were seen with nonsecretor samples than with secretor samples. This behavior could be accounted for either by the dissolution of soluble blood group substances in the antiserum in sufficient amounts to partially neutralize it, or by competition of soluble group substances for eluted antibody. In a dilution experiment, in which doubling dilutions of saliva were used to make stains, and these then tested with and without the washing step by absorption-elution, a definite prozone effect could be seen with the anti-A and anti-B reactions in the unwashed samples. It was not seen with anti-H. Thus, at very high concentrations of A or B, negative or very weak reactions are possible. Pereira (1963b) had earlier noted that there appeared to be a kind of different distribution of A and B in a stain placed on cloth, and allowed to spread out, the reactions being weak at the center and periphery, and strongest about midway in between. Pereira *et al.* noted that this effect could be an apparent one, caused by the very high concentrations of A and B substance near the center of the stain (prozone). H reactions, which did not appear to be affected by antigen concentration, were highest at the center and lower proceeding outward toward the periphery, as the concentration of H declined. The relative concentrations of antigen and antiserum are thus found to be very critical in these tests, and this fact was cited as a possible reason for the varying results of different workers, which had led to differing opinions as to the applicability of the technique to body fluid stain grouping. Fiori and Benciolini (1972) compared the inhibition procedure with the elution procedure for body fluid stain grouping. They said that false negative results could be gotten with elution tests, and that, if the procedure were used, only positive results should be interpreted. They again recommended that the last washing liquid in an elution test be checked with test cells to insure that all excess, unwanted antibody had been removed from the sample. Masis *et al.* (1973) reported that semen and saliva could be reliably grouped by the absorption-elution technique. The stains were fixed in boiling water prior to the absorption step.

Tröger *et al.* (1976) reported successful grouping of stain fragments from under the fingernails of dead bodies by a straightforward elution method. Fragments up to 11 months old were determined. Because the nail substances may contain ABH group substances, the victim's blood group must be known in order to interpret the results.

- Other Techniques

In 1962, Yamasawa reported successful grouping of semen and saliva stains on filter paper by a fluorescent-labelled antibody technique. The stains were acetone-fixed, then incubated with fluorescent-labelled rabbit immune anti-A or chicken immune anti-B. The paper was then subjected to electrophoresis to separate the antibody from the antigen-antibody complex, dried and photographed under UV light. While differentiation of positive from negative results was possible, there was a small amount of fluorescence in the negative controls. Kind and Cleevely (1970) also reported good results in grouping secretor saliva stains by fluorescent antibody technique.

In 1974, Okada *et al.* described a rather interesting, if somewhat cumbersome, technique based on prior experiments by Vos and Kirk in 1958. Vos and Kirk were interested in possible ways in which fetal red cells might be protected *in vivo* against maternal antibodies to them which cross the placenta into fetal circulation in incompatible pregnancies. They had noted that adult red cells, sensitized with anti-A or anti-B and agglutinated, then subjected to homogenization procedures which would break up the agglutinates, would reagglutinate upon being centrifuged. If the agglutinates were broken up, however, and the sensitized red cells washed in saline and treated with soluble A or B substances, reagglutination did not occur. Such cells, which had their receptors occupied by antibody, and the other antibody binding sites apparently occupied by soluble group antigen, were referred to as "protected" cells. Okada *et al.* (1974) took advantage of this behavior. A series of doubling dilutions of the body fluid to be tested was prepared, and "test cells" were added. These "test cells" were prepared by breaking up the agglutinates of red cells sensitized and agglutinated by homologous antibody. The incubations were carried out at 45° for 30 min, and the tubes read after 30 min. In the presence of soluble group substances in the body fluid being tested, agglutination (actually reagglutination) was inhibited at the higher body fluid concentrations, whereas in the absence of soluble group substances, reagglutination was seen in all dilutions. The technique was called "reagglutination inhibition".

19.10.5.3 ABO grouping of different body fluids and tissues. Of the body fluids likely to be encountered, semen and saliva are probably the most common. As a general rule, these fluids and their stains are not difficult to group by the inhibition procedure if they are from secretors. As noted above, nonsecretors do have a small amount of group substance in their secretions, and it is sometimes possible to get a group on such a sample using one of the sensitive techniques (mixed agglutination or elution). Mixtures of body fluids from different individuals lead to problems in the interpretation of these tests. The most common example is probably encountered in samples collected from victims of sexual assault. The contamination of the semen with vaginal or other secretions was discussed at some length in section 10.3.5 in connection with semen identification. The same considerations apply to grouping tests. It is essential that the victim's group and secretor status be known before the results of grouping tests on a sample from a victim can be interpreted. Hayashi (1974) claimed to have devised an acrylamide gel disc electrophoretic method whereby the seminal and vaginal fluid protein could be separated, eluted from the gel, and then separately grouped.

Various methods have been used to determine blood

groups in a variety of body fluids, secretions and tissues. A number of the older studies on this subject were discussed in section 19.8. The early studies on mixed agglutination were done on tissues, and the procedure could obviously have medico-legal value. Swinburne (1962) applied mixed agglutination to the grouping of skin fragments, such as scrapings from live persons or dead bodies, and to dandruff particles. A brief pretreatment with mild alkali, and collection of the test material by centrifugation was recommended to get rid of grease, oil and other contaminants. 39 of 40 samples could be grouped, regardless of secretor status. Poon and Dodd (1964) likewise used mixed agglutination for the grouping of epidermal cells. Slavík and Meluzín (1972) reported successful grouping of histological sections of spleen, heart, kidney, lung and liver tissue after alcohol fixation by the absorption-elution technique. Brain tissue was negative. Suyama and Imai (1975) said that ABO grouping could be done on the pulp or dentine of teeth by absorption-elution. Schaidt (1958) noted that ABO groups could be determined by the inhibition test from samples of urine or feces provided these were concentrated. He used the paper chromatographic procedure described in section 7.2 to concentrate the group substances into a zone on the filter paper. There were a number of reports in the older literature that secretor urine could be grouped by inhibition if it were concentrated. It has been known for decades that the group substance concentration in this fluid is low. Gibb and Vogt (1965) said that urine stains could not be reliably grouped by mixed agglutination. Schulz (1974), however, said that he had correctly grouped 26 urine stains from A and B secretors by mixed agglutination. Urine stains from 5 nonsecretors gave negative results. In 1958, Hayashi grouped the earwax (cerumen) of secretors by an inhibition technique. He said that the amount of ABH substance was small in this material, and that he could identify small amounts of Le^a in the earwax of nonsecretors. Yada *et al.* (1966h) could group "flakes" of earwax by absorption-elution after methanol fixation, and the group was obtained regardless of secretor status. Dillon (1971) grouped dried saline extracts of earwax by absorption-elution. Bromelin treated O cells were used to detect the eluted anti-H. 49 secretors could be correctly grouped, while 51 nonsecretors gave no reactions with any of the antisera. Trela and Turowska (1975) employed an inhibition method for the grouping of inner ear fluid (perilymph) from secretors. Saneshige *et al.* (1980) found that the amount of A substance in A secretor sweat was far less than that in A secretor saliva. Identical results were obtained with the same fluids from B secretor individuals. An inhibition test was used for these determinations.

In some circumstances, determination of the blood group of dead bodies may be desirable or necessary. When the blood has undergone hemolysis and/or putrefactive changes so as to make it untypable, one can consider attempting to determine the group from one of the tissues. Holzer examined this problem in 1937. Group substances could be found in bile, duodenal lining and in other tissues of secretors. But the richest source was the stomach. A piece of stomach wall lining about 1 cm^2 yielded group substance to 0.5 $m\ell$ saline upon overnight steeping sufficient to give inhibition of appropriate antiserum in dilutions up to 1:16. Holzer also found that the stomach contents contain enormous quantities of group substances, apparently leeched out of the walls. Inhibition testing of stomach contents yielded inhibition in some cases out to dilutions of 1:2000. Holzer rinsed out a stomach excised at autopsy, washed it, and filled it with saline. Over the course of time, samples were taken and tested, and the inhibition titer increased from 1:16–1:32 at 6 hours to 1:64–1:128 at 70 hours. A case reported by Moureau *et al.* (1963) illustrates the potential usefulness of this sort of approach. In a body which had been in the water for three weeks, and which had undergone putrefaction, the hemolyzed blood sample gave inhibition with both anti-A and anti-B. This was the body of a child whose mother was of group O, and the child could not therefore have been an AB. A culture of the hemolyzed blood samples revealed microorganisms which gave strong B reactions. A piece of the gastric mucosa, well washed, steeped in saline and tested, showed inhibition only with anti-A. Group A was thus confirmed, the B having been acquired. Moureau *et al.* said that they had tested gastric mucosa from many corpses, and were always able to diagnose the blood group. Masis (1971) said that the secretor status of bodies could be reliably determined in bile, and somewhat less reliably in urine, using an inhibition procedure. An anti-H lectin derived from *Sambucus nigra* was used for group O diagnosis.

Pereira (1973) applied the absorption-elution technique to the problem of grouping muscle tissue from decomposed bodies. The decomposition process apparently gave rise to the presence of every kind of ABH receptor, unrelated to the genetically determined blood group substance. False reactions due to A, B and H were observed, and Pereira said that this approach was utterly unreliable.

19.10.5.4 Problems in the ABO grouping of body fluids. In any ABO blood group determination on other than fresh material, the possible presence of contaminants which have A, B or H receptors must be kept in mind. This issue was discussed in sections 19.10.3.2 and 19.10.3.4 in connection with the examination of bloodstains. The receptors in adventitious substances which react with the grouping sera to give false positives are sometimes referred to as "acquired". The matter was discussed in the foregoing section in connection with the determination of blood groups in dead bodies.

It is known that the red cells of patients suffering from carcinoma of the rectum or colon, who are of blood group A, can begin to show B reactions. This phenomenon is very rare, occurs *in vivo*, and was termed "acquired B" (section 19.7.3). Not surprisingly, the culprits in these cases were bacteria, their B-like substances being able under certain circumstances to coat the red cells. Many microorganisms have surface receptors which are recognized by anti-A, anti-B or anti-H, as is clear from the experiments of Illchmann-Christ and Nagel (1954), Springer (1956) and a number of others. Jenkins *et al.* (1972) looked into the problem of "acquired B" from the standpoint of forensic serology. Their curiosity

had been raised by two cases which had come to their attention, both involving dead bodies taken from the water. In one, a body had been dismembered and the parts put into the river. The first part recovered yielded enough intact red cells to allow for grouping as an O Rh+. Another part, recovered later, yielded no red cells, but the muscle tissue grouping gave group B. Thus, although the pathologist's examination made it virtually certain that the parts were from the same body, the blood grouping results did not support the conclusion. Further study showed that *E. coli* could be isolated from the deep muscle layers, and culture filtrates could induce B receptors on red cells lacking them. The B was, therefore, acquired. In the other case, samples were received from 10 victims of an airplane disaster at sea. The bodies had been in the water for several weeks. Eight of the ten reacted as B, a most improbable result with ten persons of Western European origin.

A number of anti-B sera were examined for their reactions with ordinary B cells and with cells from two patients having *in vivo* acquired B. The acquired B cells showed very low titers with the antisera in comparison with the normal B cells. The titers of the eluates from the acquired B cells, and from stains made from the acquired B blood, were significant. The eluates from ordinary B cells were of much higher titer than those from acquired B cells, and similarly with the stains, but the eluates from the acquired B material were fully detectable with B cells. In the usual absorption-elution test, these would have been called positive. Ten seminal stains were made, and inoculated with *E. coli* O_{86} and incubated for 24–48 hours in a moist environment at 37°. Prior to inoculation, all the specimens except for one from an AB secretor, were negative in the absorption-elution test with anti-B. The tests were carried out with and without papain-treated B cells. After 24 hours incubation, 2 samples showed definite B reactions and 4 others showed weak reactions with ordinary test cells. Eight of the samples showed strong reactions with papain treated cells (not counting the AB sample). In the control (not inoculated) stains, however, some B reactions could be seen with the papain cells. While the controls were not inoculated, neither were they sterile, and the B reactions which developed were probably the result of naturally occurring bacteria in the samples. The B activity of the samples was somewhat more marked at 48 hrs of incubation. In the course of these experiments, none of the stains acquired A, but in some cases, H activity decreased or was lost after inoculation. Four samples of fresh muscle tissue showing A and H, but not B activity were placed in a river for a week, and all of them acquired B activity, which increased after another week in the water.

A possible solution to the problem would be to prepare an anti-B which reacted with ordinary B bloodstains but not with the acquired B. Jenkins *et al.* tried four approaches: (1) Absorption of anti-B with red cells having an *in vivo* acquired B; (2) Absorption of anti-B with rabbit cells—rabbits have a B antigen related to, but not identical with, human B, and closely related to the *E. coli* O_{86} B-like receptor; (3) Absorption of anti-B with human group B cells; and (4) Serial dilutions of anti-B. The various antisera were tested with group A and B bloodstains, a bloodstain made from the *in vivo* acquired B blood, a seminal stain from an A secretor inoculated with *E. coli* O_{86} and incubated, and a sample of muscle tissue which had acquired B reactivity in the river. Results of the elution experiments are shown in Table 19.9.

It was found to be more difficult to induce acquired B in a bloodstain than in a seminal stain. Acquired B can be detected by the inhibition test. The use of the considerably more sensitive elution test will detect it all the more readily. Acquired B seems most frequently to be a problem in seminal (and probably in vaginal secretion) stains, and in muscle tissue from bodies under certain circumstances. Jenkins *et al.* noted that not every anti-B would stand all the procedures carried out to try and render the antiserum specific, and that the simplest procedure was probably absorption with the rabbit red cells. There is no reason to believe, however, that acquired B antigens will behave identically in all cases, and an anti-B absorbed so as to be inactive with one specimen could still be active with a different one.

Pereira and Martin (1975 and 1976) discussed a number of problems involved in body fluid grouping. They pointed out that pseudo-A and pseudo-B reactions (i.e., acquired) were not uncommon in saliva samples. They had been found to be more common in saliva and vaginal secretion samples than in uncontaminated semen samples. Changes in the ABH specificity of saliva can be brought about by incubating the specimen at 37° for 24 hours, thus implicating bacteria in the transformation. The changes could be caused by receptors on the bacteria themselves, or by blood group enzymes (those which transfer terminal sugars involved in the immunodeterminant oligosaccharides—section 19.9.3) elaborated by the bacteria. The concentrations of pseudo-A and pseudo-B receptors are very much lower than those of the genetically determined substances in secretors, and discrimination can be achieved in some cases by using short absorption times or by diluting the sample 1:1 with saline. If corresponding blood samples are available, these simple procedures were said to be adequate in most cases, but not in cases of saliva stains or samples of unknown origin. It was noted too that AB secretors do not always secrete A and B substances to the same extent, and that occasional individuals may be encountered who secrete in one fluid but not in another. It was recommended that inhibition and elution tests be carried out in parallel, and on a series of dilutions of antigen-containing material. With whole saliva, tests were done on a series of 10-fold dilutions. True group substance reactions from secretors will be detected by inhibition, and confirmed by elution. Further, the sensitive elution test may show a prozone effect at very high antigen concentrations which can be of diagnostic value. This prozone effect was discussed in section 19.10.5.2 in connection with the experiments of Pereira *et al.* (1969). Nonsecretor material will be negative in the inhibition test, but the group may sometimes be detected by elution. It was considered unwise to rely on an elution result for diagnosis of the group in nonsecretor

Table 19.9 Reactions of Several Acquired B Samples with Anti-B Antisera, Variously Treated to Remove the Acquired B Reactions (after Jenkins et al., 1972)

	Reaction of B Cells With Eluate From:				
Antiserum	B Bloodstain	A Bloodstain	Acquired B Bloodstain	Acquired B Semen Stain	Acquired B Muscle Tissue
Neat	+++	–	+++	+++	++
1:16	+++	–	+	++	++
1:64	+++	–	–	–	+
Absorbed with acquired B cells	+++	–	(+)	nt	nt
Partially absorbed with B cells	++	–	–	–	–
Absorbed with rabbit cells	++	–	–	–	–

+++ macroscopic agglutination; ++ strong microscopic agglutination; + less strong microscopic agglutination; (+) weak agglutination; nt not tested

material, unless the nonsecretor status could be confirmed by Lewis typing (secretions from nonsecretors group as Le(a+b−), except in cases of Le(a−b−) individuals). A spurious reaction can be observed as weak in the inhibition test, and will disappear in the elution test as the dilutions become greater, while a true group substance reaction from a secretor gives strong inhibition reactions and persists in the elution test throughout the dilution series. With saliva stains, aqueous extracts were used. The neat sample was used for the inhibition test, and a series of dilutions (neat, 1:5, 1:10, 1:20 and 1:40) were subjected to the elution test. Lewis typing was carried out on 2 × 2 mm stains by an inhibition test, using a substratum control and control saliva stains from Le(a+b−), Le(a−b+) and Le(a−b−) persons. Elution had not been successful for Lewis typing. Pereira and Martin concluded by noting that the results of the two procedures, inhibition and elution, had to agree with one another before they would report the results. Pereira and Martin (1977) published a full paper on Lewis grouping in body fluid stains and in saliva by inhibition technique. Results were very good, and the procedure was found to be quite useful in certain cases in the determination and confirmation of secretor status.

19.10.5.5 Fractionation of soluble ABH substances from secretor fluids (and red cells). In 1969, Fiori *et al.* reported that the soluble substances in human saliva could be fractionated on molecular sieve media (Sephadex, Sepharose). Three fractions could be obtained: Fraction 1 was of high MW, and fractions 2 and 3 were considerably smaller. Not every secretor saliva had the same combination of fractions. All secretors had fraction 1, but, depending on the person, it could be present alone, in combination with fraction 2, in combination with fraction 3, or in combination with both. There were thus distinguished four categories or "types" of secretors: namely, I, having fraction 1; II, having fractions 1 + 2; III, having fractions 1 + 3; and IV, having all 3 fractions. These characteristics were found in A, B and O secretors, the fractions in each group having the respective blood group activity. A series of papers followed in the *Journal of Chromatography* in which the studies were expanded (Fiori *et al.*, 1971a and 1971b). Nonsecretors (in the usual sense) were studied, and it was found that they always lacked fraction 1. They could, however, possess either of fractions 2 and 3, or both of them, or neither of them, giving four "types" of nonsecretors, which were called V (fraction 2), VI (fraction 3), VII (fractions 2 + 3) and VIII (neither fraction 2 nor 3). The type VIII would be a "true nonsecretor". A person could be any one of the eight types for A, B or H, depending upon which substances were secreted. A nomenclature was suggested to indicate the combination present. Group O is the simplest, since only H is secreted. An O person found to have H fractions 1, 2 and 3 would be designated O(H-1,2,3), while an O person having only fraction 1 would be called O(H-1), and so on. An A person secreting A fractions 1 and 2 and H fractions 1, 2 and 3 would be A(A-1,2/H-1,2,3), and an AB person secreting all three A fractions, B fraction 1 and H fractions 1 and 3 would be AB(A-1,2,3/B-1/H-1,3), and so forth. Human red cell stroma were solubilized with Triton-X-100 and subjected to the fractionation procedure. All three fractions were found in all subjects, corresponding to the phenotype of the donor, i.e., all three H fractions in O cells, all three H and A fractions in A cells, etc. Fiori *et al.* (1972) reported that population and family studies had been carried out to see whether they supported the notion of genetic control of these phenomena. Detailed data were not given, but it was said that the best genetic model was one in which two structural genes controlled the synthesis of fractions 2 and 3, with two additional gene pairs regulating the expression of these structural genes in secretions. There was said to be agreement with this hypothesis in the results of the analysis of mating combinations. It was also reported that two more low MW fractions had been isolated from red cell stroma (fractions 4 and 5) which inhibited anti-A, anti-B and anti-H equally. A full paper on fractions 4 and 5 followed (Fiori *et al.*, 1973), in which it was said that fractions 4 and 5, or both, or neither, could be found in saliva in association with fraction 3. This meant that type III, IV, VI and VII people could each be subdivided into four subtypes on the basis of fractions 4 and 5. The subtypes were called α(fraction 3 alone), β(fractions 3 + 4), γ(fractions 3 + 5) and δ(fractions 3 + 4 + 5)

Quite clearly, confirmation of these observations would have far reaching implications for the present understanding of the ABH substances on red cells and in secretions, and of the secretor system itself. Rutter and Whitehead (1975 and 1976) have reported that they could not confirm the observations, and were able to find only the higher MW fraction with blood group activity in the Sephadex fractions. Saliva from 12 nonsecretors and 16 secretors was studied. In the meantime, Panari *et al.* (1976) reported that the molecular heterogeneity seen in saliva is also present in the group substances in semen. Fiori *et al.* (1978) found the heterogeneity in group substances of urine as well. They said that there were four "types", and that this system was independent of the one in saliva. Fiori (1976) replied to the Rutter and Whitehead findings by stressing the importance of using the standardized technique which had been carefully devised by trial and error, not only for the fractionation, but also for the hemagglutination inhibition tests used to locate the activity. The smaller fractions were not all that easy to detect, he said. Ueda (1974) was said to have confirmed the saliva ABH fractions. Ueda's paper is written in Japanese, but it contains a table showing the various ABH fractions apart from which I could not read it. The salivary fractions as reported by Fiori and collaborators have also been confirmed by Hamilton and Kimberling (1973), Hamilton *et al.* (1974) and by Kimberling (1979). Hamilton and Kimberling (1973) had not yet found all eight of the phenotypes, but had essentially confirmed Fiori's findings. By 1974, Hamilton *et al.* reported that they had observed all the phenotypes, and that family studies suggested the involvement of more than one genetic locus in this polymorphism. Kimberling (1979) gave a genetic explanation for all the

findings and the family studies based upon three separate genetic loci, called Sec_1, Sec_2 and Sec_3. The Sec_1 locus was the "traditional" secretor locus (*Se/se*), he said, and controlled the high MW component. The other loci were distinct, but closely linked, and were said to control the low MW components of the polymorphism. These interesting developments prompted the Editor of the *Journal of the Forensic Science Society* of England (Editorial, 1976) to call for some other laboratories to take an interest in this question, and attempt to decide upon an explanation for the disparate observations. Kind *et al.* (1979a) and Lang *et al.* (1979a) from the Central Research Establishment group in England have recently noted that they find only a single fraction of blood group active substance upon fractionation of nonsecretor salivas, and that they could not, therefore, agree that "fraction 1" was absent in these samples.

The most recent series of papers from the C.R.E. group (Kind *et al.*, 1979b; Lang *et al.*, 1979b; Rothwell, 1979) gives the details of the efforts which have been made to repeat the observations reported by Prof. Fiori's group. It has not been possible to confirm the results, the papers say in effect. Apparently, Prof. Fiori visited the C.R.E. laboratories and demonstrated the procedures on four occasions. On two of them, there seems to be agreement that they worked, while on the other two occasions, the procedures did not work. These efforts are discussed by Kind *et al.* (1979b), who cautioned that they did not think these procedures were at all ready for use in forensic cases until the discrepancies could be worked out.

Perhaps other workers will become interested in the problem so that some understanding can be reached. For the moment, the whole matter remains something of a puzzle.

19.10.6 Factors affecting the success of medico-legal blood group determinations and unusual samples

Experienced forensic serologists all recognize the obvious fact that success or failure in group determination depends to a large extent on the condition of the evidence submitted or collected. Bhatnagar *et al.* (1974) gave an analysis of their success in grouping 1295 stains from 166 cases in the Delhi region in India, and tried to correlate it with the condition of the evidence. Almost 2/3 of the stains were on textile material, and about 3/4 of them could be grouped. Most of the remainder were badly contaminated, and in a few cases, the substratum control reacted. Only 23% of putrefied liquid blood samples collected at scenes (either as liquid, or on swabs), or samples mixed with soils, could be grouped. 41% of samples on weapons could be grouped; the remainder were contaminated. Only 8% of putrefied postmortem bloods could be grouped. Bhatnagar *et al.* gave some recommendations for the proper preservation of biological evidence.

Occasionally, it could be necessary to try and determine the blood group of a badly burned or charred body. Nagano *et al.* (1975a, 1975b and 1976) attempted to get some information about this situation by studying the thermal stability of the red cell blood group active glycolipids. Partially purified A, B, AB and H active glycolipids from red cells were heated, and tested for agglutinin binding activity. Heating to 120–130° for an hour lessened all the activities, the AB material being slightly more stable. A, B and H activities were abolished by 185° heat for 5 min, and AB activity by 200° heat for 5 min. Thermostability was higher if heating was carried out in the presence of serum. If tooth material (dental pulp) is used as a source of material for ABO grouping in burned bodies, the results of Korszun *et al.* (1978) are relevant, and indicate that the tooth enamel and dentine do not adequately insulate the ABH substances in pulp when the external temperature rises above 200° (see in section 19.10.7.2).

Finally, a few reports in the literature have dealt with samples having weak receptors or unusual antibodies. These matters have been discussed in various parts of section 19. Morgan (1964) had a case in which both whole blood and stains were submitted. The stains grouped as A, the cells as A_2, but the Lattes test on the stain and the agglutination tests on the serum showed that both A_1 and B cells were agglutinated. A_2 cells, however, were not agglutinated by stain extract or by serum, showing that the sample was of group A_2, with an unusual anti-A_1 in serum (see in section 19.3.1.1). Ishigu (1968a and 1968b) encountered a blood of group B_m (section 19.3.2). The cells did not agglutinate with anti-A or with any example of natural or immune anti-B, even if bromelin treated, or by the Coombs test. The B receptor was demonstrated by absorption and elution. Serum contained an anti-A. A bloodstain made from the blood grouped as O by inhibition, elution and mixed agglutination tests, and as B by the Lattes test. Saliva contained B substance, which could be detected in a saliva stain by inhibition or mixed agglutination. Masis and Ol'khovik (1970) had a case of an AB blood with a very weak B receptor, and in which the serum contained an anti-B. B-substance was present in the person's secretions.

19.10.7 ABO grouping of hair, nails and teeth

Determination of ABO groups in hair, fingernails or toenails, and various tissues of the teeth is a matter of medico-legal interest. Hair is relatively common physical evidence, and the hair shaft has few if any individualizing characteristics apart from its morphology. While there are reports in the literature on methods for the ABO grouping of hair that are claimed to be reliable, there are also many indications that the techniques have not been found to be reproducible in other laboratories. Determination of the ABO blood group from nails or from teeth can be of some help in the identification of persons under various circumstances, the primary value being in demonstrating non-identity.

19.10.7.1 ABO grouping of hair. In 1953, Krefft investigated the possibility of determining the group substances in hair. An inhibition procedure employing 0.1 g of pulverized hair was used. The hair from 35 people was tested and the correct results obtained in each case. High titered anti-A and anti-B was reduced about 6 to 7 dilutions in the presence of the respective antigen. "Anti-O" of titer 1:32 was re-

duced to 1:8 by an O sample, but there was no effect on anti-A or anti-B. Tesař (1954) in Prague carried out similar experiments. The hair was extensively washed in ether, and then pulverized into powder, sometimes using glass powder. 0.2 g of this material was then extracted with 5 mℓ saline in a 100° water bath for an hour, and the extract was used for the test. A three dilution reduction in antiserum titer using high titer anti-A and anti-B was taken as the minimal criterion for a positive result. Correct results were obtained with all samples. Disruption of the hair structure is apparently necessary to expose the group specific substances, since Vogt *et al.* (1965) got no inhibition of anti-A or anti-B in the absence of it. McWright (1961) applied a sonication procedure, which Thoma (1954) had developed for fingernails (see in section 19.10.7.2), to the grouping of hair. Sonication was carried out on about 7 mg hair material in the presence of anti-A or anti-B. Correct results could be obtained by the inhibition technique with donors from all four groups. Sonication time was critical.

The mixed agglutination technique for hair grouping was investigated by Lincoln and Dodd (1968a). Hairs were washed in ethanol, ether, acetone, and finally in saline containing 0.3% albumin. About 10 small lengths of hair were used with each antiserum. Absorption was for 2 hours, followed by at least four washes with saline, and a last wash in saline-albumin. Papain treated test cells in 1% suspension were used for detection. 17 examples of shavings were grouped correctly, but only 19 of 45 samples of head hair corresponded to the blood group of the donor. It was said that the blood group activity of the hair might reside in the sebaceous layer, and that too much preliminary washing with solvent might remove this layer, leading to false negatives, while too little washing might leave behind adventitious substances, leading to false positives.

Most studies have employed absorption-elution procedures. Beginning around 1966, Yada and his collaborators in Japan published an extensive series of papers on hair grouping by elution. Hairs were washed in ether, and crushed with a hammer and anvil. Two 1 cm lengths were incubated each with anti-A and anti-B. After two hours at room temperature, the samples were washed once in a large volume of cold saline, and elution carried out at 55° for 10 min. Test cell suspensions of 0.2% were employed. Forty-four samples were correctly grouped in this way, and secretor status appeared to have no effect on the presence of the blood group substances in hair (Yada *et al.*, 1966a). These studies were extended to include formalin-fixed hairs, some of them decades old (Yada *et al.*, 1966c) axillary, pubic and crural hairs (Yada *et al.*, 1966b), eyebrows, eyelashes and vibrissae (Yada *et al.*, 1966f), and an 88 year old hair rope (Yada *et al.*, 1968d). In all cases, correct results were obtained in all the samples. Hairs from Caucasian donors gave similarly correct results (Yada *et al.*, 1968c). A detailed description of the technique was published (Yada *et al.*, 1968b), and a double roller device was found to be very suitable for crushing the hair (Yada *et al.*, 1968e). A blind trial study was carried out in eight laboratories (Yada *et al.*, 1968d) in four of which the examiners had been trained in the procedure. Four errors were made in 250 hair samples by the experienced personnel, while in the four laboratories in which the examiners were previously unfamiliar with the technique, there were 33 incorrect groupings in 240 samples. Yada *et al.* (1966g) reported that rabbit hairs, subjected to the procedure, give a B result in most cases, with an occasional A. Yada *et al.* (1968a) said that scalp hair from persons with very weak forms of red cell B (B_m and AB_m cells) gave correct grouping results with the elution procedure.

In 1971, Wynbrandt and Chisum reported successful results with an elution procedure. Hairs were washed in shampoo and in methanol, and then crushed by subjection to 12,000 psi in a press for 30 sec between steel plates. The hair was then boiled in McIlvaine's buffer, pH 7.4, and dried. Absorption was for 24 hrs at 4°, after which the hair sample was washed with 7 to 11 mℓ ice cold saline and blotted dry. Elution was carried out for 10 min at 55° into an 0.25% test cell suspension, and the results read every 10 min for an hour. 50 samples could be correctly grouped in a blind trial, with the test on two of them having had to be repeated because of technical errors in handling. Anti-H reacted with all the samples, regardless of secretor status.

Gramer and Tausch (1973) gave a detailed elution procedure with which 42 of 43 samples were correctly grouped. Hairs were ether-washed and physically disrupted, and then incubated with antisera overnight in the cold. They were then washed with ice cold saline by means of a vacuum suction device, like the one used by Wynbrandt and Chisum (1971). Elution was allowed to proceed at room temperature for an hour into 1% test cell suspensions that had been bromelin treated. Technique was critical, and not all commercially obtained bromelin preparations gave satisfactory results. Cegla and Popielski (1976) said that they had used a microelution procedure with trypsin treated test cells to test 120 hair samples from living and dead persons. The group obtained corresponded with the blood group in 90% of the cases, was uncertain in 6.6%, and did not agree in 3.4%.

Boettcher and Kay (1973) reported success in hair grouping using ^{125}I-labelled antibody and autoradiography. The positives gave distinguishably darker images than the controls, and *Ulex* anti-H was not found to be satisfactory in the test. The presence of group specific substances in hair was independent of the secretor status of the donor.

Oepen and Noever (1980) carried out a study on 346 hair samples from 195 donors using a modification of the elution procedure. The group determined from the hair failed to match the red cell type in a substantial fraction of the cases (about 20%). A good review of the literature was given in this paper.

Kishi and Iseki (1977) recently reported isolating a glycolipid from hair which had A activity. The material gave all the reactions to be expected from an isolated A substance. H and B substances were isolated by these same workers in 1978.

19.10.7.2 ABO grouping in nails and teeth. In 1954,

Thoma reported on ABO group determination in fingernails and toenails by an inhibition technique. Sonic oscillations were found to expose the group substances in some way. From 25 to 50 mg nail substance was sonicated in saline for 30 min at 1000 KHz. The nail material was then dried, and overlaid with antiserum for 24 hrs at 5°. Anti-A,B (O serum) was used for the test. Reliable results were obtained with samples from all four groups, which were collected only from secretors. Sonication time was found to be very critical. McWright (1961) applied the procedure to 15 mg amounts of nail material, except that sonication was for 10 min and in the presence of the antisera. Reliable results were obtained, the sonication step being required for any inhibition of the antisera.

Outteridge (1963c) reported successful results in the grouping of 105 samples from 28 donors belonging to all four groups. The amount of antigen was variable, and was varied accordingly from 10 to 30 mg. Longer (36 hr) absorption times were used, and the anti-H reaction showed peculiar variations. Yada *et al.* (1966d) reported successful grouping of fingernail samples of about 1 mg from 62 persons of all four groups and including nonsecretors by their elution procedure (see in Section 19.10.7.1). The nail material was first scraped, and then crushed prior to absorption.

Schiebe *et al.* (1961) noted that the soft tissue of teeth, the dentine and pulp, could be grouped for ABO if the material came from secretors. An inhibition test was used. Takata (1973) showed that the elution technique could be applied to crushed tooth enamel for determination of ABO, secretor status being immaterial. The test needed 6–10 mg of material. In 1974, Takata said that the test was equally applicable to teeth which had been in the sand or in water for 2 years, or in air for 3 years, and from teeth that had been exposed to high heat (100° for 4 hrs, 150° for 2 hrs, or 200° for 15 min). Funatsu (1975) said that dental calculus could be used in an elution test for group determination. Mukai *et al.* (1975) tried a number of tooth tissues using elution, and could not get results from enamel, but they always got reliable results from pulp. Dentine and cementum were variable. Anti-H lectin and eel serum anti-H were used, with papain treated O indicator cells, for H antigen. Korszun *et al.* (1978) studied the thermostability of the ABH antigens in pulp, and estimated the protection afforded by enamel and dentine against heat by mathematical methods. At temperatures of 200° or greater, the hard tissue could not effectively insulate the pulp antigens from denaturation. Typing of pulp was reliable by elution technique, but typing of the hard tissues was not. Mukherjee and Chattopadhyay (1976) were successful in grouping the deeper hard tissue of teeth by elution. Yada *et al.* (1966e) noted that their elution procedure (Section 19.10.7.1) was applicable to bone fragments, if these were soaked in water for a couple of days, etherwashed, and then crushed prior to absorption.

19.11 Distribution of ABO Groups and Secretors in U.S. Populations

The data are shown in Tables 19.10 and 19.11. The criteria used in the selection of the data shown in these all subsequent population distribution tables were given in the Preface of this book.

Table 19.10 Frequency of ABO Groups in U.S. Populations

Population	Total	Frequency of Occurrence — Number (Percent) O	A A_1	A A_2	B	AB A_1B	AB A_2B	Reference
CAUCASIAN								
Iowa City, IA	49,979	22,392 (44.8)	21,144 (42.31)		4,695 (9.39)	1,748 (3.5)		Buckwalter & Knowler, 1958
Iowa City and Des Moines, IA	6,313	(45.8)	(41.6)		(9.0)	(3.6)		Buckwalter et al., 1958
Brooklyn, NY								
Leukemia patients								
Jewish	665	226 (34)	261 (39.2)		120 (18.0)	58 (8.7)		
Non-Jewish	639	291 (45.5)	235 (36.8)		90 (14.1)	23 (3.6)		
Blood donors								Macmahon and Folusiak, 1958
Jewish	375	142 (37.9)	165 (44.0)		46 (12.3)	22 (5.9)		
Non-Jewish	548	281 (51.3)	184 (33.6)		62 (11.3)	21 (3.8)		
Seattle, WA								
University students	5,657	2,399 (42.4)	2,458 (43.4)		599 (10.6)	201 (3.6)		Van Arsdel and Motulsky, 1959
St. Louis, MO								
Veteran's Hospital	359	169 (47.1)	149 (41.5)		29 (8.1)	12 (3.3)		Sievers, 1959
Control Donors	32,945	14,918 (45.3)	13,611 (41.3)		3,248 (9.9)	1,168 (3.5)		
New Haven, CT								
Yale students	1,000	431 (43.1)	422 (42.2)		110 (11.0)	37 (3.7)		Niederman et al., 1962
New York, NY								
Memorial Hospital								
Donors	4,738	2,029 (42.82)	1,828 (38.58)		636 (13.42)	245 (5.17)		
Transfused patients	2,332	976 (41.85)	932 (39.97)		291 (12.48)	133 (5.7)		Osborne and DeGeorge, 1962
Tumor patients	525	192 (36.57)	233 (44.38)		70 (13.33)	30 (5.72)		
Southeast Georgia	300	172 (57.3)	97 (32.3)	39 (13.0)	20 (6.7)	4 (1.3)	1 (0.3)	Cooper et al., 1963
Boston, MA								
Rheumatoid	*608*	276 (45.4)	183 (30.1)	63 (10.4)	55 (9.0)	25 (4.1)	6 (1.0)	Dublin et al., 1964
Non-rheumatoid	605	281 (46.4)	173 (28.6)	52 (8.6)	76 (12.6)	16 (2.6)	7 (1.2)	

Table 19.10 Cont'd.

Population	Total	O	A		B	AB		Reference
			A_1	A_2		A_1B	A_2B	
Pittsburgh, PA								
School population	1,578	609 (38.6)	630 (39.9)		250 (15.8)	89 (5.6)		
County Fair sample	3,871	1,586 (41.0)	1,595 (41.2)		489 (12.6)	201 (5.2)		Kaplan et al., 1964
Male blood donors	1,959	840 (42.9)	742 (37.9)		253 (12.9)	124 (6.3)		
Salt Lake City, UT								
College age population	247	111 (45.0)	108 (43.7)		23 (9.3)	5 (2.0)		Mayeda, 1966
Oakland - Eastern San Francisco Bay area, CA								
Mothers	4,928	(44.91)	(40.93)		(10.33)	(3.84)		Reed, 1967
Newborns		(45.43)	(39.77)		(11.24)	(3.55)		
San Francisco Bay area, CA								
"Caucasians"	8,962 ★	4,067 (45.4)	2,856 (31.9)	783 (8.7)	968 (10.7)	228 (2.5)	70 (0.8)	
"Caucasians of Western European origin"	5,056	2,361 (46.7)	1,557 (30.8)	476 (9.4)	507 (10.0)	118 (2.3)	37 (0.7)	Reed, 1968
New York, NY	500 ☆	229 (45.8)	134 (26.8)	37 (7.4)	62 (12.4)	29 (5.8)	5 (1.0)	Wiener, 1969
Denver, CO	3,648	1,717 (47.1)	1,473 (40.4)		324 (8.9)	134 (3.7)		Charney, 1969
West Virginia - central and southern area	1,412 ✪	(45.9)	(41.3)		(9.1)	(3.7)		Juberg, 1970
Tecumseh, MI	8,965	3,916 (43.68)	3,048 (34.0)	859 (9.5)	811 (9.05)	234 (2.61)	97 (1.08)	Schreffler et al., 1971
Brooklyn, NY								
Kings County Hospital								
Adults	599	265 (44.24)	220 (36.73)		76 (12.69)	38 (6.35)		
Newborns	253	114 (45.05)	102 (40.31)		29 (11.46)	8 (3.16)		Robinson et al., 1971
Patients	68	32 (47.06)	28 (41.18)		8 (11.76)	0		
Los Angeles, CA	205	79 (38.5)	63 (30.7)	25 (12.2)	31 (15.1)	3 (1.5)	4 (2.0)	Sturgeon et al., 1973
California and Hawaii	6,004	(48.2)	(35.8)		(12.1)	(4.0)		Grunbaum et al., 1978
Bexar County, TX	200	(54.0)	(34.0)		(8.0)	(4.0)		Ganaway and Lux, 1978
Detroit, MI	507	(41.2)	(33.5)	(1.4)	(17.0)	(4.1)	(2.8)	Shaler, 1978 ■
Baltimore, MD								
Postmortum samples	1,095	(44.1)	(39.7)		(11.7)	(4.5)		Profili and Hurley, 1979
Miami/Dade Co., FL	366	189 (51.6)	111 (30.3)		44 (12.0)	22 (6.0)		Stuver, 1979 and see Shaler, 1978
Los Angeles, CA Case material	419	190 (45.3)	169 (40.3)		52 (12.4)	8 (1.9)		Siglar, 1979 and see Shaler, 1978

Table 19.10 Cont'd.

Population	Total	O	A		B	AB		Reference
			A_1	A_2		A_1B	A_2B	
NEGRO								
New York, NY	200	(50.0)	(15.0)	(7.0)	(23.5)	(2.5)	(2.0)	Miller et al., 1951
Miami, FL	502	244 (48.6)	122 (24.3)		117 (23.3)	19 (3.8)		Butts, 1955
Washington, D.C.								
Howard University	1,188	(53.03)	(27.2)		(17.26)	(2.52)		Moore, 1955
Students	937	(49.3)	(20.7)	(6.5)	(20.0)	(2.9)	(0.6)	
Iowa City and Des Moines, IA	6,722	(49.1)	(26.5)		(20.1)	(4.3)		Buckwalter et al., 1958
St. Louis, MO								
Veterans Hospital	99	53 (53.5)	24 (24.2)		19 (19.2)	3 (3.0)		Sievers, 1959
Control Donors	1,395	713 (51.1)	353 (25.3)		269 (19.3)	60 (4.3)		
Iowa City, IA								
Controls	1,261	563 (44.65)	424 (33.6)	88 (6.98)	144 (11.42)	31 (2.46)	11 (0.87)	Newman et al., 1961
Southeast Georgia	333	162 (48.6)	50 (15.0)	24 (7.2)	58 (17.4)	1 (0.3)	5 (1.5)	Cooper et al., 1963
Oakland - Eastern San Francisco Bay area, CA								
Mothers	1,453	(49.28)	(26.84)		(19.13)	(4.75)		Reed, 1967
Newborns		(46.59)	(27.24)		(21.40)	(4.27)		
San Francisco Bay area, CA	3,146	1,540 (49.0)	603 (19.2)	267 (8.5)	605 (19.2)	84 (2.7)	47 (1.5)	Reed, 1968
Birmingham, AL								
School children	610	302 (49.5)	163 (26.7)		122 (20.0)	23 (3.8)		Casey et al., 1968
New York, NY	500 ☆	242 (48.4)	100 (20.0)	23 (4.6)	110 (22.0)	10 (2.0)	8 (1.6)	Wiener, 1969
West Virginia - central and southern area	133 ✪	(55.0)	(27.0)		(16.0)	(2.0)		Juberg, 1970
Brooklyn, NY								
Kings County Hospital								
Adults	1,150	580 (50.43)	271 (23.57)		246 (21.4)	53 (4.61)		Robinson, et al., 1971
Newborns	2,933	1,441 (49.13)	790 (26.93)		592 (20.18)	110 (3.75)		
Patients	628	298 (47.45)	171 (27.23)		137 (21.82)	22 (3.5)		
California and Hawaii	1,025	(47.7)	(25.9)		(21.5)	(5.0)		Grunbaum et al., 1978
Bexar County, TX	200	(55.0)	(21.0)		(19.0)	(5.0)		Ganaway and Lux, 1978
Detroit, MI	507	(53.1)	(16.0)	(0.2)	(26.2)	(2.8)	(1.8)	Shaler, 1978 ■
Baltimore, MD								
Postmortum samples	1,289	(46.6)	(27.5)		(22.3)	(3.6)		Profili and Hurley, 1979
Miami/Dade Co., FL	346	180 (52.0)	75 (21.7)		76 (21.9)	15 (4.3)		Stuver, 1979 and see Shaler, 1978
Los Angeles, CA								
Case material	185	87 (47.0)	56 (30.3)		35 (18.9)	7 (3.8)		Sigler, 1979 and see Shaler, 1978

Table 19.10 Cont'd.

Population	Total	O	A		B	AB		Reference
			A_1	A_2		A_1B	A_2B	
HISPANIC								
Southern Texas								
Mexican Surname	1,597	962 (60.2)	452 (28.3)		144 (9.0)	39 (2.5)		King et al., 1955
San Francisco Bay area, CA								
"Mexicans"	335	186 (55.5)	74 (22.1)	17 (5.1)	44 (13.1)	13 (3.9)	1 (0.3)	Reed, 1968
Brooklyn, NY –								
Kings County Hospital								
Puerto Rican								
Adults	288	161 (55.9)	90 (31.25)		27 (9.38)	10 (3.47)		
Newborns	928	533 (57.44)	275 (29.63)		96 (10.34)	24 (2.59)		Robinson et al., 1971
Patients	248	131 (52.82)	78 (31.45)		29 (11.69)	10 (4.03)		
California and Hawaii ◇	1,696	(56.5)	(30.9)		(10.8)	(1.9)		Grunbaum et al., 1978
Bexar County, TX	200	(61.0)	(26.0)		(11.0)	(2.0)		Ganaway and Lux, 1978
Miami/Dade Co., FL	357	204 (57.1)	117 (32.8)		29 (8.1)	7 (2.0)		Stuver, 1979 and see Shaler, 1978
Los Angeles, CA								
Case material □	215	133 (61.9)	60 (27.9)		19 (8.8)	3 (1.4)		Siglar, 1979 and see Shaler, 1978
CHINESE								
New York, NY								
Blood donors	103	(45.6)	(27.2)	(0)	(22.3)	(4.9)	(0)	Miller et al., 1951
New York, NY	400	172 (43.0)	108 (26.0)	0	101 (25.25)	19 (4.75)	0	Wiener, 1969
New York, NY	946 *	395 (41.75)	263 (26.74)		240 (25.37)	58 (6.13)		Wiener, 1974
ASIAN								
California and Hawaii	3,053	(32.5)	(37.8)		(21.0)	(8.8)		Grunbaum et al., 1978

★ Includes the 5,056 "*Caucasians of Western* European origin" in the next row.

☆ 2 A_1 and 2 A_1B were found in Caucasians; 4 A_1, 2 A_1B and 1 A_2 were found in Negroes.

✪ In 485 Caucasian A types, 76% were A_1 and 24% were A_2; in 34 Negro A types, 82% were A_1 and 18% were A_2; in 36 Caucasian AB types, 72% were A_1B and 28% were A_2B; in 3 Negro AB types, 1 was A_1B and 2 were A_2B.

* Includes the 400 previously reported in the row above (Wiener, 1969)

◇ "Chicano/Amerindian"

□ Primarily Mexican

■ *Data of* Stolorow and collaborators

Table 19.11 Frequency of Secretors in U.S. Populations (Caucasian)

		Frequency — Number (Percent)		
Population	Total	Secretor	Nonsecretor	Reference
Iowa City, IA	1,261	971 (77.0)	290 (23.0)	Newman et al., 1961
New Haven, CT				
Yale Students	1,000	773 (77.3)	227 (22.7)	Niederman et al., 1962
Boston, MA	1,194	857 (71.8)	337 (28.2)	Dublin et al., 1964
Tecumseh, MI	8,664	6,461 (74.57)	2,203 (25.43)	Schreffler et al., 1971
Los Angeles, CA	205	141	64	Sturgeon et al., 1973

SECTION 20. THE LEWIS SYSTEM

20.1 Introduction

The Lewis system is rather complex, related in a number of ways to the ABO and Secretor systems, and not yet completely understood. Some discussion of the Lewis groups appeared in parts of Section 19, and the nature of biosynthesis of Le^a and Le^b was covered in Sections 19.9.2 and 19.9.3. The Lewis groups may be thought of as a body fluid group system, the Lewis substances being absorbed onto the red cells under certain conditions.

20.2 Discovery and Development

The antigen Le^a was identified in nonsecretor saliva in 1939 by several Japanese workers. It was called "T", and the chicken immune precipitating serum which detected it was called "anti-T". This work was overlooked in the West until recently. It is discussed by Race and Sanger (1975) and by Prokop and Uhlenbruck (1969). There is another antigen at present which is called "T" (the Thomsen-Friedenreich T—see in section 21.8.2), and these two should not be confused. Wiener *et al.* (1964a) mention a number of examples of Lewis antibodies which were found, but were not named, prior to 1946.

In 1946, Mourant described a new agglutinin in a serum from a woman whose baby was suspected of having hemolytic disease, and which agglutinated about 25% of group O adult red cells from English people. The woman, Mrs. Lewis, and her husband gave Dr. Mourant permission to call the new antigen "Lewis". In 1947, Andresen found an antibody with similar characteristics in a woman called Anna Erikson. The antigen being detected was called "L", and about 21% of adults were L+. The percentage was much higher in children, depending upon their age. It was noted that L− parents could have L+ children, and Andresen supposed two alleles, *L* and *l*, where *LL* and *Ll* children were L+, but where only *LL* adults were L+. Somehow, the *L* gene was expressed in adults only in the homozygous state. In 1948, Andresen noted that Anna Erikson serum contained the antibody which Mourant had called "anti-Lewis", and which he now called anti-L_1. The serum of an A, MN man had been found to contain another agglutinin, called anti-L_2. L_2 behaved almost, but not quite, as if it were the product of the allele of the L_1 gene (e.g. 21.4% of adults were L_1-L_2-). There was a relationship between A_1 and L_2, but not between A_1 and L_1, when the L-system phenotypes were looked at within the ABO blood groups. Mourant's Lewis and Andresen's L_1 are now called Le^a, and L_2 is called Le^b.

In 1948, Grubb made the important observation that persons whose red cells were Le (a+) were nonsecretors of ABH, while the majority of people with Le(a−) cells were secretors. He found too that the saliva of Le(a+) people inhibited anti-Le^a. Brendemoen (1949) found that the serum of all Le(a+) people he tested, and most of the salivas, inhibited anti-Le^a. In 1949, Grubb and Morgan noted that Le^a and Le^b were present in the secretions of persons of appropriate genotype, and that these substances were very similar in their properties to the ABH substances. Some human ovarian cyst fluids were found to be rich sources of these substances. Their serological properties were destroyed by incubation with filtrates from cultures of *Clostridium welchii*. Brendemoen (1950) noted that Le^a was secreted in the saliva of people whose red cells were Le(a+b−), and in some people whose cells were Le(a−b+) as well, but to a variable extent. People whose cells were Le(a−b−) did not secrete Le^a in saliva. In 1950, Grubb said that he did not think of the Lewis system primarily as a blood group system, but as a serological system of water soluble mucoproteins. Consideration of Lewis as a blood group system, he said, was perhaps due to the ease with which red cells could be tested.

As a general rule, then, Le(a+b−) red cells came from ABH nonsecretors, Le(a−b+) red cells came from ABH secretors; and Le(a−b−) cells may be from ABH secretors or nonsecretors, the proportion of each being the same as in the rest of the population. There are exceptions. Andresen (1948) described a few people whose cells were "L_1 L_2" with a weak "L_1", i.e., Le(a+b+) where the Le^a was weak. Cutbush (1956) described other examples of these cells in adults and in infants. In populations other than Europeans, secretors of ABH may be found whose cells are Le(a+). Lewis *et al.* (1957) found this circumstance in two Japanese, and Boettcher and Kenny (1971) found that 10–15% of Australian aborigines have Le(a+) red cells and are ABH secretors.

In 1951, Grubb set forth an explanation for the Lewis system which, with minor modifications, still forms the basis of the way the system is looked at. Essentially, the presence of Le^a in saliva was regarded as being independent of the secretion of ABH substances in saliva. Le^a is found in the saliva of about the same proportion of secretors as of nonsecretors. The Secretor and Lewis genes were thought of as being independently segregating, but as interacting in some of their phenotypic expressions. Ceppellini's studies have tended to confirm Grubb's ideas, and helped to form the basis for the general framework. Ceppellini and Siniscalco (1955) analyzed data from family material which had been collected for another purpose, with the idea of understanding the relationship between Lewis and Secretor. ABH secretor status was determined, as was the presence or absence of Le^a in saliva and on red cells. Some of the results had been presented elsewhere, and details are given in the paper. The data indicated that two genes, called *Le* and *le*

comprised an allelic pair which controlled the synthesis of Le^a (and thus its appearance on red cells and in body fluids). Persons of genotypes *LeLe* and *Lele* had Le^a; persons who were *lele* lacked it. The *Le/le* genes were inherited independently of the genes controlling the ABH secretion characteristic (*Se/se*). The *Se* gene, however, exerts an effect on the phenotypic expression of the *Le* gene, in that *Le* expression is suppressed in saliva and nullified in red cells in the presence of *Se*. Thus, *LeLe* and *Lele* people are Le(a−) on cells and have some Le^a in saliva if they are *SeSe* or *Sese* as well, but are Le(a+) on cells and have more Le^a in saliva if they are *sese* (see Table 19.8). In this view, Le(a+) parents can produce Le(a−) children (*Lele sese* × *Lele sese* → 1/4 *lele sese*), and such families have been found. Le^b is now regarded as the product of the interaction of *Le* and *H*, as shown by the biochemical studies of Morgan and Watkins (section 19.9.3).

20.3 Lewis Antigens on the Red Cell

In 1955, Sneath and Sneath made the important observation that Le(a+b−) cells transfused into an Le(a−b+) recipient, the transfused cells and "native" cells later being separated by differential Rh agglutination, had become Le(a+b+) (Sneath and Sneath, 1955a). Thus, Le(a+b−) cells had acquired Le^b *in vivo* in an Le(b+) person. The transformation could be brought about *in vitro* as well: Le(a+b−) cells + Le(a−b+) plasma → Le(a+b+) cells, and Le(a−b+) cells + Le(a+b−) plasma → Le(a+b+) cells. Mäkelä and Mäkelä (1956a) confirmed this observation for Le^b, and said that plasma Le^b did not inhibit the agglutination reaction, but could transform Le(b−) red cells into Le(b+). The work was extended (Mäkelä *et al.*, 1967), and the plasma substances found to be stable to 100° heating for 2 min, and nondialyzable. Plasma substances could transform the red cells, but salivary ones could not, and there was no evidence for enzymatic involvement in the transformation. Nakajima *et al.* (1968) noted that there were no differences between the abilities of fetal and adult red cells to take up plasma Lewis substances, and uptake did not appear to depend on the amount of the substance present in the plasma.

20.4 Lewis Substances in Saliva and Other Body Fluids

It is now accepted that the symbol "Les" stands for the presence of Le^a in saliva, and the symbol "nL" for the absence of Le^a in saliva (Race and Sanger, 1975). From what has been said, the general scheme predicts that nL × nL matings will not produce any Les children, and no exceptions to this rule have been found in family studies (Race and Sanger, 1975).

Brandemoen (1949) showed that the serum of Le(a+) people contains Le^a, but that the serum of Le(a−) people does not. This was found independently by Grubb and Morgan (1949) and has been confirmed.

Brown *et al.* (1959) found that the Le^a antigen in the saliva of Le(a+b−) people is immunologically different from that in the saliva of Le(a−b+) people. The former is precipitated by an anti-Le^a precipitin serum prepared in rabbits. The latter is not, even though it may very strongly inhibit an anti-Le^a agglutinin serum tested with Le(a+) cells. The same observation was made by Baer *et al.* (1959) using antisera prepared in chickens by injection of certain ovarian cyst fluid or nonsecretor saliva substances. Some Le^a substance in the saliva of Le(a−b−) nonsecretors has been reported (Sturgeon and Arcilla, 1970; Andresen, 1972; Gunson and Latham, 1972). This perplexing observation could be related to the kind of anti-Le^a used for the tests, or to Le^c (Race and Sanger, 1975).

In 1964, Kaklamanis *et al.* proposed that it should be possible to distinguish homozygous from heterozygous secretors (*SeSe* from *Sese*) on the basis of the Le^a/H ratio in saliva. Based on prior studies (Brown *et al.*, 1959), they had the view that Le^a and H specificities reside on the same macromolecule, and that phenotypic expression of the genes gives rise to different numbers of the different determinants on the molecular surface. Kelso (1968) agreed that H and Le^a expression in saliva was competitive, but said that it was not strictly reciprocal. He denied that the genotype at the secretor locus could be determined. The amount of Le^a in saliva in various ABO, secretor and Lewis phenotypes has been studied by a number of investigators, and is variable (Kaklamanis *et al.*, 1964; Kelso, 1968; Sturgeon and Arcilla, 1970; Boettcher and Kenny, 1971; Randeria and Bhatia, 1971; Arcilla and Sturgeon, 1973).

There is evidence that the Lewis substances in plasma are glycosphingolipids, whereas those in the saliva and ovarian cyst fluids are glycoproteins (Marcus, 1970). The plasma Lewis antigens are found in both high and low density lipoprotein fractions, isolated from plasma. These preparations, but not the residual plasma, can transform red cells with respect to Lewis antigens. It was noted that all Le^b glycoprotein preparations have H activity, whereas Le^b glycosphingolipids do not. Concentrated solutions of HLe^b glycoprotein preparations had a weak transforming effect on Lewis negative red cells. These data help to explain some of the prior observations on red cell Lewis transformation.

Grubb (1951) found no Le^a in five seminal samples. Lodge and Usher (1962), however, found Lewis substances in semen in concentrations parallel to what is found in plasma. McConnell (1961) said that Lewis substances were present in semen at lower concentrations than in saliva, and that occasionally a man may have Lewis substances in saliva but not in semen. Lewis substances are present, however, in fairly high concentrations in both gastric juice and in urine.

20.5 Some Complexities of the Lewis System

20.5.1 Antigens of Le(a−b−) red cells

In 1957, Iseki *et al.* described an immune antiserum which contained an incomplete, cold antibody reacting with Le(a−b−) cells from secretors as well as from nonsecretors. The antiserum was obtained by immunizing rabbits with saliva from a secretor whose red cells were Le(a−b−)

and then absorbing with Le(a−b+) and Le(a+b−) red cells. The antibody was called anti-Le^c, and the antigen it was detecting was Le^c. 1.86% of Japanese people tested reacted as Le(a−b−c+), and a three allele scheme, Le^a, Le^b and Le^c, where Le^c was dominant over Le^b and Le^a, and Le^b dominant over Le^a in red cell expression, was needed to explain the family studies. Race and Sanger (1975) have noted that this antibody, in view of the fact that it reacts with Le(a−b−) cells from *both* secretors and nonsecretors, may be a mixture of the anti-Le^d of Potapov (1970b) and the anti-Le^c predicted by Potapov (1970b) and described by Gunson and Latham (1972) (see below). Lodge *et al.* (1965) did a rather involved analysis of five Lewis antisera, which were said to be anti-Le^{ab} (equivalent to anti-Le^x - see below), and one of which reacted with Le(a−b−) and $O_hLe(a-b-)$ cells. Andersen (1958) found an antibody in the serum of a 12 year old male cancer patient who was $A_2Le(a-b+)$ and had no history of transfusions. This "Magard" serum reacted with $A_1Le(a-b-)$ cells from secretors in preference to $A_2Le(a-b-)$ cells from secretors, and Andersen said that the *Se* gene modified the A substance on the cells, in the absence of *Le*, so as to make them reactive with the Magard serum. Such an interpretation would not be compatible with the overall scheme of gene action indicated in Fig. 19.9 (Race and Sanger, 1975).

In 1970, Potapov prepared an antiserum by injecting goats with saliva from an OLe(a−b−) secretor, and absorbing the serum with trypsin treated Le(a+b−) cells (Potapov, 1970b). The antiserum contained an anti-Le^b, and an incomplete, cold antibody which did not react with Le(a+b−) or Le(a−b−) cells from nonsecretors, but did react with Le(a−b−) cells from secretors. The new agglutinin was called anti-Le^d, and its antigen Le^d. The designation "Le^c" was reserved for the predicted antigen that would characterize Le(a−b−) red cells of nonsecretors. Gunson and Latham (1972) found anti-Le^c in a woman who had been transfused and who had had four pregnancies. It reacted with the cells of Le(a−b−) nonsecretor people, and the reaction was inhibited by saliva from Le(a−b−) nonsecretors, less strongly by saliva from Le(a−b−) secretors, and very little by saliva from Le(a−b+) secretors. It was also inhibited by purified glycoprotein from ovarian cyst fluids of Le(a−b−) nonsecretors, and by 3-fucosyl-lactose. These tests were done by Dr. Tippett and Dr. Sanger in London (Race and Sanger, 1975), who also confirmed that a goat anti-Le^c prepared by Dr. Potapov in 1973 had the same specificity as Gunson and Latham's human one. Dr. Winifred Watkins commented in the discussion section of Gunson and Latham's paper that the anti-Le^c reaction inhibition by 3-fucosyl-lactose suggested that Le^c had a terminal chain configuration corresponding to "X" in Fig. 19.11. It could be expected, too, that Le^d would be equivalent to "Y" in Fig. 19.11, since in secretors, the second α-fucosyl residue would be transferred to the galactosyl residue. It is not necessary to postulate any new alleles to understand these new Lewis antigens in this way, since Le^a and Le^b are derived from type 1 chains, while Le^c and Le^d (X and Y) would be derived from type 2 chains (Figs. 19.10 and 19.11). Table 20.1 shows some of the relationships that were given in Table 19.8, extended to include the Le^c and Le^d factors. Hirsch and Graham (1980) have recently shown that Le^c and Le^d, like Le^a and Le^b, are adsorbed onto the red blood cells from plasma.

20.5.2 Le^x

In 1949, Andresen and Jordal described an antibody, which they termed "anti-X", and which later became known as anti-Le^x. Some have considered anti-Le^x to be anti-Le^a + anti-Le^b, or anti-Le^{ab}, but the discoverers do not so consider it. Andresen (1961) said that anti-Le^x is a specific agglutinin, detecting a specific red cell receptor Le^x, closely related to Le^a. Sturgeon and Arcilla (1970) studied families having members with red cell phenotype Le(a+b+x+), and the data suggested to them that the condition could be explained by a variant *Se* gene, in which Le^a is not completely converted to Le^b. In their view, and in Andresen's, Le^x is considered a product of the *Le* gene, but independent of secretor status. Sturgeon and Arcilla (1970) said that the *Le* gene might have cistrons, coding for the presence of Le^a and Le^x. Andresen (1972) and Arcilla and Sturgeon (1973) studied subjects with Le(a−b−x−) cells, and small quantities of Le^x and of Le^a could be found in the saliva of nonsecretors. These findings are not readily understandable at the present time.

20.5.3 Anti-A_1Le^b

Seaman *et al.* (1968) described an antibody in a serum "Siedler" from an $A_1Le(a-b-)$ subject which reacted only with $A_1Le(b+)$ cells, but not separately with A_1 or Le(b+) cells. A few additional examples of this antibody have been reported.

20.6 Lewis Antisera

20.6.1 Anti-Le^a

Many examples of human anti-Le^a have been found since Mourant found the antibody in the serum of Mrs. Lewis, most of them very weak, and, according to Race and Sanger (1975), frequently hemolytic. They are nearly always found in Le(a−b−) subjects, and may contain some anti-Le^b. Potent anti-Le^a is rare. The use of papain treated cells is often helpful.

Anti-Le^a may be prepared in animals by injecting a variety of Le^a containing fluids or red cells. A number of examples have been mentioned in section 20. Potapov (1976) prepared a variety of Lewis antisera in goats.

20.6.2 Anti-Le^b

Some of the confusion in the literature is undoubtedly because of the fact that there seem to be two types of anti-Le^b. One of these, the anti-Le^{bH} of Ceppellini *et al.* (1959) or anti-$Le_1{}^b$ of Sneath and Sneath (1955b), is inhibited by all secretor saliva, and behaves very much like anti-H in an inhibition test. The red cell Le^b detected by this antiserum shows considerable suppression in the presence of A_1. The other, anti-Le^{bL} (Ceppellini *et al.*, 1959) or anti-$Le_2{}^b$

Table 20.1 Saliva and Red Cell Relationships for the Four Lewis Factors

Saliva		Red Cell Reactions With			
ABH	Le^a	Anti-Le^a	Anti-Le^b	Anti-Le^c	Anti-Le^d
Secretor	Les	−	+	−	−
Nonsecretor	Les	+	−	−	−
Secretor	nL	−	−	−	+
Nonsecretor	nL	−	−	+	−

(Sneath and Sneath, 1955b) is not inhibited by saliva from Le(a−b−) secretors, but only by saliva which contains (inhibits) anti-Le^a. The red cell Le^b detected by this antiserum does not show epistatic suppression by A_1. Thus, many examples of anti-Le^b react with O and A_2 cells. Bird (1959) has suggested that "anti-H" may be regarded as anti-H + anti-H_1, where anti-H_1 would be equivalent to anti-Le^b, presumably to anti-Le^{bH}. Wiener *et al.* (1964a) shared this view of anti-Le^b.

20.6.3 Other Lewis Antisera

Lewis antisera other than anti-Le^a and anti-Le^b have been discussed in section 20.5.

20.7 Theories About the Lewis System

The ideas of Grubb and of Ceppellini about the Lewis system were developed in section 20.2. As will be evident, there are things about the Lewis system and its interaction with other genes that are not very clear. Some other ideas about the Lewis system will be mentioned here. Andresen and Henningsen (1951) and Andresen *et al.* (1950) developed explanations to take care of the reactions of anti-Le^x. These ideas have been extended (Andresen, 1961) to include the two types of anti-Le^b and the "Magard" antibody. Andresen's paper (1961) must be read. It is much too complicated to summarize easily.

Pettenkofer (1953a and 1953b) put forward a three allele hypothesis which included the genes Le^a, Le^b and Le^c. Le^c was dominant over Le^b and Le^a, and Le^b was dominant over Le^a. The theory explained the anti-Le^a, anti-Le^b and anti-Le^x reactions, and their relations to the *Se/se* locus. Wiener *et al.* (1964a) are for the most purposes in agreement with Grubb and Ceppellini, except that Le^b is regarded as a form of H, as suggested by Bird (1959). Wiener *et al.* suggest that if Le^b is really a variant of H, it should be ignored in the Lewis system notation. A review of the ABO, Secretor and Lewis systems is given by Andersen (1969), and Hossaini (1977) reviewed a number of aspects of the Lewis system.

20.8 Biochemical Studies

Most of this material was presented in Section 19.9. Studies on fractionated Le^a-active ovarian cyst fluid glycoprotein were done by Bhaskar and Creeth (1974). Hanfland *et al.* (1978) isolated and purified a number of Le^a-active and some related glycolipids from the serum of ALe^a people. Prohaska *et al.* (1978) demonstrated the *in vitro* enzymatic synthesis of Le^a and Le^b from the glycolipid Lewis precursor found in the sera of Le(a−b−) people. They showed further that the precursor is present on the red cells of Le(a−b−) people. Staal *et al.* (1977) carried out studies on a family with a rare congenital disease called fucosidosis, in which an α-L-fucosidase specific for α1→2 linkages is congenitally absent, causing Le^b to accumulate in tissues. The condition is inherited as an autosomal recessive trait.

20.9 Medico-legal Applications

The Lewis system is not used in disputed affiliation cases because its mode of inheritance does not allow conclusions to be drawn. Good Lewis antisera are relatively uncommon, and the system has only one major medico-legal application, that of confirmation of secretor status in body fluids and body fluid stains. Pereira and Martin (1975, 1976 and 1977) have discussed this application (see in Section 19.10.5.4), which was carried out using an inhibition technique. They said they had not had success with the elution method. Piner and Sänger (1980) recently reported successful results in grouping body fluid stains for Le^a and Le^b by inhibition technique. Paired saliva stain-vaginal secretion stain samples from women and paired saliva stain-semen stain samples from men were tested and shown to give identical Lewis results. The Lewis substances in body fluid stains could be detected for a matter of weeks in these experiments. It was said that the finding of Le(a+b−) results in such a stain could be used to verify ABH nonsecretor results. The finding of Le^b in mixtures of semen and vaginal secretions might be useful in confirming the presence of secretor semen in vaginal material which was Le(b−) as well.

In 1974, Yudina in Moscow reported that Le^a and Le^b could be determined in bloodstains up to a year old using an absorption-elution procedure modified from that of Nickolls and Pereira (1962). Anti-Le^a and anti-Le^b from goats was used, test cells were trypsin-treated, and the detection reaction was carried out in 3% serum albumin (human or bovine). The antisera had a titer of 1:32–1:64, and elution was carried out at 52–54° for 30 min. Davie (1979) described a procedure for Lewis grouping on microtiter plates, which was applicable to blood or body fluids, and had the advantage of conserving material.

20.10 Distribution of Lewis Phenotypes in U.S. Populations

These data are presented in Table 20.2.

Table 20.2 Distribution of Lewis Plenotypes in U.S. Populations

Population	Total	Frequency — Number (Percent) Le(a+b−)	Le(a−b+)	Le(a−b−)	Le(a+)	Le(a−)	Reference
CAUCASIAN							
New York, NY	460	(22.8)	(71.5)	(5.7)			Miller et al., 1951
Boston, MA	1,194	307	666	221			Dublin et al., 1964
South Central West Virginia	1,412				(25.9)	(74.1)	Juberg, 1970
Tecumseh, MI	7,775				7,296 (93.84) saliva	479 (6.16)	Schreffler et al., 1971
Los Angeles, CA	205				189 saliva	16	Sturgeon et al., 1973
NEGRO							
New York, NY	211	(23.2)	(54.5)	(22.3) ☆			Miller et al., 1954
New York, NY	397	44 (16.95)	138 (58.5)	54 (24.5) ★			Ceppellini et al., 1959
South Central West Virginia	133				(20)	(80)	Juberg, 1970
CHINESE							
New York, NY	85	(23.5)	(70.6)	(5.9)			Miller et al., 1951

☆ Of 23 Le(a−b−), 17 were ABH Secretors and 6 were ABH Nonsecretors

★ Of 54 Le(a−b−), 40 were ABH Secretors and 14 were ABH Nonsecretors

SECTION 21. THE MNSs SYSTEM

21.1 Discovery and Mode of Inheritance of the MN Blood Groups

In 1927, when ABO was the only blood group system known, Landsteiner and Levine were looking for other antigenic differences in human red cells. Cells of different people were injected into rabbits, and the rabbit antisera absorbed with human cells of various types. Antibodies were being looked for which would discriminate cells from different individuals on the basis of other than ABO distinctions. An antibody was found which agglutinated the cells of about 83% of Caucasians and about 69% of Blacks, independent of their ABO groups (Landsteiner and Levine, 1927a). The antigen being detected was designated "M". Soon afterward, two additional factors, called N and P, were found (Landsteiner and Levine, 1927b), and it was established that M was an inherited characteristic. P will be discussed in Section 24.1. In the papers which followed (Landsteiner and Levine, 1928a and 1968b), further studies on the population distribution of the characteristics were done, along with studies on 161 families. N was clearly inherited as well, and both M and N behaved as dominants, since parents who both possessed a factor could have children who lacked it, but parents who both lacked a factor could not have children who possessed it. If the M and N characteristics were inherited independently, it would have been expected that some M-N- people would have been found in the number of people grouped, and none were. Assuming that there was not some peculiar irregularity (e.g. M-N- being lethal, etc.), the easiest explanation of the observations was a two allele hypothesis, in which the homozygotes were M or N, and the heterozygotes were MN. This hypothesis was very soon confirmed by Schiff (1930) in 42 families, and by Wiener and Vaisberg (1931) in 131 families. Thus, M and N are inherited as a Mendelian codominant pair of alleles *M* and *N*, where the genotypes *MM, MN* and *NN* give rise to the phenotypes M, MN and N, respectively. This mode of inheritance has been confirmed by studies on thousands of families, and is widely accepted. There have arisen a number of complexities with MN over the years, and these will be described in a subsequent section.

21.2 The S and s factors

In 1947, Walsh and Montgomery found an antibody in a puerperal patient which reacted with about 48% of randomly selected cells, and whose reactions appeared to be independent of ABO, Rh, P, Lutheran, Kell and Lewis systems. A sample of the serum was sent to Sanger and Race in London, who tested it and agreed that it was detecting an antigen independent of the systems noted above, but it was not independent of MN (Sanger and Race, 1947). The possibility that the antigen, called "S", was an allele of MN was considered, along with the possibility that it was governed by a separate locus. In the latter case, a corresponding allele for *S*, namely "*s*", would be predicted. It became clear that *S* was not an allele of *M* and *N*, and it appeared to be related to MN as a linked locus. A joint report by all four investigators appeared in 1948 (Sanger *et al.*, 1948). It was predicted that an anti-s would eventually be found. In 1951, Levine *et al.* did find the anti-s in the serum of a Mrs. Guth, who had had a child that suffered from hemolytic disease (Levine *et al.*, 1951b). They conducted a number of studies on it, and sent some of it to Sanger and Race in London, who tested it in connection with their ongoing MN studies. These studies (Sanger and Race, 1951) indicated that the Ss locus was separate from, but closely linked to the MN locus, and the MNSs system genotypes, phenotypes and reactions using anti-M, anti-N, anti-S and anti-s are indicated in Table 21.1.

21.3 Recombination and Mutation in the MNSs System

The genetic evidence clearly shows that *S* and *s* are not alleles of *M* and *N*, but the linkage is apparently very close. If this were not so, recombination would be relatively common, for crossover frequency is directly proportional to the distance between loci. When recombination is rare, it can be difficult to distinguish between recombination and mutation. A most interesting family study was reported by Gedde-Dahl *et al.* (1967). The family was pretty large (12 sibs in the second generation), and data for most members of four generations was available. The family was being studied because a rare, inherited disease called Epidermolysis bullosa simplex was segregating in it. In a male of the second generation was found the phenotype MNS, and his genotype could be diagnosed through his descendants as *MS/NS*. The genotypes of his parents, established by the informative sibs in his generation, were the same: *MS/Ns*. The possibility of his being illegitimate was considered, but it is virtually ruled out by the other blood groups, and by the fact that he, like his father, had the rare variant form of Epidermolysis bullosa. Affected male relatives of the propositus were excluded as possible fathers by other blood groups. The choice as to how this observation should be interpreted came down to one between recombination and mutation, and the investigators favored the former. In a careful survey of the literature, three other possible recombinations were discussed. Details are given in Race and Sanger (1975). Although mutation cannot be ruled out, it is known that mutation in all organisms is exceedingly rare. There is only one example of an apparent mutation in the

Table 21.1 MNSs Types Using All Four Antisera

Reaction With					
Anti-M	Anti-N	Anti-S	Anti-s	Phenotype	Genotype(s)
+	+	+	+	MNSs	MS/Ns; Ms/NS
+	+	+	−	MNS	MS/NS
+	−	+	−	MS	MS/MS
+	−	+	+	MSs	MS/Ms
+	−	−	+	Ms	Ms/Ms
+	+	−	+	MNs	Ms/Ns
−	+	+	−	NS	NS/NS
−	+	+	+	NSs	NS/Ns
−	+	−	+	Ns	Ns/Ns

blood groups, this in a case studied by Henningsen and Jacobsen (1954), in which an M mother had an N child. This child was born at home, and maternity was not in question. The mother reacted as *MM* but the child's cells reacted as having a single dose of N. A special anti-N antiserum from a normal healthy MN person, which reacted only with NN cells, but not with MN cells (Metaxis-Bühler *et al.*, 1961), was used to test the child's cells. The explanation did not appear to be any known rare variant of the MN system (see below), and it remains something of a puzzle.

21.4 The Variant S^u—The Problem of U

In 1953, Wiener *et al.* described a fatal hemolytic transfusion reaction in a 35 year old Black female, who had had three pregnancies, and was being treated for a bleeding ulcer (Wiener *et al.*, 1953d). Her serum turned out to contain a strong, saline agglutinating antibody against all cells from Caucasians and almost all cells from Negroes. The factor being detected was called "U", and 4 out of 425 Black people tested turned out to be U-. In further studies (Wiener *et al.*, 1954), the cells of 1100 Whites reacted with anti-U, and 12 out of 989 Blacks were found to be U-. Cells from U- people were injected into rabbits to see if an "anti-u" could be made, but without success. There was a suspicion that U was MNSs related in some way.

A second example of anti-U was found in another Black woman, and studied by Greenwalt *et al.* (1954). In the course of the study, it was noted that the two U- bloods available failed to react with *either* anti-S or anti-s. Anti-U could thus be thought of as anti-S + anti-s, and *U* could be interpreted as an allele of *S* and *s*, which was called S^u. This tidy explanation did not quite do, however, since it was soon found that not all S-s- samples are U-. Race and Sanger (1962) recorded this result in samples sent from various places. Further studies by Allen *et al.* (1963) and by Francis and Hatcher (1966) indicated that about 15% of S-s- samples were U+. Allen *et al.* (1963) observed too that MS-s- cells, whether U+or U−, do not contain the small amount of N that is characteristic of all other M cells. Thus, anti-U is not anti-Ss. The S-s- phenotype may be due to an allele S^u, or to an inhibitor gene operating on the Ss locus. Race and Sanger (1975) said that they would continue to use the designation S^u for the S-s- phenotype. Apparently, anti-U reactions with S-s- cells are not always that clear cut, leading to problems of interpretation. There have been suggestions that there may be two different anti-U. The U-phenotype has been found thus far only in non-white populations, mostly in Blacks. However, S^u or something like it, has been observed rarely in Europeans. An interesting example is given by Austin and Riches (1978).

21.5 Complexities of the MN System

21.5.1 Variants of M and N

The major outlines of the MNSs system, in terms of reactions with the four antisera, and in terms of inheritance, are not especially complicated. The problem of U has been discussed. For most practical purposes, the peculiarities of the system are not an issue, since the majority are rare. In terms of understanding the system, however, there will eventually have to be an explanation for the unusual observations that have built up in fair quantity. There are variants of both M and N, and a number of antigens that have their own names, but which are in some way associated with the MNSs system. Ultimately, all these pieces must be put together in a way that is consistent with the underlying biochemical basis for the system. The extensions of the MNSs system are mentioned briefly here, but are not discussed in detail.

In the older literature are found descriptions of weak M and N receptors. In 1935, Crome described an apparent M mother with an N child, and the grouping results were confirmed by a number of laboratories. Thomsen and Landsteiner apparently agreed that the mother could have a very weak N receptor which had been inherited and was fully expressed in the child. Friedenreich (1936c) had an exactly similar situation, except that the mother did react weakly with selected examples of potent, immune anti-N. The mother had two sisters in this case who shared the characteristic. Friedenreich called the normal N antigen "N_1", and this weak variety "N_2". Moskow (1935) reported the subdivision of both M and N into M_1/M_2 and N_1/N_2. Pietrusky (1937) reported studies on the N_2 receptor, as did Krah (1949a and 1949b). It is hard to know what to make of these studies today in terms of the present versatility of the system, and the wider range of antisera that are available.

The antigen M^g was discovered by Allen *et al.* (1958b), and it appears to be of very low frequency. Oddly enough, however, anti-M^g is relatively common. The M^g antigen does not react with anti-M or anti-N. M^g is a little more frequent in Switzerland. M^g is governed by a gene M^g, an allele of M and N. People who are M^gM^g are known. Brocteur (1968) mentioned the possible problem of missing M^g in paternity testing. Ikin (1966) made good anti-M^g in rabbits by injection of cells from one of the rare homozygotes.

In 1964, Metaxas and Metaxas-Bühler described an apparently silent allele of M and N, called M^k. From the data on this and subsequent families that have been studied, it is clear that M^k gives no M or N antigen, and no S or s antigen. It may not be a true allele, but some sort of operator gene variant which can affect MN or Ss. M^k is quite rare.

In 1960, Jack *et al.* found that some human anti-M reagents contain an "anti-M_1", which divides the cells of group M people in a qualitative way into M and M_1. M_1 is fairly infrequent in Caucasians (about 3% of M genes), and more frequent among Black people. In the Bantu of Africa, half the *M* genes are M_1 (Le Roux and Shapiro, 1969). Most antisera to M_1 are anti-M + anti-M_1, which gives rise to complications. Occasionally, an almost "pure" anti-M_1 can be found (Le Roux and Shapiro, 1969). Anti-M_1 is occasionally found in the serum of an individual with MN red cells (Molthan, 1980).

In 1966, Gershowitz and Fried reported an antigen called M^v. M^v is dominant and may be associated with S or s. It is quite rare. It is peculiar in that it behaves in certain ways like M, and in others like N.

Some other variants: M^c, M^r, M^z, and M^a and N^a. Coverage may be found in the classic references (Race and Sanger, 1975; Prokop and Uhlenbruck, 1969).

21.5.2 Other associated antigens

An antigen called Hu (for Hunter) occurs in some 7% of American Black people. Another antigen called He (Henshaw) occurs in about 3% of African Black people, and not in Caucasians. M. Shapiro (1956) has studied He, and MacDonald *et al.* (1967) found an anti-He in the serum of a pregnant White woman which reacted in saline at 12° and in albumin at 37°. H. A. Shapiro (1956) commented on the medicolegal significance of He. Anti-He sera can be produced in animals (Wiener *et al.*, 1964b). The Miltenberger series of antigens has an intricate history involving the antigens Vw, Gr and Mi^a. Cleghorn (1966) brought much order to the confusion, there being apparently 5 classes of antigens in the series. Some other antigens: Vr, Sul, Far, Mt^a, Cl^a, Ny^a, Ri^a and St^a. A reference to "Sul" is Konugres and Winter (1967), and for some background on Ny^a, see Schimmack *et al.* (1971). Information on Tm and Sj may be found in Issitt *et al.* (1968). Judd *et al.* (1979b) described an apparently new MN-related antigen, defined by a serum called "Can" from a 57 year old white male with cancer. All M+ cells tested had the Can antigen, although some had to be treated with neuraminidase before they would react. Some N cells reacted as well. The antiserum also reacted with a higher percentage of cells from Black donors than from White donors.

21.6 Antisera to MNSs Antigens

Anti-M and anti-N occur in human serum. Wolff and Jonsson (1933) found an anti-M in a human serum. There are many examples of these sera. Prokop and Uhlenbruck (1969) give a thorough discussion of this subject. Kao *et al.* (1978) have suggested that anti-M formation in M-people may come about as the result of bacterial infection. Antisera to M and N are often made in rabbits, and have been made in other animals as well. Kerde (1965) had no luck in trying to make the reagents in sheep. Occasionally, antibodies which appear to react with part of M, or part of N, are disclosed. Konugres *et al.* (1966) found an anti-M in an MN person which was not autoagglutinating. It had to be assumed that the antibody was detecting something of M that the possessor lacked, from whence we get the antibody being called anti-M^A, and the person's cells M^aN. A rather similar kind of anti-N, called anti-N^A, was found in Melanesians (Booth, 1971). Metaxas-Bühler *et al.* (1961) found a very interesting anti-N, called "AP", in an MN donor. Exhibiting a sort of ultimate dosage effect, this serum reacted with NN cells, but not at all with MN cells. It has been very useful in the study of peculiar MN antigens.

Smith and Beck (1979) studied 50 human sera containing saline agglutinating anti-M. 78% of these were IgG while the remainder were IgM. Ordinarily, IgG antibodies are not expected to be saline agglutinins, but anti-M is an apparent exception to this rule.

Anti-M and anti-N lectins have been found. The seeds of *Vicia gramineae* have a rather specific anti-N (Ottensooer and Silberschmidt, 1953), while those of *Iberis amara* have an anti-M (Jack *et al.*, 1960). Several species of *Bauhinia* seeds contain anti-N, but it is not usually saline reacting (Mäkelä and Mäkelä, 1956b; Mäkelä, 1957). The specificities detected by these lectins are not precisely identical, and the lectins have been used as probes to get information about the structure of the receptors.

Anti-S and anti-s are found in sera on occasion. Anti-s can be made in rabbits. Human anti-s is not as common as anti-S. Antisera to other factors in the system have been mentioned briefly in the foregoing sections.

21.7 Heterozygous Advantage and MN

There has been considerable discussion in the literature about the fact that, if all the data are looked at, there is an excess of MN offspring produced by MN × MN matings, i.e., it is significantly greater than the 50% predicted (One expects from MN × MN matings a 1:2:1::M:MN:N ratio). Wiener (1962) believed that this effect was an apparent one, caused by grouping errors made by using underabsorbed anti-N. Rabbit anti-M and anti-N reagents are made by injecting group OM and ON cells into the animal, and then absorbing with human cells of the opposite specificity. It has been known for a long time that anti-N sera often react with M cells. Overabsorption with M cells renders the sera weak and nonspecific, while underabsorption leaves them cross-reactive, i.e., MM may be grouped as MN. The matter is discussed below. Wiener (1962 and 1963) presented a compelling argument for his position, and showed that in his own studies representing a large number of families, the production of MN offspring by MN × MN matings was very close to 50%. Race and Sanger (1975) and Prokop and Uhlenbruck (1969) agreed with Wiener on this point. Prokop and Uhlenbruck (1969) pointed out the fact that this shows that accurate MN grouping can be less trivial than is perhaps widely appreciated.

21.8 Biochemical Studies on the MN System

21.8.1 Introduction

The nature of the M and N determinants has been studied intensively in a number of laboratories, and there is now substantial information on the immunodeterminants. The picture is, however, less clear than in the case of ABO, and there are a number of complications in the data which will eventually require explanation. Although the various plant agglutinins have aided in the research, their reactions are not all exactly identical, and this situation has complicated the data somewhat.

Like ABH determinants, MN determinants are not restricted to red cells. Springer (1965) regards both classes of determinants as rather generally distributed cell surface structures, which have been named "blood group substances" essentially on the basis of their having been first discovered on red blood cells. They are not specific to blood, nor even to animals.

Uhlenbruck (1965) brought up some useful biochemical distinctions, which it is well to keep in mind in discussions of the chemical nature and biosynthesis of the blood group substances. The molecules are glycoproteins, a general class of substances which contain protein and carbohydrate. Further information about glycoproteins may be found in Gottschalk (1972). Uhlenbruck (1965) distinguished the following categories of these molecules: *mucins*, consisting of a protein chain with many low MW carbohydrate units and with hexosamine-N-Ac-neuraminic acid disaccharides; *mucoids*, which are quite similar, but contain higher MW carbohydrate moieties, which are branched, and in which the same molecule may contain a number of different kinds; *serum glycoproteins*, containing both of the above type of prosthetic carbohydrate groups; and the *γ-globulin type*, in which the molecule has one or two high MW carbohydrate chains. The term "mucopolysaccharide" is equivalent to "mucoid", British and American authors tending to favor the former. Mucopolysaccharides and mucoproteins are not quite identical. Further discussion of this richly complex subject is given by Prokop and Uhlenbruck (1969). The MN substances (and the ABH and Lewis ones) are mucoids. Further information on the nature of many kinds of mucus secretions may be found in *Ann. N.Y. Acad. Sci.* **106**: 157–809 (1963).

21.8.2 The Thomsen phenomenon

This could very well be considered a separate subject, but it is brought up and discussed briefly here, since references to it arise in connection with the nature of M and N. Simply put, the Thomsen phenomenon is polyagglutinability. It was first observed in blood samples a few days old. The cells were agglutinated by the serum from blood of any ABO group, and this agglutination took place better at temperatures less than 37°. Hübener (1926) and Schiff and Halberstaedter (1926) both described this behavior. Oluf Thomsen (1927) observed the same phenomenon and studied it in more detail. He showed that the agent bringing about the change was of bacterial origin, and that exposure of cells brought about a change in them such that a previously hidden antigen was exposed. He called it "L" (for "latent"). His student, Friedenreich, studied the phenomenon in quite a bit of detail (Friedenreich, 1928a and 1928b), and wrote his doctoral dissertation on it (Friedenreich, 1930). It was found that certain bacteria produced the "transforming principle", which led to the development of the previously hidden receptor. Friedenreich re-named the receptor "T", and the serum agglutinin for it was called "anti-T". The terminology has persisted, and one must not confuse Friedenreich's T with the salivary antigen first described by the Japanese workers in 1939, but which we now call Le^a (section 20.2). Friedenreich showed that the transforming principle was an enzyme. Anti-T could be found in the serum of all adults in variable amount. Prokop and Uhlenbruck (1969) discussed the subsequent development of the T/anti-T system and panagglutinability generally. The subject has become quite complicated. The enzyme causing the transformation is now known to be neuraminidase, and the transformation of cells, such that they are anti-T agglutinable, was called the Hübener-Thomsen-Friedenreich Phenomenon by Prokop and Uhlenbruck (1969). Springer *et al.* (1979) have conducted a series of studies, the results of which suggest that T and/or related antigens may be associated with certain types of human cancer cells.

It is now clear that T is a member of a general category of receptor which Uhlenbruck (1965) has called "cryptantigens". The red cell (and other cell) surfaces are now known to contain a number of structures whose immunodeterminant specificity resides in various carbohydrate chain end groups. Removal of the end residue changes the immunospecificity, sometimes generating an antigen which was not there before. In the particular case of cryptantigens revealed by the removal of neuraminyl groups (like T), Uhlenbruck uses the term "Friedenreich antigens", and there are now a number of these known (see Kim *et al.*, 1970). They are not limited to human cells nor to red cells. Studies on the distribution of the T antigen in cells and membranes from various sources were carried out by Newman and Uhlenbruck (1977). The "true" Thomsen-Friedenreich antigen, now called T_F, has the terminal disaccharide β-D-Gal(1→3)NAc-D-GalNH$_2$ as its immunodeterminant structure. This is recognized by serum anti-T, and by a number of lectins. It is known that an agglutinin from the peanut is anti-T specific, and Skutelsky *et al.* (1977) took advantage of this property to study the distribution of T receptors and agglutinin binding to a number of cells using ferritin-conjugated lectin, and examination of the reacted material in the electron microscope.

21.8.3 The nature of the MN receptors

In 1954, Hohorst used a phenol extraction procedure to obtain an MN-active preparation from red cells, which was partially characterized and contained polysaccharide. Around 1958, Georg Springer and his collaborators took up their extensive studies of MN substances. Certain influenza viruses were found to inactivate M and N, but not other known blood group receptors. Identical effects could be produced by neuraminidase (which was called RDE, receptor destroying enzyme) from a bacterial source. The virus was known to code for neuraminidase (Springer and Ansell, 1958). These observations were independently made by Mäkelä and Cantell (1958) in Finland. In 1959, Baranowski *et al.* in Poland isolated and partially purified M and N substances from red cells and determined their carbohydrate and amino acid composition. In 1960, Lisowska showed that several proteolytic enzymes reduce, but do not abolish M and N activity. No sialic acid was released upon treatment with these enzymes (pepsin, papain, α-chymotrypsin and trypsin). Klenk and Uhlenbruck (1960) isolated NANA-containing mucoids, and could show that papain did release sialic acid from these preparations, concomitantly destroying M and N reactivity, without, however, destroying the influenza virus receptor. It is to be noted that neuraminidase

treatment of cells always generated the T agglutinogen (section 21.8.2) in cells and purified preparations. Stalder and Springer (1960) could show that preparations from red cells and from human kidneys inhibited anti-M and *Vicia gramineae* anti-N reactions. Springer and his collaborators have isolated M and N substances from red cells and other sources, and purified some of the preparations to a high degree. Isolation and partial characterization of M and N substances from red cells and N substances from meconium were reported by Hotta and Springer (1965). In 1966, Springer *et al.* purified the N substance from red cells and that from meconium (which was called Me-Vg antigen, because it reacted with *Vicia graminea* lectin) extensively. The preparations were potent myxovirus receptors, and induced specific anti-N formation in rabbits. The peptide content of the red cell preparations was 44%, and of the Me-Vg antigen, 13%. The MW of the red cell preparation was 595,000, and that of the Me-Vg was 520,000. There was evidence that the carbohydrate residues were linked to the peptide by way of serine or threonine residues.

Other information has been gathered from studies on rare variants of the MN system, and from differences in the reactivities with lectins and other agglutinins of nonhuman origin. Springer and Stalder (1961) had an opportunity to examine MM^g and NM^g cells (see in section 21.5.1). The M^g was not destroyed by influenza virus nor by neuraminidase. Trypsin and papain did inactivate M^g. The evidence on M^g had suggested that it was an allele of M and N, but these studies clearly indicated that, at minimum, the arrangement of sialic acid residues in the M^g receptor differed from what it is in M and N receptors.

It has been known for a long time that M cells will react with anti-N (Landsteiner and Levine, 1928b). Rabbit anti-N has to be absorbed with M cells in order to obtain specificity, but overabsorption can remove too much anti-N and make the serum useless. Many investigators have confirmed this finding. The same behavior characterizes the *Vicia graminea* anti-N reagent. Levine *et al.* (1955a) found that, although the *Vicia* reagent does not agglutinate M cells too well—although it does to some extent, and especially if they are centrifuged—it is absorbed onto M cells and can be eluted from them. On this basis, and on the basis of the finding that mucoid preparations from M cells inhibited anti-N reactions, Uhlenbruck (1960) suggested that N, or something like N, served as the precursor substance in the biosynthesis of the MN substances. Allen *et al.* (1960) interpreted this behavior to mean that the *M* gene produces a small amount of N substance. There are, however, some M cells that do not react with anti-N reagents, including *Vicia* lectin. Allen *et al.* (1960) showed that an example of MU cells did not react with anti-N, and they tested an example of MU- cells and found the same behavior. These cells, which were S-s-, did not, therefore, appear to make the N substance found in most other M cells. Uhlenbruck and Krupe (1965) reported, however, that MuMu cells do in fact have the receptor for *Vicia* anti-N, but as a cryptantigen, and that neuraminidase treatment is required in order to reveal the reaction. Romanowska (1964) showed that the reactions of several lectins were a little bit different. Neuraminidase treatment of M and N substances inhibited but did not abolish their ability to inhibit the reactions of *Iberus amata* anti-M and *Bauhinia variegata* anti-N. Exposure of M and N substances to receptor destroying enzyme from *T. fetus* abolished their reactions with *Vicia* and *Iberus* reagents, but not the *Bauhinia* reagent. Springer *et al.* (1972) have shown that isolated M antigens inhibit most anti-N reagents tested (rabbit, human, and *Vicia*). Mild acid hydrolysis of M substance releases sialic acid, and leads to an increase in anti-N inhibition, such that in the course of hydrolysis, M substance becomes indistinguishable from N substance. Springer and Huprikar (1972) noted that the virus receptor and the isoantigen were thought to be controlled by a single gene, leading to a branched terminal structure possessing β-galactose and N-Ac-neuraminic acid, both of which are required for N activity. β-galactosidase destroys N activity without affecting M. Cohen *et al.* (1972) studied the reactions of an agglutinin from the horseshoe crab (*Limulus polyphemus*). Absorption of the agglutinin onto MN cells is inhibited by prior sensitization with anti-M or anti-N, and MN mucoid substance inhibits *Limulus* reagent agglutination of cells. This evidence suggested that *Limulus* reagent was reacting with a receptor common to M and N determinants, but not determining the difference between them.

As noted previously, the increased number of plant and animal agglutinins and the sometimes subtle differences in their reactivity has complicated the picture to some degree. At the same time, any general theory of the biochemical genetics must be able to explain the reactions. Uhlenbruck has proposed to try and put some order to the dozens of "reactivities" with various "receptors" of sera and of lectins and protectins. The effort needs to be appreciated in the broad terms of looking at the red cell (and other cell) surface as a structurally complex mosaic of antigenic determinants. Uhlenbruck accepts the concept of Wiener that many of the antigens may be complex, i.e., that they may have a number of determinants (which Wiener calls blood factors). The nomenclature is fairly involved, but it is covered in Prokop and Uhlenbruck (1969). It is descriptive, and general, in that it allows for expansion as new reactions are found, and does not carry with it any mechanistic or genetic implications. There is much to recommend a standardized set of nomenclature, but, as Prokop and Uhlenbruck (1969) themselves noted, such an effort ". . . . occasionally leads to strained relationships between serologists!" Two examples will illustrate the idea: $A^{hum}(A^{pig})$ indicates the A antigen of *human* red cells, which contains the A factor of *pig* red cells that is detected with *human* anti-A. It also contains the so-called sheep cell Forssman antigen, which can be detected with rabbit serum. Thus, A^{she}_{ra}. And the full designation of human red cell A would be $A(A^{pig}_{hu}, A^{she}_{ra})$. Cryptantigens are denoted by square brackets. Thus human red cell M substance is $M(M_{ra}, M_{la}, M_{Vg}, [N_{Vg}])$, where "ra" indicates

rabbit, "Ia" indicates *Iberus amata* and "Vg" indicates *Vicia graminea*.

As to the biochemical genetics of MN, it is not yet as clear as in the case of ABO, as pointed out at the beginning of the discussion. Uhlenbruck's original suggestion that N, or something like it, was a precursor of M has been modified to take into account the reactivity with the various phytagglutinins, and the studies on the rarer kinds of MN variants (Uhlenbruck, 1969). The model is quite involved, but invokes a precursor substance which has the *Vicia*, *Bauhinia* and *Iberus* reagent receptors. The precursor is the molecule to which sialic acid is added, thus generating the M and N receptors. The U/u genes are postulated to act next in the sequence, and the S/s genes last. Springer and Huprikar (1972) and Springer *et al.* (1972) took the view that N gene product is the immediate precursor of M gene product, and that the difference between M and N resides in the carbohydrate chain. In this view, M and N are not alleles, and the allele of *M* is an amorph. More recently, Springer and Desai (1974 and 1975) have said that the difference in M and N structures is one NANA residue attached to a β-galactopyranosyl residue, as indicated in Fig. 21.1, which also indicates the proposed relationship of M and N with T and Tn. The results of Sadler *et al.* (1979) and of Judd *et al.* (1977a) with M and N reactivity as functions of sialic acid content are not in agreement with the view that sialic acid is the integral determinant of the antigen structure.

There is now considerable biochemical evidence that the substantial difference between M and N resides in the structures of the N-terminal peptides of the glycoprotein molecules, and does not depend on carbohydrate. At the same time, there is no doubt that carbohydrate, especially N-acetyl-neuraminic acid, plays an important role in defining the structure of the antigens, some anti-M and anti-N reagents being more capable of detecting NANA-dependent differences than others (Sadler *et al.*, 1979; Judd *et al.*, 1979a; Judd *et al.*, 1979b; Springer and Yang, 1978). In 1967, Lisowska and Morawieki showed that blocking the free amino groups of M and N substances (mostly ϵ-NH_2 groups of lysine were involved) interfered with their reactivity toward rabbit antisera. Activity toward *Vicia* reagent was, however, not affected. They suggested that the peptide moieties were important features in determination of the basic differences between the structures. Dahr *et al.* (1975a

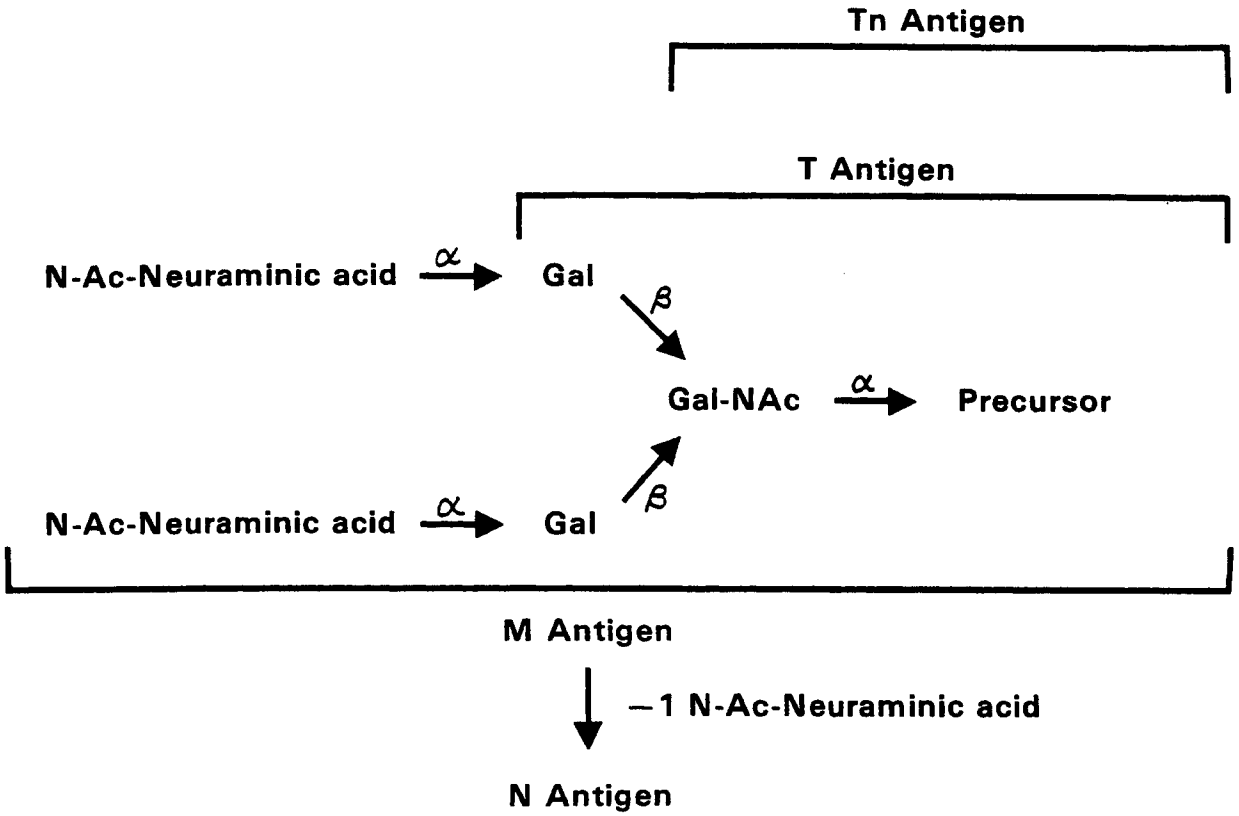


Figure 21.1 Terminal Oligosaccharide Structure of M, N, T and Tn Immunodominant Groups (according to Springer and Desai, 1974)

and 1975b) did a number of similar experiments and reached similar conclusions. Lisowska and Duk (1975) expanded the previous studies, and suggested that the peptide moieties in the molecule conferred certain steric configurations on the end groups which explained the available data. The *Vicia* receptor was said to be more internal, accessible in N cells, but not in M cells. Dahr *et al.* (1975a) agreed with this view. Waśniowska *et al.* (1977) have now shown that the N terminal amino acid of the peptide chain in M substance is Ser, whereas in N substance it is Leu. Dahr *et al.* (1977) reported identical results, and additionally that MN cells yield both amino acids. Waśniowska *et al.* found another amino acid substitution—probably at position 5—where M has Gly and N has Glu. These results have been confirmed in a number of different laboratories (Blumenfeld and Adamany, 1978; Dahr and Uhlenbruck, 1978; Furthmayr, 1978). Lisowska and Kordowicz (1977) have shown that antisera can be prepared in rabbits against desialyzed (sialic acid removed) M and N glycoproteins. These sera agglutinate desialyzed red cells, and precipitate desialyzed glycoprotein, but do not react with native cells or glycoprotein. After appropriate absorption of the anti-desialo-M and N, the reagents can be rendered specific, and this is taken to mean that sialic acid is not an essential feature in determining the difference between M and N structures. Springer *et al.* (1977) have said that only serine is found as the N-terminal amino acid of the peptide in gently isolated M and N glycoproteins. A partially purified glycopeptide fraction from M cells showed N-terminal serine predominantly, but sometimes leucine, while the reverse was true in the case of the fraction derived from N cells.

Walker *et al.* (1977) studied the reactions of normal M and N cells, MU cells and of the cells of an M/- patient and his family quantitatively, and they also determined the electrophoretic mobility of the cells. The data did not support the idea that M and N differ only in sialic acid residue content (Fig. 21.1). They did not support the idea of an N precursor relationship to M either, because if this were the case, MU cells—which do not cross react with rabbit anti-N—would be expected to have more M receptors, and they did not. The previously reported "generation" of rabbit anti-N receptor by neuraminidase treatment is explained on the basis of the presence in rabbit anti-N of an antibody which reacts better with desialized red cells. A theory was proposed to account for these findings and to take into account the evidence suggesting that the peptide moieties from M and N specific structures were different. It was called the "acceptor site" theory. The *M* and *N* genes, in this view, determine only the peptide sequences. The M and N specificities are produced by transferases which put together the oligosaccharides, but which are coded for by genes unrelated to *M* and *N*. It was suggested that the terminal oligosaccharide structure may be identical in M and in N, the conditions for antigenic specificity being brought about by configurational differences dictated by the peptide, which by its primary structure, determines the location of the attachment sites for the first sugar, N-Ac-Gal-NH_2. At least two such attachment sites would be specified by the primary structures in each MN protein, and possibly many more. Springer's data, suggesting that M specificity needs two closely juxtaposed sialic acid residues, could be explained by supposing that the *M* gene yields a peptide in which acceptor sites (i.e., amino acids) are close by, or next to one another. The difference in M and N would reside, therefore, in the distance between the amino acids to which the terminal oligosaccharide chain became attached. The *M* and *N* genes are thought to dictate the synthesis of distinct and independent glycoprotein, thus explaining the M^k and M/- hemizygous conditions. MU and Mu phenotypes are thought to represent alterations in the sequence.

The structural studies on the glycoproteins of the red cell membrane in a number of laboratories appear to have revealed that the difference in M and N lies in two amino acids in the N-terminal glycopeptide of the glycoprotein. Marchesi (1979) has reviewed the studies on the red cell membrane glycoproteins. This work is closely related to the structural studies discussed in section 21.9.4. The glycoproteins which carry MNSs specificities have been called "glycophorins" by Marchesi, Furthmayr and their collaborators at Yale. There are now known to be three separate glycophorins, designated A, B and C. Glycophorin A has been purified, and the complete amino acid sequence of its peptide moiety determined (Tomita *et al.*, 1978). It is in amino acids 1 and 5 (counting from the N-terminus) that the glycophorin A molecules from MM and NN cells differ (Waśniowska *et al.*, 1977; Dahr *et al.*, 1977; Dahr and Uhlenbruck, 1978; Furthmayr, 1978; Blumenfeld and Adamany, 1978; Tomita *et al.*, 1978). The data of Blumenfeld and Puglia (1979) fully support the structure for glycophorin A set forth by Tomita *et al.* (1978) (see Fig. 21.2). Furthmayr (1978) has shown that the N-terminal sequence of glycophorin B is identical to that of the glycophorin A from NN cells in at least the first 23 residues. This accounts for the fact that anti-N reacts with MM cells. Glycophorin B also carries the Ss antigens. Investigations on S-s- cell glycoproteins have shown that the glycopeptide carrying these specificities is very much reduced (Tanner *et al.*, 1977) or perhaps absent altogether (Dahr *et al.*, 1978). Red cells of type M^g have the same amount of glycophorins A and B as normal cells, but MM^k cells have only 50% of the normal complement (Furthmayr, 1978). Anstee and Tanner (1978) and Anstee *et al.* (1979) showed that cells from M^g and Miltenberger types have altered sialoglycoproteins.

21.9 Medico-legal Applications

21.9.1 Introduction—disputed parentage applications

As noted, M and N factors were first found in 1927. Within a short while, it was established that these were consistent serological characteristics of the human population, and the mode of inheritance was clear (Landsteiner and Levine, 1928; Schiff, 1930; Wiener and Vaisberg, 1931). The potential for use of the system in disputed affiliation cases was immediately recognized (Wiener and Vaisberg, 1931; Schiff, 1932b and 1933a). According to Schiff and

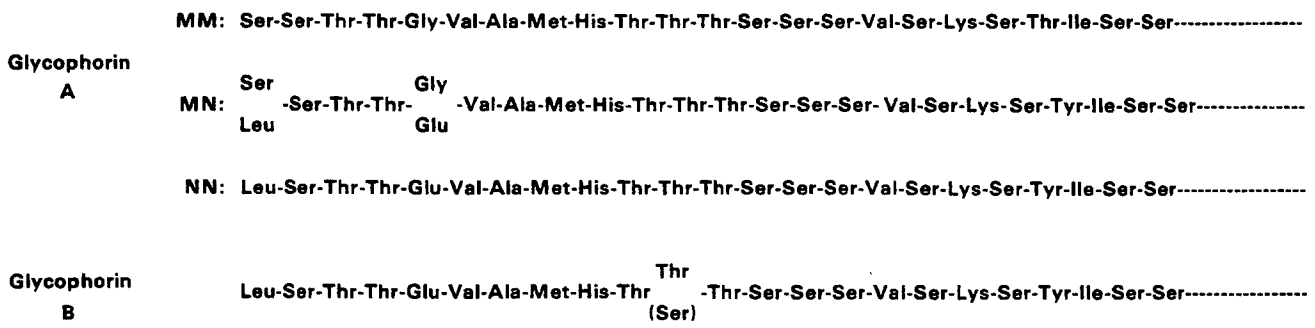


Figure 21.2 Amino Terminal Sequences of the First 23 Amino Acid Residues in the Peptide Moieties of Glycophorin A from MM, MN and NN Red Cells and of Glycophorin B from Red Cells, regardless of MN Type (after Furthmayr, 1978 and Tomita et al., 1978).

Boyd (1942), the first legal case in which MN was employed was in Potsdam in 1931. Wiener (1935) reported an exclusion in a case in this country involving a mother, three children, and two different men, based on MN grouping, but he did not say when it was done, nor whether it went into court. The system is well established as a marker in disputed affiliation cases, and S and s are regularly included as well by many laboratories (see, for example, the case of Silveira, 1967). Application of the system to the grouping of bloodstains developed more slowly.

If anti-M and anti-N are used in paternity tests, the probability of excluding a falsely accused father is close to 18% (AABB, 1978). Should anti-S and anti-s be employed in the tests as well, the probability value is increased to about 31% for Whites and 32% for Blacks (Chakraborty *et al.*, 1974; AABB, 1978). MN is the most powerful two allele system for paternity exclusion, and exclusion probabilities if all four antisera are used are higher than for any other red cell antigen system. It is important to be aware of the rare variants discussed in section 21.5.1 when carrying out these tests. On the one hand, it is not expected that these variants would be encountered very often, if ever; on the other, the presence of one of them (e.g. M^k or M^g) in a sample being tested could cause one to misinterpret the results of the in vestigation. If it could be shown that a rare allele were passed from a putative father to a child, this fact would greatly increase the probability of his being the true father. The peculiarities of MNSs must be appreciated and considered in interpreting exclusions obtained with the system (Morel, in AABB, 1978). Problems of interpretation may be encountered if S^u types are present, and Black populations are more likely to show them than White populations (Moores *et al.*, 1980).

21.9.2 Detection of MN in bloodstains

Landsteiner and Levine (1928a) noted in the original paper that blood dried on a glass surface would absorb appropriate anti-M or anti-N and reduce its titer, and they noted that bloodstains might, therefore, be amenable to MN typing. In 1933, Lauer presented an inhibition technique for MN grouping in dried blood. Quite a bit of blood was needed for the tests (about 10 mg dried blood was used), and care had to be exercised in preparing and selecting antisera. Proper controls had to be run as well, because of dosage effects with some sera—homozygous bloods giving stronger reactions than heterozygous samples—and the problem of cross-reactivity. It has been known since Landsteiner and Levine's work that M cells can absorb some anti-N. M grouping was more reliable than N grouping. Lauer presented his results in two criminal cases. In 1933, Clausen carried out studies on MN grouping in stains, and gave a slightly modified inhibition technique. His results did not look terribly promising. he said that he could find no trace of MN factors in saliva, semen, serum or urine. Schiff (1932b) mentioned that MN determination in bloodstains would be very uncommon, because in most cases, the rather large amount of material required for the test would be unavailable. Therkelsen (1934) reported a modified inhibition technique for M. He used a large amount of material (50 mg dried blood) in order, he said, to get a large quantity of specific binding with M containing stains, and minimize the role of nonspecific binding. Results with N were not very good, and its determination was not recommended. M was determined in a 14 month old stain. The subject was covered again in Therkelsen's lengthy paper in 1936, the conclusions being the same. Moureau (1935a) reported results quite similar to Therkelsen's in the grouping of MN in bloodstains by inhibition technique. In 1936, Holzer tested animal material, including blood and tissues, for absorption of anti-M and anti-N, and found it to be minimal where it occurred at all. He did not think that this was an important consideration in MN grouping in bloodstains. Wiener (1939) was in agreement with Therkelsen and Moureau that M could be determined reliably, but that N could not be. Schiff and Boyd (1942) briefly mentioned Therkelsen's (1934 and 1936) procedure for M in bloodstains. Harley (1943) said

that he had been unsuccessful in devising a technique for MN grouping in "smaller stains". Mueller (1953) noted that Balgairies and Christiaens had had only 20–30% successful results in their studies on bloodstains up to 4 days old in 1938, and he said that the success rate with MN in his own laboratory was probably still less.

21.9.3 More recent developments in MN grouping in bloodstains

In 1961, Sylvia and Kirk investigated the problem of MN grouping in stains, using an anti-M serum, and the anti-N lectin derived from *Vicia graminea*. An inhibition procedure was used for stains. The antiserum and lectin preparations were selected and calibrated for use in grouping on the basis of the time dependence of agglutination of different cells, which was itself a function of the titer of the antiserum or lectin. M cells could be agglutinated by *Vicia* reagent, but much more slowly than MN cells. In 100 bloodstains, M and N could be correctly grouped in all the samples.

In 1963, Pereira reported success in the determination of MN in bloodstains by the elution technique (Pereira, 1963a). Carefully selected rabbit antisera were used in 2 hr absorptions in a moist chamber at room temperature. Elution was at 56° for 15 min following three washes with saline. Cloth controls gave no reactions. N stains did not react with anti-M. M stains often reacted weakly with anti-N. A full series of controls, and caution in interpretation of results was recommended. Pereira said that if all the reactions were weak, no conclusions should be drawn. Stains up to 3 months old could be grouped, but the reactions tended to be weak in 9 month old stains. Fiori *et al.* (1963) also reported successful results in grouping MN in bloodstains by elution. Absorption was at 4°, as for ABO, and high titer ($\geq$1:64) antisera were required. Budvari (1963) confirmed these results. In 1969, Howard and Martin reported successful results with M and N by elution, using the technique in which the threads are affixed to a cellulose acetate backing, and the entire procedure carried out on the backing sheet (see in section 19.10.3.4). Katsura *et al.* (1971) proposed a solution to the problem of antiserum specificity. Rabbit antisera, raised against OM or ON cells, were absorbed onto boiled red cells of appropriate type. The material was washed, and the absorbed antibodies then eluted, the eluate consisting of "specific" antiserum. The eluates had titers of about 1:32 for anti-M and 1:16 for anti-N in saline. The titers were improved by the use of an antiglobulin test. These preparations could be successfully employed for MN grouping of blood-stained threads by elution technique (Sagisaki *et al.*, 1971). Elution of the antibody and addition of appropriate test cells was followed by the addition of anti-rabbit globulin serum raised in goats to effect agglutination. Stains a year old, and sometimes up to twice that, could be determined reliably. Yudina (1972) reported an absorption-elution method for grouping M and N in bloodstains.

Mixed agglutination procedures have been found to be satisfactory as well. Nickolls and Pereira (1962) employed a mixed agglutination procedure, which was a modified version of that of Coombs and Dodd (1961), for MN determination. Fiori *et al.* (1963) agreed that mixed agglutination was a suitable procedure for MN grouping, although somewhat more delicate and involved than elution. Akaishi (1965) got good mixed agglutination (GSDCM) results for M and N as well. Maresch and Wehrschütz (1963) confirmed the successful results obtained by Nickolls and Pereira (1962) using mixed agglutination. They followed the mixed agglutination determination with a 50° elution step which gave agglutinates in the field no longer adhering to the fibril. They regarded this step as giving additional certainty to the determination, and with certain kinds of materials (e.g., Nylon), it was essential to obtain a good result. Driesen and Keller (1973) followed a procedure rather similar to that of Maresch and Wehrschütz with good results.

Schwerd (1978) examined several procedures for MN grouping in stains. Elution techniques similar to those of Kind (1960b) and of Lincoln and Dodd (1973) were employed, along with a mixed agglutination procedure similar to that of Coombs and Dodd (1961), as modified by Kobiela *et al.* (1972). Methanol fixation of the threads was always done. It was found to be important to carefully arrange the quantities of antigen and antibody, as Lincoln and Dodd had shown, and to do the washing steps at 4°. The use of rabbit anti-globulin did not improve the results. Selection of antisera was stressed as the most important consideration. Useful anti-N were the most difficult to find. With carefully standardized test conditions and antiserum selection, Schwerd said that false N reactions should not ordinarily be observed. Failure to detect N (false negative) could be avoided by the use of several anti-N sera. The use of *Vicia graminea* lectin was found to be useful as well, and rabbit antisera worked better on the whole than did human derived antisera.

Denault *et al.* (1978) found that M and N were detectable for the most part in stains on a number of substrata for up to 26 weeks by the elution procedure. There was a hint that N was slightly affected by high humidity when the bloodstain was on wool. Both M and N could be missed, and there were occasional false positive M and N reactions.

21.9.4 The problem of anti-N cross reactivity

All investigators familiar with the MN system are aware of the reaction of anti-N with M cells. The reasons for this "cross-reactivity" are not simple, nor yet fully understood. It has as its basis the fundamental immunochemical structures of the M and N determinants, and their arrangement on the surface of the cell membrane. These matters were discussed in section 21.8. The problem must be dealt with in practice by carefully screening antisera for specificity, and in bloodstain grouping, control bloodstains of all three types must be used. In spite of precautions, errors in MN grouping are possible. N antigen can be missed, and MM stains can be misgrouped as MN because of their cross reactivity with anti-N. A recent blind trial study in this country, in which many laboratories participated, indicated that the

MN types in bloodstains were not correctly determined in a significant number of samples. Although the subject has not been widely discussed in the published literature, a number of laboratories that we know about have taken MN testing out of their routine bloodstain examination procedures.

Shaler *et al.* (1978) have recently employed a different approach to this problem, based on what is known about the biochemical composition of the MN-active glycoproteins. Some additional background material is introduced here as a basis for understanding these studies. These studies closely relate to the material discussed in section 21.8.3. In 1971, Fairbanks *et al.* fractionated the major polypeptides of the human erythrocyte membrane by electrophoresis in polyacrylamide gels containing the detergent sodium dodecylsulfate (SDS). Coomassie Blue staining of the gels located six fractions containing protein. If the gels were stained with so-called periodic acid-Schiff reagent, four bands could be seen, one of which was regarded as being a (glyco)lipid fraction. Periodic acid is known to oxidize structures containing neighboring C-OH groups to the level of aldehydes, and these compounds can form colored products with Schiff reagent. For details of the reaction schemes, see Pigman (1957), Lillie (1977) and Clark (1973). Periodic acid-Schiff staining can thus be used for histochemical location of glycoproteins. The major glycoproteins located on the polyacrylamide gel by Fairbanks *et al.* (1971) were called PAS-1 through PAS-3. The arrangement of these proteins and glycoproteins on the membrane was studied in a subsequent paper (Steck *et al.*, 1971) using proteolytic enzymes to partially hydrolyze the molecules on membrane vesicles—both "right side out" and "inside out"—from erythrocytes. Hamaguchi and Cleve (1972) looked at the serological specificities of the glycoprotein fractions and said that PAS-1, with MW about 58,000, was probably the MN glycoprotein. PAS-3 contained most of the S activity. Interestingly, rabbit anti-N inhibition was seen with PAS-3 as well as with PAS-2 on occasion. Dahr *et al.* (1975a) showed that trypsin and α-chymotrypsin had differential effects on the MNSs activities as assessed with different antisera. Rabbit anti-N reactivity with M cells was abolished by chymotrypsin, its reactivity with N cells being unaffected. Anti-S or anti-s reactions were greatly inhibited or abolished by chymotrypsin treatment, while trypsin had no effect or slightly enhanced them. This, and other data, suggested that the "cross-reacting N" receptor of M cells is present on the same glycoprotein as the Ss activity (PAS-3), but not on the MN glycoprotein (PAS-1). Dahr *et al.* (1975c) looked at the glycoproteins from M or N S-s-U- cells, and PAS-3 was absent. S-s-U+ cells showed the same profile. These observations constituted compelling evidence for the assignment of MN activity to PAS-1, and "cross reacting N" and Ss activity to PAS-3. It is now clear that PAS-1 and PAS-2 comprise glycophorin-A, and that PAS-3 comprises glycophorin B (Furthmayr, 1978). It may also be noted that Tanner *et al.* (1977) find *some* PAS-3 in S-s- cells. Furthmayr (1978) has pointed out that there are great technical difficulties in these SDS-polyacrylamide gel fractionations which could account for differences in results. These studies help to explain some of the observations in the literature on the serological effects of proteolytic enzyme treatment of different types of red cells (Hirsch *et al.*, 1957; Morton, 1962). Judson and Anstee (1977) have examined the effects of enzyme treatment on various red cell types, and have correlated these with alterations seen in the glycoprotein profile. Shaler *et al.* (1978) took advantage of the fact that cross-reacting N activity, which resides on a different glycoprotein than does MN, is differentially sensitive to treatment by α-chymotrypsin. M cells, previously determined to possess N cross reacting activity, were used to make bloodstain, along with control M and N cells. These stains, treated with appropriate concentrations of chymotrypsin (4 mg/mℓ) for up to 15 min at 37° showed selective destruction of cross reacting N in the subsequent elution test, without affecting normal M and N activity. Incubation time and chymotrypsin concentration were both critical variables. Shaler *et al.* emphasized that this delicate procedure, while showing promise and illustrating the value of fundamental biochemical approaches to practical problems, was not being recommended as a routine procedure in casework for the time being.

21.9.5 The detection of the Ss and other antigens in bloodstains

In 1968, Lincoln and Dodd first reported the detectability of S in bloodstains by elution technique (Lincoln and Dodd, 1968b). Four anti-S, containing complete antibodies active at room temperature, were tested in the elution procedure, all operations being carried out at 20°. None of these were satisfactory. One serum was found, however, that contained incomplete, AHG detectable antibodies which were most active at 37°. Absorption at 37°, elution, and detection of the antibody with test cells at 37° with this serum yielded excellent results using the anti-human globulin technique. S+ and S− stains up to 6 months old could be grouped reliably. Kobiela *et al.* (1972) confirmed that S could be reliably grouped in stains up to 4 months old, the details of their procedure being beyond our ability to decipher the Polish text. Lincoln and Dodd (1975a and 1975b) recently added s to the list of antigens that can be grouped reliably in bloodstains. As in the case of S, the anti-s contained incomplete antibodies, and 37° absorption and reaction temperatures were employed, detection being by means of anti-human globulin technique. Washing steps were carried out at 4°. The two useful anti-s reagents had AHG titers of 1:8 against cells. 1 cm long bloodstained threads could be grouped for s in samples up to 7 months old, SS stains giving uniformly negative reactions. McDowall *et al.* (1978b) compared an auto-analyzer procedure with a manual one for the detection of anti-S eluted from bloodstains. Autoanalyzer procedures are briefly discussed in Section 22.8. The manual detection method was found to be superior to the automated one.

Denault *et al.* (1978) found that S survived aging in bloodstains less well than did s. The effect did not seem to be specifically attributable to humidity differences, nor to any particular substratum. It also appeared that s could be missed in

stains more easily than could S. A single false positive S reaction was seen with denim.

Some time ago, Ducos *et al.* (1969a) reported that the MNSs related antigen Vw could be detected in stains up to 6 months old in most cases. An inhibition technique was used, and a human anti-Vw was available for the experiments. Vw is a low incidence antigen, classified by Cleghorn (1966) as part of Class I of the Miltenberger series of antigens (see section 21.5.2 and Race and Sanger, 1975).

21.10 The Frequency of MNSs Phenotypes in U.S. Populations

Frequences of MN blood groups in some U. S. populations using anti-M and anti-N are shown in Table 21.2, and MNSs groups determined using anti-M, anti-N and anti-S and/or anti-s are shown in Table 21.3.

Table 21.2 Frequencies of MN Groups in U.S. Population

Population	Total	Frequency — Number (Percent) M	MN	N	Reference
CAUCASIAN					
New York, NY	3,263	1,037 (31.73)	1,621 (49.6)	603 (18.6)	Wiener and Gordon, 1951
New York, NY	954	287 (30.1)	481 (50.4)	186 (19.5)	Wiener et al., 1953
Western Alaska	784	(36.61)	(45.03)	(18.37)	Pauls et al., 1953
University of Iowa "Controls"	2,186	587 (26.85)	1,208 (55.26)	391 (17.89)	Buckwalter et al., 1962
Boston, MA					
Rheumatic	606	200 (33)	293 (48.4)	113 (18.6)	Dublin et al., 1964
Non-rheumatic	600	182 (30.3)	305 (50.9)	113 (18.8)	
Eastern San Francisco, CA Bay Area					
Mothers	4,928	(31.29)	(48.86)	(19.85)	Reed, 1967
Babies		(28.37)	(52.42)	(19.22)	
New York, NY	500	158 (31.6)	249 (49.8)	93 (18.6)	*Wiener, 1969*
Miami/Dade Co., FL	366	108 (29.5)	181 (49.5)	77 (21.0)	Stuver, 1979 and see Shaler, 1978
NEGRO					
Baltimore, MD	580	136 (23.45)	280 (48.25)	164 (28.3)	Glass and Li, 1953
Washington, D.C. Howard University Students	937	(39.7)	(36.0)	(24.3)	Moore, 1955
Eastern San Francisco CA Bay Area					
Mothers	1,453	(24.36)	(50.79)	(24.85)	Reed, 1967
Babies		(23.19)	(51.34)	(25.47)	
Birmingham, AL	610	154 (25.2)	298 (48.9)	158 (25.9)	Casey et al., 1968
New York, NY	500	119 (23.8)	242 (48.4)	139 (27.8)	Wiener, 1969
Miami/Dade Co., FL	345	81 (23.5)	176 (51.0)	88 (25.5)	Stuver, 1979 and see Shaler, 1978
CHINESE					
New York, NY	400★	141 (35.3)	192 (48.0)	58 (14.5)	Wiener, 1969
New York, NY	946☆	321 (33.93)	460 (48.62)	148 (15.64)	Wiener, 1974
HISPANIC					
Miami/Dade Co., FL	359	116 (32.3)	167 (46.5)	76 (21.2)	Stuver, 1979 and see Shaler, 1978

★ Includes 9 MN_2 types ☆ Includes the 400 people reported by Wiener, 1969, and there were 17 (1.79) MN_2 types

Table 21.3 Frequencies of MNSs Groups in U.S. Populations

Population	Total	Frequency — Number (Percent) MS	MSs	Ms	MNS	MNSs	MNs	NS	NSs	Ns	Reference
CAUCASIAN											
New York, NY	394★	88 (22.1)		37 (9.6)	106 (26.9)		87 (22.0)	27 (7.0)		49 (12.4)	Wiener et al., 1953
Southeastern GA	333	13 (3.9)	39 (11.7)	34 (10.2)	11 (3.3)	76 (22.8)	79 (23.7)	1 (0.3)	21 (6.3)	59 (17.7)	Cooper et al., 1963
New York, NY	332	12 (3.6)	44 (13.3)	39 (11.7)	7 (2.1)	75 (22.6)	77 (23.2)	4 (1.2)	26 (7.8)	48 (14.5)	Issitt et al., 1965
New York, NY	900	50 (5.6)	121 (13.4)	109 (12.1)	29 (3.2)	194 (21.6)	206 (22.9)	11 (1.2)	53 (5.9)	127 (14.1)	Issitt et al., 1968
San Francisco, CA Bay Area	8,962	575 (6.4)	1,327 (14.8)	853 (9.5)	331 (3.7)	1,992 (22.2)	2,088 (23.3)	41 (0.5)	457 (5.1)	1,298 (14.5)	Reed, 1968
South Central West Virginia	1,051	(6.4)	(13.8)	(9.6)	(3.6)	(24.1)	(24.1)	(0.6)	(4.5)	(13.4)	Juberg, 1970
Tecumseh, MI	8,447	587 (6.95)	1,262 (14.94)	697 (8.25)	278 (3.29)	1,959 (23.19)	1,954 (23.13)	29 (0.35)	423 (5.01)	1,258 (14.89)	Schreffler et al., 1971
Detroit, MI	461	(6.3)	(17.6)	(13.4)	(2.4)	(24.3)	(22.3)	(1.7)	(3.0)	(10.0)	Shaler, 1978 ◇
Miami/Dade Co., FL	370	28 (7.6)	50 (13.5)	30 (8.1)	19 (5.1)	82 (22.2)	80 (21.6)	3 (0.8)	19 (5.1)	59 (15.9)	Stuver, 1979 and see Shaler, 1978
NEGRO											
New York, NY	580★	(6.9)		(17.2)	(16.6)		(33.1)	(26)		(20.2)	Miller et al., 1951
Ann Arbor, MI	96★	10 (10.4)		18 (18.8)	12 (12.5)		31 (32.3)	8 (8.3)		17 (17.7)	Neel and Henig, 1951
Southeastern GA	304☆	6 (2.0)	22 (7.2)	42 (13.8)	9 (3.0)	31 (10.2)	113 (37.2)	4 (1.3)	15 (4.9)	58 (19.1)	Cooper et al., 1963
New York, NY	204●	10 (4.9)	16 (7.8)	28 (13.7)	4 (2.0)	33 (16.2)	64 (31.4)	5 (2.5)	10 (4.9)	34 (16.7)	Issitt et al., 1965
Houston, TX	263○	–	12 (4.6)	48 (18.3)	4 (1.5)	49 (18.6)	79 (30.0)	–	15 (5.7)	56 (21.3)	Francis and Hatcher, 1966
New York, NY	493□	10 (2.0)	32 (6.5)	78 (15.8)	8 (1.6)	71 (14.4)	163 (33.1)	9 (1.8)	23 (4.7)	99 (20.1)	Issitt et al., 1968
San Francisco CA Bay Area	3,148	57 (1.8)	201 (6.4)	482 (15.3)	98 (3.1)	349 (11.1)	1,132 (36.0)	33 (1.0)	167 (5.3)	627 (19.9)	Reed, 1968
South Central West Virginia	100	(2.0)	(6.0)	(16.0)	(4.0)	(10.0)	(34.0)	(1.0)	(4.0)	(24.0)	Juberg, 1970
Detroit, MI	485	(3.1)	(6.2)	(13.4)	(2.7)	(11.0)	(38.1)	(2.3)	(4.0)	(19.4)	Shaler, 1978 ◇
Miami/Dade Co., FL	337	7 (2.1)	23 (6.8)	49 (14.5)	11 (3.3)	40 (11.9)	122 (36.2)	6 (1.8)	11 (3.3)	68 (20.2)	Stuver, 1979 and see Shaler, 1978
CHINESE											
New York, NY	103★	(3.9)		(36.0)	(5.8)		(37.9)	●	(1.0)	(16.5)	Miller et al., 1951
HISPANIC											
San Francisco CA Bay Area "Mexican"	335	23 (6.9)	62 (18.5)	41 (12.2)	18 (5.4)	77 (23.0)	78 (23.3)	1 (0.3)	12 (3.6)	23 (6.9)	Reed, 1968
Miami/Dade Co., FL	363	24 (6.6)	57 (15.7)	36 (9.9)	12 (3.3)	77 (21.2)	81 (22.3)	2 (0.6)	12 (3.3)	62 (17.1)	Stuver, 1979 and see Shaler, 1978

★ Tests with anti-M, anti-N, and anti-S only

☆ 4 people were S—s—

● 1 person was MS—s—U— and another was MNS—s—U—

○ Anti-M and anti-M, were used. M and M, combined in the table.

□ Two people were Mu, one was MNu and four were Nu

◇ Data of Stolorow and collaborators

SECTION 22. THE Rh SYSTEM

21.1 Introduction

The Rh System is one of the most complicated cell or serum group polymorphisms in human beings, a distinction which it now shares with the Gm and HLA systems. In its practical essentials, Rh is not too complex, but there are many pecularities and intricacies of Rh if all the information is taken into account. There are two systems of nomenclature (three, if the numerical system is counted) for Rh in common use, which reflect real differences in the opinions of the people who proposed them about the genetic basis of the system. Efforts to standardize Rh nomenclature have never been successful. Very much is known about Rh serology, but very little illuminating work has been done on the biochemistry and biochemical genetics of Rh. It is clear that the Rh antigenic determinants are not carbohydrate in nature, and it has not been possible to use the very enlightening studies on the ABH, Lewis (and to a lesser extent, MNSs) as models.

22.2 Discovery and Development of the Rh System

In 1940, Landsteiner and Wiener reported that a new human red cell agglutinogen was recognizable using an immune serum prepared by the immunization of rabbits with the red cells of Rhesus monkeys. The factor appeared unrelated to the known blood factors (ABO, MN and P), and was called "Rh". Wiener (1952) traced the beginning of this work to 1937. In 1941, 60 families with 237 children were investigated by Landsteiner and Wiener, and the "Rh factor" behaved as a simple Mendelian dominant characteristic, there being no Rh+ children from Rh− × Rh− matings.

In 1939, Levine and Stetson had found an agglutinin in the serum of a mother who had given birth to a macerated, stillborn fetus as the result of her second pregnancy. The fetus could be shown to have the antibody. Fetal death was the result of a hemolytic reaction, and the mother had serious hemolytic reactions following transfusions with ABO compatible blood. The agglutinin in her serum agglutinated about 80% of random red cells, and Levine and Stetson thought that this antibody was the result of frank immunization of the mother by the cells of her fetus in response to an antigen which had been inherited from the father. This work represented one of the first major steps in the understanding of hemolytic disease of the newborn. In 1940, Wiener and Peters reported on three cases of hemolytic transfusion reactions in cases of transfusion of apparently compatible (for ABO) blood. The best explanation for the incidents was the formation in the patients, who were Rh−, of antibodies in response to transfusion by Rh+ blood. The antibody in the patients' sera closely resembled the anti-Rh serum prepared in rabbits by immunization with Rhesus monkey red cells. It was, therefore, clear by 1940 at least that the new Rh factor could be immunogenic in human beings (Wiener and Peters, 1940; Landsteiner and Wiener, 1941; Wiener, 1942). In 1941, Levine and his collaborators put forward the notion that the Rh immunization of Rh− mothers by Rh+ fetuses was the principal etiologic factor in the pathogenesis of a number of cases of erythroblastosis fetalis (Levine *et al.*, 1941a and 1941b). This substantially correct explanation of certain types of hemolytic disease associated with pregnancy could not, at the time, account for the fact that the incidence of erythroblastosis fetalis was considerably lower than the number of Rh+ infants delivered from Rh− mothers, nor for the fact that hemolytic disease of the newborn was sometimes seen in cases where the mothers were Rh+. Recognition of a number of Rh antigens in the system, of individual variation in isoimmunization phenomena, and of the fact that agglutinogens in other than the Rh system can be responsible for hemolytic disease associated with pregnancy, has made the explanations much clearer (see Wiener, 1946).

In 1943, Wiener and Landsteiner had looked at the sera of a number of mothers of infants born with hemolytic disease, and had found some antibodies detecting further variants of Rh, which, at the time, were called anti-Rh_1 and anti-Rh′, these in addition to the original anti-Rh (or human sera which behaved just like it). People who were "Rh positive" could be "Rh_1" and react with all the antisera, "Rh_2" and react with anti-Rh and anti-Rh′, or "Rh′" and react with anti-Rh_1 and anti-Rh′. "Rh negative" people reacted with none of the antisera. Wiener and Sonn (1943) reported a newly found anti-Rh antiserum, and the nomenclature in this paper changed a little bit from that just described. Wiener (1943b) described six allelic genes, called rh, Rh_1, Rh_2, Rh′, Rh″ and Rh on the basis of what was then known. Not all combinations had been found, and some different genotypic combinations gave identical phenotypic reactions. There were only three known agglutinins in Wiener's view, but there were some sera (which had names) that contained more than one of them.

In England, Race and his collaborators were uncovering additional Rh antisera as well. At first, the sera were designated by descriptive combinations of letters derived from the donors' names. A serum reacting with "Rh negative" cells was described by Race and Taylor (1943). Two further examples of sera with an additional Rh specificity were described by Race *et al.* (1943). By 1944, there were four antisera defining seven alleles (Race *et al.*, 1944a). In 1944, Prof. R.A. Fisher (see in Race, 1944) formulated a genetic hypothesis based on the results with the four antisera then known. Two of the sera defined antithetical reactions, and

Fisher postulated that the genes responsible for the synthesis of the two antigens were alleles. The antigens and the genes were called C and c. The remaining sera were said to be defining antigens D and E. Fisher supposed that the genes D and E would have alleles, d and e, and that, ultimately, antisera would be found to them as well. At the time, the antisera were given Greek letter designations by Fisher, Γ being "anti-C", γ "anti-c", Δ "anti-D", H "anti-E", and the predicted "anti-d" and "anti-e" were, respectively, δ and η. Fisher supposed that the three gene loci, if separable at all, must be very closely linked, for no crossing over had been observed (Race *et al.*, 1944b). The possible Rh gene complexes or assemblages were, therefore, CDe, cDE, cde, cDe, Cde, cdE, CDE and CdE. Only CdE remained to be found. In 1945, Murray *et al.* found an individual possessing the CDE complex, and the reactions with the four antisera were as predicted. Mourant in 1945 found the predicted antibody η (anti-e) in a person suffering from hemolytic anemia. The reactions were as predicted. The CdE complex was convincingly demonstrated in the mother of a child with erythroblastosis fetalis by van den Bosch in 1948. Fisher carried the genetic argument for the scheme a step further. Noting that the complexes CDe, cDE and cde were relatively common in Britain, that cDe, cdE, Cde and CDE were quite rare, and that CdE was extremely rare, Fisher said that the less common types were maintained by occasional crossing over in the more usual heterozygotes. A crossover event between the C/c and D/d loci in cDE/CDe would produce cDe and CDE combinations. All the so-called second order combinations (low frequency) could originate in this way, but the rare complex CdE would have to be produced by a crossover event involving a second order combination, which is itself a product of a crossover event, if the theory is correct. Carrying the argument still another step, Fisher used the frequencies of the combinations, which, according to this thinking, should be related to the crossover frequencies between the loci, to postulate that the locus order on the chromosome was D/d, C/c, E/e. These arguments were developed by Fisher and Race (1946) and by Fisher (1947). The antiserum notations, anti-D, anti-C, etc., were put forth by Cappell in 1944, and by Fisher and Race in 1946.

Wiener's conception of the system was different from the outset. He looked upon the locus responsible for the Rh factors as a single, immutable locus which could be occupied by a number of different alleles. Wiener and Landsteiner (1943) identified four allelic genes using two antisera, anti-Rh (presently anti-Rh_o), and anti-Rh_1 (presently anti-rh'), and a serum which contained both of these. A new antiserum was mentioned, containing an antibody, then called anti-Rh_2 (presently anti-rh''), and a full paper appeared describing its characteristics (Wiener, 1943a). Wiener (1943b) gave a full description of the eight Rh phenotypes (only seven of which had been found) based on six genes, which could give rise to 21 different genotypes. Family studies were carried out by Wiener *et al.* in 1944 to confirm the six allelic gene hypothesis. Levine (1943) mentioned that he and Javert had encountered an antiserum reacting with some Rh negative bloods, very similar to Race and Taylor's (1943) "St" serum. He called it an anti-Hr serum, to indicate that it seemed to be the reverse of the different "anti-Rh" sera. Levine (1945) noted that the "Hr" designation was a good one for the gene producing the antigen that reacted with the "anti-Hr" sera, and he said that he had first proposed this designation in 1941. Wiener adopted the "Hr" symbolism and incorporated it into his system of Rh nomenclature (Wiener, 1945; Wiener *et al.*, 1945). In 1948, Wiener and Hyman described families in which the rare genes R^z (CDE in Fisher-Race terms) and r^y (CdE) were segregating. Around 1945, Wiener began taking to task both the Fisher theory of inheritance, and the Fisher-Race nomenclature scheme, and he continued to do so for the next 30 years.

22.3 Rh Nomenclature

The basic structure of the Rh system was worked out in the 1940's (see, for example, Race, 1948). Numerous additional complexities within the system have come along since that time, and some of these will be discussed below. The subject of nomenclature is considered here because it will be needed for the remainder of the discussion, and because it has always been something of an issue in discussions of Rh. The nomenclature has changed a number of times, and it would be more confusing than informative to review its history in detail. Some of the older designations are mentioned in the text. Much has been written about Rh nomenclature, because the two schemes reflected real differences in the conception of the system at the genetic level. Wiener has argued strenuously and long for the universal adaptation of his system of nomenclature. Some of his papers on the subject are collected in his several books (Wiener, 1954, 1961, 1965a and 1970). The Fisher-Race nomenclature is clearly presented and used in Race and Sanger (1975), and in all previous editions, and it is used by most English workers. In Table 22.1 are shown the genes, gene products and antigens, or blood factors, along with the reactions of these products with the five common antisera. Fisher-Race usage is indicated in square brackets. The more common phenotypes of the Rh system detectable with the five common antisera, and the genotypes with which they are associated, are indicated in Table 22.2 The "usual" or shorthand notation for the phenotypes is given in the Table as well. The nomenclature has become somewhat more complicated as unusual Rh factors and phenotypes have been found. These will be mentioned briefly below.

Efforts to arrive at a universally acceptable symbolism for the Rh factors and their antisera have been made, but without success. Some of the difficulty derives from the fact that neither system has been able to digest very easily the multitude of complexities that have come along since the eight "basic" Rh factors were discovered in the 1940's. Nomenclature discussions are always inextricably tied directly, or by implication, to the genetics of the system. The subject will be returned to again in a subsequent section.

Table 22.1 Genes, Gene Products and Reactions in the Rh System With the Five Common Antisera

Gene	[Gene Complex]	Agglutinogen	Blood Factors	[Antigens]	Anti-Rho [Anti-D]	Anti-rh' [Anti-C]	Anti-rh'' [Anti-E]	Anti-hr' [Anti-c]	Anti-hr'' [Anti-e]
r	cde	rh	hr', hr''	c,e	−	−	−	+	+
r'	Cde	rh'	rh', hr''	C,e	−	+	−	−	+
r''	cdE	rh''	hr', rh''	c,E	−	−	+	+	−
R^0	cDe	Rh_0	Rh_0, hr', hr''	c,D,e	+	−	−	+	+
R^1	CDe	Rh_1	Rh_0, rh', hr''	C,D,e	+	+	−	−	+
R^2	cDE	Rh_2	Rh_0, hr', rh''	c,D,E	+	−	+	+	−
R^z	CDE	Rh_z	Rh_0, rh', rh''	C,D,E	+	+	+	−	−
r^y	CdE	rh_y	rh', rh''	C,E	−	+	+	−	−

Usage according to Fisher-Race indicated by square brackets.

Table 22.2 Rh Phenotypes and Genotypes ★

Name ☆	Usual Designation ○	Reaction with Anti- Rh_o [D]	rh' [C]	rh'' [E]	hr' [c]	hr'' [e]	Genotypes ☆	[Genotypes]
Rh_o	R_or,R_oR_o	+	−	−	+	+	R^or,R^oR^o	cDe/cde, cDe/cDe
Rh_1rh	R_1r,R_1R_o,R_or'	+	+	−	+	+	R^1r,R^1R^o,R^or'	CDe/cde, CDe/cDe, cDe/Cde
Rh_1Rh_1	R_1R_1,R_1r'	+	+	−	−	+	R^1R^1,R^1r'	CDe/CDe, CDe/Cde
Rh_2rh	R_2r,R_2R_o,R_or''	+	−	+	+	+	R^2r,R^2R^o,R^or''	cDE/cde, cDE/cDe, cDe/cdE
Rh_2Rh_2	R_2R_2,R_2r''	+	−	+	+	−	R^2R^2,R^2r''	cDE/cDE, cDE/cdE
Rh_1Rh_2	R_1R_2,R_1r'',R_2r'	+	+	+	+	+	R^1R^2,R^1r'',R^2r'	CDe/cDE, CDe/cdE, cDE/Cde
Rh_2rh	R_2r,R_oR_2,R_or^Y						R^2r,R^2R^o,R^or^Y	CDE/cde, cDe/CDE,cDe/CdE
Rh_2Rh_1	R_2R_1,R_2r',R_1r^Y	+	+	+	−	+	R^2R^1,R^2r',R^1r^Y	CDE/CDe,CDE/Cde,CDe/CdE
Rh_2Rh_2	R_2R_2,R_2r'',R_2r^Y	+	+	+	+	−	R^2R^2,R^2r'',R^2r^Y	CDE/cDE, CDE/cdE, cDE/CdE
Rh_2Rh_2	R_2R_2,R_2r^Y	+	+	+	−	−	R^2R^2,R^2r^Y	CDE/CDE, CDE/CdE
rh	rr	−	−	−	+	+	r^or	cde/cde
rh'rh	r'r	−	+	−	+	+	$r'r$	Cde/cde
rh'rh'	r'r'	−	+	−	−	+	$r'r'$	Cde/Cde
rh''rh	r''r	−	−	+	+	+	$r''r$	cdE/cde
rh''rh''	r''r''	−	−	+	+	−	$r''r''$	cdE/cdE
rh'rh''	r'r''	−	+	+	+	+	$r'r''$	cdE/Cde
rh_yrh	rY_r						r^Yr	CdE/cde
rh_yrh''	rY_r''	−	+	+	+	−	r^Yr''	CdE/cdE
rh_yrh'	rY_r'	−	+	+	−	+	r^Yr'	CdE/Cde
rh_yrh_y	rY_rY	−	+	+	−	−	r^Yr^Y	CdE/CdE

★ The number of genotypes and phenotypes can be expanded if anti-rh^W [anti-C^W] is included – see sections 22.5.2 and 22.6.1

☆ According to Wiener

○ Designations not restricted to either system. Until recently, Race and Sanger used R' for r' and R'' for r'', but have now gone to the lower case r for those phenotypes which do not react with anti-D (anti-Rh_o), a practice long favored by Wiener.

[ ] Square brackets denote Fisher-Race symbols and designations

22.4 The Incomplete Rh Antibody

In the early years of Rh serology, investigators were puzzled by the observation that mothers of children with erythroblastosis fetalis were mainly Rh negative, and that in many cases no anti-Rh could be found in their sera. In 1944, Race and Wiener simultaneously elucidated the nature of this phenomenon. Race (1944) showed that some sera contained what he called "incomplete" antibody. The presence of incomplete anti-D could be demonstrated by sensitizing D+ cells with this antibody, washing and then showing that the cells were no longer agglutinable by an anti-D agglutinin. The "incomplete" antibody could, thus, bind the red cell receptor, but did not bring about agglutination in saline. Wiener (1944b) obtained identical results in his experiments, and referred to the nonagglutinating antibody as a "blocking" antibody. The inhibition of agglutination by sensitization of cells with the nonagglutinating anti-Rh_o was referred to as the "blocking test".

Somewhat better approaches to the detection of incomplete antibodies were soon found. Diamond and Abelson (1945) devised a rapid slide test for the detecton of anti-Rh in serum. During these studies, they noticed that multiple saline washing of Rh+ cells sometimes inhibited agglutination, i.e., the reaction seemed to be better in the presence of some serum or plasma. It was soon shown that serum albumin medium, as well as serum or plasma, allowed the agglutination of the cells by some "incomplete" antibodies (Diamond and Denton, 1945; Cameron and Diamond, 1945). The use of colloidal media for the detection of incomplete antibodies has since been widely confirmed. Wiener thought for a time that an additional component of plasma, which he called "conglutinin" was required for agglutination by the blocking antibody in colloidal media, and that this was

the basis for the observations (Wiener and Hurst, 1947; Wiener *et al.*, 1947).

Detection of "incomplete" Rh antibodies by means of anti-human globulin serum was introduced by Coombs *et al.* (1945a and 1945b). Cells sensitized with the incomplete antibody and washed were agglutinated by the addition of the serum of a rabbit immunized with human serum or human serum globulin. The test is often called the "anti-human globulin test" (AHG test) or the "Coombs test", and the AHG serum is sometimes called "Coombs serum".

In 1946, Pickles discovered that the treatment of Rh+ cells with the filtrate of a culture of *Vibrio cholera* made them agglutinable by incomplete anti-Rh. Pickles believed that an enzyme in the filtrate was responsible for this effect, and in 1947, Morton and Pickles showed that trypsin treatment had a similar effect. In 1950, Kuhns and Bailey found that papain treatment was effective in rendering Rh+ cells agglutinable by saline-incomplete antibodies. Stratton (1953) confirmed the papain findings, and devised a slide method with papainized cells, primarily for screening Rh negative mothers for Rh antibodies. In 1955, Löw proposed a routine papain cell test which is still sometimes quoted. A number of workers prefer papain to other proteolytic enzymes (e.g. Boorman *et al.*, 1977). Detailed instructions for the preparation of papain solution, papain treatment of cells, and trypsin, bromelin and ficin procedures may be found in Boorman *et al.* (1977) as well.

Many antibodies have since been found that are "incomplete" in the original (Race, 1944) sense of the term. There is further discussion of this subject in section 1.3.4.

22.5 Complexities of the Rh System—Further Rh Factors

Some of the complexities of the Rh system will be described. Many of the unusual Rh factors or conditions are very rare, but instructive in yielding information about the nature of the system. Not enough detailed information about the biochemical and immunological nature of the system is yet available to reconcile the complicated array of facts that have accumulated.

22.5.1 Subdivisions of D or Rh_0

In 1946, Stratton described a blood whose cells were agglutinated by anti-c, anti-E and anti-e, but not by anti-C. The results with a number of anti-D reagents were variable, 12 of 32 sera giving agglutination. Some of the anti-D reagents which did not cause agglutination contained incomplete anti-D. Stratton suggested that this weak sort of D was caused by a new allele which was called D^u, and the reactions in this case indicated that these cells were cD^uE/cde. This characteristic was studied in detail by Stratton and Renton (1948) and independently by Race *et al.* (1948a and 1948b). There was variability in different examples of D^u blood, some being agglutinable by some anti-D reagents, and others being detectable only by an AHG test with incomplete anti-D. It is possible to miss D^u in typing, so that a blood grouped as cde/cde (rr), for example, might in reality be cD^ue/cde. Wiener denotes the D^u condition in his nomenclature by a Germanic script upper case R (ℜ). At the other extreme, there are cells with abnormally strong D antigen. A case was reported by Renton and Hancock (1955) in which the cells were agglutinated by some incomplete (against ordinary cells) anti-D. There was something peculiar about the C in the cells as well.

In 1951, Shapiro reported an anti-D in the serum of the blood of a South African Bantu mother, whose cells grouped as D^u. Argall *et al.* (1953) reported another case of anti-D in the serum of a patient whose cells were D^u. Other examples of this behavior were soon found, and since the people did not have hemolytic anemia, the concept of complexity of the D ($\mathbf{Rh_0}$) antigen had to be invoked. Allowing the antigen to be complex, that is, to possess more than a single potential kind of antigenic specificity then allowed a person to have the "parts" of the antigen to which the serum did not contain antibodies, and to lack those "parts" to which it did. A number of examples of this sort of blood have been described, and have been studied by Dr. P. Tippett in London, and by Wiener and his collaborators in New York (Tippett and Sanger, 1962; Wiener *et al.*, 1957b; Unger and Wiener, 1959; Wiener and Unger, 1959; Unger *et al.*, 1959; and see Wiener and Gordon, 1967). The reactions of people with $\mathbf{Rh_0}$ (D)-like receptors on their cells, and an anti-$\mathbf{Rh_0}$ (D)-like antibody in serum, turn out to be different in different cases. Tippett and Sanger (1962) could classify the different forms of D antigen they studied into six categories. Detailed descriptions may be found in Race and Sanger (1975). Wiener enlarged his nomenclature to accommodate the several forms, which were regarded as further factors belonging to the Rh_0 agglutinogen. These were designated $\mathbf{Rh^A}$, $\mathbf{Rh^B}$, $\mathbf{Rh^C}$, etc. A person of type Rh_1rh who could be shown to lack $\mathbf{Rh^A}$ was designated $Rh_1^?rh$. If the $\mathbf{Rh_0}$ factor is weakly reacting (D^u) as well, the Germanic R indicates this in the symbol, e.g. a woman in the studies of Unger and Wiener (1959) was classified $ℜh_2^?rh$, indicating that her cells had a weak $\mathbf{Rh_0}$ and lacked the $\mathbf{Rh^C}$ factor associated with Rh_0.

Alter *et al.* (1962 and 1967) reported an antibody in the blood of a mother detecting an antigen which was called Go^a (Gonzales). Further studies by a number of workers indicated that Go^a is associated with Rh. Anti-Go^a apparently reacts with a part of D, the part that is missing in Tippett and Sanger's Category IV. The antigen has also been called D^{cor}, and is somewhat more common in Blacks than in Western European Caucasians.

22.5.2 Variations in C or rh′

In 1946, Callender and Race found a number of antibodies in the serum of a multiply transfused woman, one of which was an Rh agglutinin. The antibody was first called "anti-Willis", and later, anti-C^w. It was detecting an antigen C^w, which was present in the cells of one of the donors to the patient. The antigen was regarded as being due to an allele C^w at the C/c locus. Wiener calls the antibody anti-$\mathbf{rh^w}$ (or anti-$\mathbf{rh^{w1}}$). The common alignment C^wDe is

designated R^{1w} by Wiener, and the less common alignments (C^wde, C^wDE and C^wdE are designated r'^w, R^{2w} and r^{yw}, respectively. Anti-C^w is more common than it once was, and some laboratories use it in their routine testing procedures. Many anti-C contain at least some anti-C^w and are designated anti-CC^w by Race and Sanger (1975). Frequencies of C^w in different populations vary from less than 1% to as high as 9%. C^w (r'^w) is inherited. Wiener *et al.* (1957a) studied a family in which it was segregating.

In 1954, Stratton and Renton, investigating a case of hemolytic disease of the newborn, found an antibody in the mother which reacted with the cells of her husband and baby. Tested with a selection of anti-C reagents, the cells gave variable results. The antigen was named C^x and the antibody anti-C^x. Prokop and Uhlenbruck (1969) said that a number of anti-C contain anti-C^x. C^x is quite rare in Caucasians of Western European origin.

Race *et al.* (1948c and 1948d) reported what appeared to be two new antigens related to C/c and attributed at the time to alleles of C/c which were called C^u and c^v. C^u was a variably reacting kind of C, and more was said about it by Race and Sanger (1951). It turned out later that c^v reactions represented a kind of "position effect", that is, the anti-c reactions which characterized c^v were, in fact, characteristic of CDE/cde cells, and Race *et al.* (1960) said that the symbol c^v was not needed.

Huestis *et al.* (1964) described a serum from an R_1R_1 donor which had an anti-**hr**′ (c) reacting with most cells containing **hr**′. Occasionally, though, cells could be found which reacted with all anti-**hr**′ except this one. This antiserum was assigned the number 26 in the numerical nomenclature system (see in Section 22.6.2), and the phenotype it identified would be called Rh: w4, w6, -26, meaning that the cells react weakly with anti-c and anti-f (see below), but not with anti-Rh 26.

22.5.3 Variations in E or rh′′ and e or hr′′

Greenwalt and Sanger (1955) found an antibody detecting an antigen which was called E^w. The anti-E^w had caused hemolytic disease in one family. E^w cells react with anti-E^w and with selected examples of anti-E. The antigen is apparently quite rare. In Wiener nomenclature, it is designated rh^{w2}. A family in which E^w was segregating was reported by Henke and Kasulke (1976).

Another form of E, called E^u, which reacted weakly and variably with strong anti-E reagents, was described by Ceppellini *et al.* in 1950.

In 1955, DeNatale *et al.* described an Rh antigen which was relatively common in Blacks and relatively rare in Whites, and called it V. A further antibody, anti-VS (from a Mrs. VS) was described by Sanger *et al.* (1960). It reacted with cells having V, and the characteristic r'^s, both of which are more common in Black than in White people. The r'^s gives rise to weak or negative reactions with anti-C reagents whose main component is anti-Ce (see further below). The anti-VS appears to detect the product of an allele of e called e^s. The anti-V detects e^s as well as a "complex" antigen ce^s in the r'^s type (see below). Shapiro (1964) studied three examples of anti-VS, and one of them did not react with bloods that were V+. Such cells are said to have $\mathbf{hr}^v$ in Wiener nomenclature, which Shapiro uses. These results indicated that "anti-VS" might sometimes be anti-$\mathbf{hr}^v$ and anti-$\mathbf{hr}^H$. In the Bantu of South Africa, Shapiro found that $\mathbf{hr}^v$ and $\mathbf{hr}^H$ occurred with different frequencies.

In 1960, Shapiro found in the serum of a Mrs. Shabalala two antibodies, one of which was called anti-$\mathbf{hr}^s$. The anti-$\mathbf{hr}^s$ reacted with all bloods from Caucasians tested, and all but a few Bantu bloods. The antibody reacts generally like anti-**hr**′′, except in the unusual cases where it gives negative reactions with cells that are otherwise **hr**′′ positive. The exceptional bloods were denoted $\hat{R}h_o$ phenotypically, the characteristic being attributed to an allele denoted $\hat{R}^o$. Shapiro said that most anti-**hr**′′ contain anti-$\mathbf{hr}^s$. It is unfortunate and accidental that $\mathbf{hr}^s$ and e^s designations appeared, and they should not be confused for they are not the same thing.

In 1972, Shapiro *et al.* reported another antibody from the mother of a child with hemolytic disease, detecting a different hr′′-associated factor called $\mathbf{hr}^B$. The antiserum detects what would be regarded by the British workers as an e variant, more common in Black than in White people. Shapiro denotes the phenotypes of cells that fail to react with anti-$\mathbf{hr}^B$ by a dot above the R in Rh, e.g., $\dot{R}h^N$, $\dot{R}h_o$, $\dot{R}h_2rh$, etc. Rosenfield *et al.* (1973) assigned the number Rh 31 to $\mathbf{hr}^B$. The original serum of Mrs. Bastiaan contained an additional antibody which could be absorbed out with R_2R_2 cells. The "total immune response" of Mrs. Bastiaan (the antiserum before absorption) is assigned the number Rh 34 in the numerical designations (see below).

22.5.4 Compound or complex antigens and/or antisera

The terms "compound" and "complex" are used in describing these antigens, because they appear to result from some sort of combined interaction of more than one of the "basic" antigens in the present conception of the Rh system.

In 1953, an antibody was found in the serum of a much transfused hemophiliac man which reacted with bloods having cde, cDe and cD^ue composition (Rosenfield *et al.*, 1953b). The original thinking seemed to be that the antiserum might be detecting an antigen due to an additional closely linked locus of C/c, D/d and E/e, and it was called "anti-f". The possibility of "f" being some product of c and e when they are "cis", i.e., located on the same chromosome, was considered. Jones *et al.* (1954) describing a further example of anti-f, tended to favor the "cis-ce" explanation, but had nevertheless found a few cells that were R_1r (CDe/cde) which were not agglutinated by anti-f. Rosenfield and Haber (1958) favored the suggestion that f was ce (**hr** in the Wiener nomenclature). Although anti-f (anti-**hr**) behaves in most cases as an "anti-ce", there is undoubtedly more to it, because it reacts with cells in rare cases that it would not be expected to react with if it were simply "anti-ce".

In 1954, Race *et al.* described what they called "position effects" with regard to C and E in reactions with various anti-C and anti-E. The terms "cis" and "trans" refer to the genes being located on the same, or on opposite members of the homologous pair of chromosomes, respectively. The terms have meaning only in the Fisher closely linked allele concept of Rh. It was observed that, with selected antisera, C appeared to be inhibited when C and E were cis (as in CdE/cde or CDE/cDe), while E was less strong when they were trans (as in Cde/cdE or CDe/cDE). In 1958, Rosenfield and Haber found a serum "Ba." which detected an antigen called $\mathbf{rh_i}$, the product of R^1 and r'. The $\mathbf{rh_i}$ would be equivalent to Ce. Some anti-$\mathbf{rh'}$ (C) sera contained anti-$\mathbf{rh_i}$. Race and Sanger (1975) said that they now favor the idea that cis C and e give rise to the $\mathbf{rh_i}$ (Ce) antigen, while trans C and e do not, essentially as suggested by Rosenfield and Haber. The c reactions in the original "position effect" studies could be understood if some anti-E contained anti-CE, as has indeed proved to be the case. Examples of anti-cE have been reported as well.

Sturgeon (1960) studied the relationship between what was called anti-$\mathbf{rh'^N}$ and anti-$\mathbf{rh_i}$. The $\mathbf{rh'^N}$ was designated rh'^s (or r'^s for the gene) by Race and Sanger (described above in section 22.5.3) to avoid the racial label to which objections had been raised, and because the type is not limited to Blacks. Sturgeon found that so-called anti-$\mathbf{rh'^N}$ and anti-$\mathbf{rh_i}$ were identical. Anti-$\mathbf{rh_i}$ reacts generally with $\mathbf{rh'}$ when it is the product of R^1 and r', but not when it is the product of R^2 (or r^y). It fails to react with $\mathbf{rh'}$ in many Black and a few White people, which are then called $\mathbf{rh'^N}$. Rosenfield *et al.* (1960) reported an $\mathbf{rh'^N}$ blood in a family which had other Rh peculiarities.

In 1958, Allen and Tippett described the reactions of the cells of a Mrs. Crosby in Boston. They reacted as Rh negative with anti-D, anti-C and anti-E, but reacted with a number of "anti-CD" sera. The explanation was given that the cells possessed an antigen "G", and that "anti-CD" reagents had "anti-G" in them. Her genotype was written $r^G r$. The "anti-G" could be isolated by absorption and elution with these cells, and was used to show that all the common genes except r and r'' gave rise to G. So-called anti-CD reagents were assumed to have a G component, and could be anti-C + G, anti-D + G or anti-C+D+G. This finding could explain a number of previously puzzling cases of the immunization of mothers by their fetuses, e.g., rr mothers making apparent anti-CD in response to fetuses whose cells had D but not C (the antibody was anti-D+G), and could also explain some previously puzzling absorption results with anti-CD. Samples having D or C without G have been reported, as have samples with G which lack C and D. Levine *et al.* (1961c) reported a case of an $r^G r^G$ homozygote. The r^G appeared to condition the antigens G, e, $\mathbf{Hr_o}$, **Hr** and C^G, but not D, C, c, E, C^w, C^x, f, Ce, V or $\mathbf{hr^s}$. C^G is like C but not identical to it, and $\mathbf{Hr_o}$ and **Hr** are very high frequency antigens.

In 1953, Davidsohn *et al.* found an antibody in a Mrs. Berrens, who had given birth to a baby suffering from hemolytic disease. The antibody reacted with the cells of her husband and baby, and detected a rare antigen which was called Be^a. The factor was inherited and was segregating in Mr. Berrens' family. The factor is highly immunogenic, and Be(a+) cells caused production of anti-Be^a when injected into volunteers (Stern *et al.*, 1958). A family called Koz., studied by Ducos *et al.* (1974) demonstrated that Be^a belongs to the Rh system. Be(a+) people have an unusual cde complex, which produces weak e, c and ce (f).

In 1971, Giles *et al.* reported a new gene complex of Rh, giving rise to a hitherto undescribed antigen, and for which a specific antibody was found. The complex is of the R^o type, with a weak D and a weak E, a kind of c(D)(E), where the parentheses indicate a weak or depressed antigen (see further below). The antibody, contained in the serum "Hawd.", was designated anti-Rh 33 in the numerical system, and the antigen may also be designated as R^{oHar} after a British blood donor in whose cells it was found.

In 1960, Rosenfield *et al.* discovered two new alleles in a Black family. One of them, r'^N (or r'^s) was prevously discussed. The other, called $\bar{\bar{R}}^N$, had ordinary D, but much depressed C and e. It was, thus, a kind of (C)D(e), where the parentheses denote depressed antigen. The symbol was chosen because $\bar{\bar{R}}^o$ denotes a complex having only $\mathbf{Rh_o}$, with no $\mathbf{rh'}$-$\mathbf{hr'}$ or $\mathbf{rh''}$-$\mathbf{hr''}$ factors (–D–). This last will be discussed below. Antisera have been found which, after appropriately absorbing out other antibodies, have anti-$\bar{\bar{R}}^N$ activity. There are only a few examples of these sera. In 1971, Chown *et al.* reported a young Mennonite man who was $\bar{\bar{R}}^N R_2$. His parents were R_1R_2 and R_1R_1 and six sibs were of the parental types. Legitimacy was all but certain, and Chown *et al.* preferred mutation, probably at some control gene locus, but affecting structural gene products, as the explanation. Race and Sanger (1975) thought that not enough families with $\bar{\bar{R}}^N$ have been studied as yet to justify mutation as an explanation. The $\bar{\bar{R}}^N$ was assigned the numerical designation Rh 32 by Allen and Rosenfield in 1972. $\bar{\bar{R}}^N$ is more common in Black than in White people. In 1963, Broman *et al.* described a number of (C)D(e) Swedish people, and the characteristic was shown to be inherited. The (C)D(e) cells are not serologically identical to $\bar{\bar{R}}^N$ cells from the Black families, as indicated by testing with many anti-C and anti-E (P. Tippett, see in Race and Sanger, 1975).

Heiken (1967) has reported several people having an Rh complex with ordinary D, but with suppressed c and E, i.e., (c)D(E).

22.5.5 The LW antigen

The antibody which is now called anti-$\mathbf{Rh_o}$ (anti-D) was first described by Levine and Stetson (1939). In 1940, Landsteiner and Wiener published the studies on the rabbit immune anti-Rhesus monkey red cell serum. The rabbit immune serum appeared to have identical properties to the antibody observed by Levine and Stetson, and the human example acquired the name anti-Rh (later, anti-$\mathbf{Rh_o}$). It is

now known that the antigen in human cells detected by antisera raised against Rhesus monkey cells in rabbits or guinea pigs is not identical to the clinically significant antigen **Rh_o** or D, detected by anti-**Rh_o** of human origin. By the time this fact was recognized, however, it was too late to change the name.

The first indication that anti-Rhesus monkey red cell serum and human anti-**Rh_o** were not the same came in 1942, but was not understood at the time. Fisk and Foord (1942) observed that the cells of newborns, whether Rh+ or Rh−, as indicated by antiserum of human origin, all reacted with guinea pig immune serum against Rhesus monkey red cells. The antibody raised in guinea pigs is now known to be anti-LW. In 1952, Murray and Clark looked into the possibility of preparing anti-Rh sera in animals using heat extracts of Rh+ red cells. It had been shown that heating saline suspensions of Rh+ red cells to 50° made them "Rh−", and yielded up "Rh substance" to the medium. They were successful in preparing anti-D in guinea pigs using the heat extracts, but observed to their surprise that about half the heat extracts of Rh negative cells caused production of anti-D in the guinea pigs as well. Levine *et al.* (1961a and 1961b) were able to show that human red cells, whether Rh+ or Rh−, as well as Rhesus monkey red cells, possessed what they called a "D-like" antigen. Antibodies prepared in guinea pigs with these cells, or heat extracts of them, reacted as "anti-D-like". Levine *et al.* (1963) suggested that the "D-like" antigen be called "LW" in honor of Landsteiner and Wiener, and that the antibodies detecting it be called "anti-LW", or "anti-Rh(LW)". It turns out that the gene conditioning LW is not part of the genes conditioning the Rh complex. This matter is discussed in the next section.

22.5.6 Suppressions, deletions and modifiers

In 1950, Race *et al.* found a blood with no detectable C, c, E or e on the red cells. The person was homozygous for the deficiency, and the mother was heterozygous for it. The condition was designated -D-/-D-. A number of further examples of the phenotype have since been found. Wiener denotes such cells, missing the **rh′-hr′** and **rh″-hr″** factors, but having **Rh_o**, as $\bar{R}h_o$. People with the -D- or $\bar{R}h_o$ characteristic have more D (**Rh_o**) than people of ordinary D-containing phenotypes. There is also a rather high rate of consanguinity among the parents of these propositi. Cells have been described, too, which have C or c, and D, but which lack E/e, e.g. cD-/cD-. Such a blood is designated $\bar{R}h_o$ by Wiener (see Sachs *et al.*, 1960).

Among the rarest but most interesting findings in Rh serology has been the description of red cells which lack the Rh antigens altogether. There appears to be more than one kind of basis for the lack of Rh antigens. In 1961, Vos *et al.* found an Australian Aboriginal woman, 37 years old, with no living parents or children, who lacked Rh antigens of any kind regardless of antisera or technique used. The condition was first designated "—/—", but was later called Rh_{null}, as suggested by Ceppellini (Levine *et al.*, 1964). One explanation for the condition is that the person lacked a very common gene which codes for the precursor to all Rh substances on the cell membrane. It was, of course, of great interest to find out whether this person had the LW antigen (section 22.5.5). Levine *et al.* (1962) showed that she did not. Boettcher and Watts (1978) carried out serological studies on the Rh types of a relative of the original Rh_{null} proposita and his immediate family. He and one of his daughters are believed to be heterozygotes for Rh_{null}, and they exhibit weak expression of Rh antigens. The data were consistent with the idea that Rh_{null} represents homozygosity at a genetic locus controlling the synthesis of precursor for LW and Rh antigens. In 1964, a second example of Rh_{null} blood was found in a White American woman (Mrs. LM). Her husband was rr, and her daughter R_1r, and she therefore had at least one, and probably two normal Rh gene complexes which were unexpressed. She was LW− as well. The finding that both known Rh_{null} persons were LW− tempted the suggestion that LW antigen was some sort of basic precursor substance for the Rh antigens. In 1955, however, Cahan and Wallace (see in Race and Sanger, 1975) had found two people, both R_1r, who had a weak, but identifiable, kind of "anti-D", which turned out to be anti-LW. Both families of these LW− people have since been studied, and the results reported in Race and Sanger (1975). Although LW− people are extremely rare, they do have normal Rh antigens, and LW cannot, therefore, be a direct precursor for the Rh substances, nor can the gene responsible for LW be part of the Rh gene complex (Tippett, 1963). The third example of Rh_{null} was found in a Japanese boy (Ishimori and Hasekura, 1967), and was called —/—, or $\bar{\bar{r}}h$. A number of family members were heterozygous for the condition, and the pedigree indicated that this case was different from the other two, most readily explained by the presence of a silent allele for Rh ($\bar{r}$). Race and Sanger (1975) refer to the first kind of Rh_{null} as the "regulator" type, caused by a regulator gene, while the kind that appeared in the Japanese family is called the "amorph" type. These findings make more clear Henningsen's (1958) case, in which a ccDEE mother had a CCDee child. This was not a baby mix-up, and the grandmother of the child was apparently rr. The best explanation was that the grandmother was cde/—, the mother cDE/—, and the child CDe/—.

There can be a number of serological abnormalities associated with Rh_{null}, apart from the absence of Rh antigens. Expression of the antigens of the MNSsU system may be affected (Schmidt and Vos, 1967). There is evidence that the condition is associated with membrane abnormalities, as noted by Smith and Sinclair (1977), who used the most unfortunate designation "Rh_o" to refer to Rh_{null}, as though the nomenclature situation in this system were not already complicated enough. A number of cases of Rh_{null} are reviewed by Race and Sanger (1975), and there are not always abnormalities in the MNSsU expression. Likewise, some subjects are anemic while others are healthy. Rh_{null} is reviewed by Seidl (1967).

22.6 Inheritance of the Rh Factors and Further Nomenclature Considerations

22.6.1 Rh Inheritance

It is important to realize that the differences of opinion about the mechanism of Rh inheritance do not affect the routine use of the system at a practical level. The arguments have been over the relationship between "gene" and "antigen". At the level of testing for most routine purposes, the results are translatable from one system to the other, and most importantly, the results are the same. Translation is provided in Tables 22.1 and 22.2. The number of genotypes and phenotypes in Table 22.2 can be enlarged if tests are carried out with anti-C^w (anti-**rh**^{w}). In any one of the genotypes having the "C" of Fisher-Race, C^w can occur instead. Two examples will suffice: Suppose in line 2 of Table 22.2, the cells reacted with anti-C^w (anti-**rh**^{w}) and not with anti-C (anti-**rh**′). The name of the phenotype would then be Rh^wrh, with usual designations R^wr, R^wR_o and r'^wR_o, corresponding to genotypes $R^{1w}r$, $R^{1w}R^o$ and $R^or'^w$ or C^wDe/cde, C^wDe/cDe, and cDe/C^wde, respectively. As a second example, suppose cells reacted only with anti-**rh**^{w} (C^w), anti-**hr**′ (c) and anti-**hr**′′ (e). It would have the name rh'^wrh, the usual designation r'^wr, and the genotype r'^wr or C^wde/cde.

Fisher originally conceived of Rh as being controlled by a series of three closely linked loci, at which codominant alleles C and c, D and d, and E and e operated. Each allele was imagined to code for an antigen on the red cell. Two predictions are made by this hypothesis that have not been supported experimentally. One is that there should have been found anti-d. It appears now that anti-d does not exist, the few reports of it in the literature having been discredited. The other is that crossing over, although expected to be very rare event, should be observed on occasion. If the hypothesis is correct, d must be regarded as a silent allele. There is only one report in the literature of a possible crossover (Steinberg, 1965). The observation was made in a family in a small, culturally and genetically isolated population called the Hutterites. An R_1r father and an rr mother had eight children, of whom four were rr and three were R_1r. The 6th child born, however, was r′r (Cde/cde). The sociological circumstances of these people and the other blood groups essentially excluded illegitimacy as an explanation. Crossing over would provide an explanation for this child, but mutation cannot be excluded either. Steinberg considered a "D suppressor" as a possibility, but thought that it would have been seen more frequently in grouping thousands of these people if it were around. In view of what is now known of suppressors in the Rh system, however, Race and Sanger (1975) were inclined to think it the most likely explanation.

Wiener's conception of the system is that the Rh locus is not complex, that is, that the locus is occupied by only one "gene" at a time. The system, however, is coded for by a series of multiple alleles. The allele codes for what is termed an "agglutinogen", and this occupies the red cell surface. Agglutinogens can be thought of as complex antigens in a way, i.e., they have more than one blood group determinant specificity. The structures in which the blood group specificity resides, and to which antibodies are directed, are called blood factors. The blood factors are denoted by bold face type.

For Fisher, there was a one-to-one correspondence between "gene" and "antigen", and the genetic locus was complex. For Wiener, the Rh locus was not complex, but had multiple alleles, and the agglutinogens for which they coded were complex antigens. Neither of the systems has been able to assimilate easily the growing list of complicated new phenomena, and in some of the arguments over nomenclature, it is not clear whether the issue is notation or genetics.

It seems quite clear that an understanding of the biochemical genetics of Rh will ultimately settle the issues involved. The membrane structures responsible for Rh activity have been more resistant to study than have their counterparts in the ABO, Lewis and MNSsU systems, in part because they are apparently lipoproteins, and in part because soluble forms of them do not exist.

22.6.2 The numerical system of nomenclature

The notion of a numerical system of notation which describes the serological reactions observed with the Rh antisera used is not a new one. Murray (1944a and 1944b) proposed it as a simple means of recording reaction results. The system was simple, reflecting the state of knowledge at the time. Antisera would be assigned standard numbers, and one would then indicate by subscripts of the symbol "Rh" which ones had agglutinated the cells, e.g. Rh_4 would mean that the cells had reacted only with serum number 4. Murray's suggestions were apparently not very widely adopted.

In 1962, Rosenfield *et al.* resurrected the idea of numerical designation, taking into account a number of complexities of Rh which were by then apparent. Their major point was that a notation should give the reader the essential information about the reactions observed with the antisera without carrying implications about fundamental assumptions about the genetics of the system. The paper should be read, for it points up clearly the deficiencies of both Fisher-Race and Wiener nomenclature, and the underlying assumptions which gave rise to them. The assumptions about the relationships between mutational sites, antigenic structural groupings and specificities in either view could not be supported, it was argued, by the available immunochemical data. The term "agglutinogen" to denote a total gene product, and "blood factor" to denote a serological specificity were regarded as undesirable, because this terminology is not used anywhere else in general immunology or immunochemistry, and because they represent assumptions about the immunochemical nature of the Rh system which have yet to be proven. Further, since the pathway between the genetic locus and the antigen may prove to be complex, a one-to-one correspondence between "gene" and "antigen", as postulated by Fisher, is not yet warranted. Twenty-one

antisera defining Rh specificities were given in the 1962 paper. By 1972, Allen and Rosenfield could list 33 Rh antisera, to which has been added the serum of Mrs. Bastiaan before absorption (see in Section 22.5.3), Rh 34 = Bas (Rosenfield *et al.*, 1973). Race and Sanger (1975) said they thought that the designation Rh 35 had been given to the product of the (C)D(e) complex detected by the FBC serum (serum 1114) of Giles and Skov (1971).

The numerical designations are given in Table 22.3, along with Wiener and Fisher-Race equivalents. There are a few simple rules for the use of the numerical nomenclature system. Antisera are simply called anti-Rh 1, anti-Rh 2, etc. A phenotype is denoted by the symbol "Rh", followed by a colon, and a list of the factors tested for, separated by commas, and preceded by a minus sign if the results were negative. Thus, the types rh (cde/cde) and R_1R_2 (CDe/cDE), if tested with the five common antisera, would be designated Rh:-1,-2,-3,4,5 and Rh:1,2,3,4,5, respectively. If an unexplained weak reaction is encountered, a lower case "w" may precede the number. Thus, (C)D(e)/cDE may be designated Rh:1,w2,3,4,w5,-6,-7,-8,-9,-10,12. Alleles are indicated by an italic capital R, with the factors determined (and not determined) by the allele shown as superscripts. Thus the allele R^{1w} (or C^wDe) would be designated $R^{1,2,-3,-4,5,8}$, and, if necessary, could be designated $R^{1,2,-3,-4,5,-6,7,8,-9,-10,12,(19),(21)}$. Factors given in parentheses mean that only a single example of the specificity has been described so far. Examination of Table 22.3 illustrates the present complexity of the Rh system, and the problems of equivalency between Fisher-Race and Wiener usage.

In 1979, Rosenfield *et al.* (1979a) transferred the task of keeping track of new numerical assignments in the Rh system to Mr. P.D. Issitt then in Cincinnati, now in Miami. Observers of "new" Rh specificities were urged to consult Mr. Issitt concerning the assignment of numbers. Some recently assigned numbers, and the antigens they represent: Rh 36 is Be^a, an antigen defined by Mrs. Berrens serum, and discussed in section 22.5.4. The informative Koz family (Ducos *et al.*, 1974) showed that Be^a belonged to Rh. Rh 37 is the antigen 'Evans', studied by Contreras *et al.* (1978). Rh 38 is 'Duclos', described by Habibi *et al.* (1978). Rh 39 has no other name, and was briefly described by Issitt *et al.* (1978) and more fully by the same authors in 1979. Rh 40 is 'Targett', first described in 1975. The family Nie allowed the assignment of 'Targett' to the Rh system (Lewis *et al.*, 1979).

The nomenclature issue, which was brought up frequently by Wiener, has been the subject of many papers. Wiener felt that his nomenclature should be adopted internationally, as had been done for the ABO system nomenclature years earlier. But efforts to bring this about always failed, and at times led to strong reactions in print. In 1957, the Committee on Medicolegal Problems of the A.M.A. reported out a set of recommendations, which included exclusive use of the Rh-Hr nomenclature. The subcommittee responsible for the recommendations consisted of Wiener and three adherents of his nomenclature. A great number of other workers objected to this report (see Allen *et al.*, 1958c). Wiener (1965b) objected to the numerical designation system as well, and Rosenfield and Kochwa (1965) answered the objections.

22.7 Biochemical Studies on the Receptors—Biochemical Genetics

22.7.1 The number of Rh antigenic sites on red cells

It has been known for quite some time, and has been demonstrated quantitatively, that the amount of Rh antibody with a particular specificity that can be bound to the cells is a function of the Rh genotype of the cell. Although there are a number of different quantitative methods for assessing the amount of antibody bound, the results that have been obtained with radioactive iodine-labelled Rh antibodies will be mentioned here.

Masouredis (1960) showed that more ^{131}I-anti-D was bound to DD than to Dd cells (about 1.6 times more) when both types of cells were also cc. Cells having a CD makeup took up less anti-D than those with a cD one as well. The data indicated that there was heterogeneity in the D antigen, and Masouredis said that the data could be regarded as accurate only for the particular antibody actually used in the experiments, since different antibody might give very different results. Kochwa and Rosenfield (1964) and Rosenfield and Kochwa (1964) carried out similar kinds of studies, and found that there were large differences in the uptake of ^{131}I-anti-D by cells of various D-containing phenotypes. The magnitude of the differences was seen to increase as the amount of available antibody increased. In 1965, Rochna and Hughes-Jones found that there were no differences in the equilibrium constant for the binding of ^{125}I-anti-D to cells of various phenotypes, indicating that there was no qualitative difference in the D receptor. The number of D sites per cell was found to be 9,900–14,600 for CcDee, 12,000–20,000 for ccDee, 14,000–16,600 for ccDEe, 14,500–19,300 for CCDee, 23,000–31,000 for CcDEe and 15,800–33,300 for ccDEE. Masouredis and Sturgeon (1965) found that the uptake of ^{131}I-anti-D by D^u cells was less than 10% of that in control cells, but that papain treatment of the D^u cells resulted in a 3-fold increase in anti-D uptake. Several techniques were used to measure anti-D uptake and the results were in accord, but the values varied according to the technique used. Hughes-Jones *et al.* (1971) measured the number of c, D and E sites with ^{125}I-labelled antibodies and found that the homozygotes always gave higher values than the heterozygotes, except that with anti-E, the results were somewhat variable. The number of D sites on -D- cells was measured, and found to be very much higher than on ordinary D cells (110,000–202,000 sites/cell). Skov and Hughes-Jones (1977) measured available C sites with radioactive anti-C, and found 45,700–56,400 for CDe/CDe, 42,200 for Cde/Cde, 25,500–39,700 for CDe/cDE, 31,100 for Cde/cde, 8,500–9,800 for CDE/cDE, 21,500–40,000 for C^wDe/cde and 7,200 for Cde^s/cde.

Table 22.3 Numerical Designations of Rh Factors and Their Equivalents *

Antigen	Wiener Equivalent	Fisher-Race Equivalent	Antigen	Wiener Equivalent		Fisher-Race Equivalent
Rh1	Rh_o	D	Rh19	hr^s		
Rh2	rh'	C	Rh20	—		VS; e^s
Rh3	rh''	E	Rh21	—		C^G
Rh4	hr'	c	Rh22	—		cis-CE
Rh5	hr''	e	Rh23	—		D^W (Wiel)
Rh6	hr	f; cis-ce	Rh24	—		E^T
Rh7	hr_i	cis-Ce	Rh25	—	LW	—
Rh8	rh^{W1}	C^W	Rh26	—	Deal	"c-like"
Rh9	rh^x	C^x	Rh27	—		cis-cE
Rh10	hr^v	V; ce^s	Rh28	hr^H		—
Rh11	rh^{W2}	E^W	Rh29	—	"total Rh"	—
Rh12	rh^G	G	Rh30	Rh_o^{Cor}	Go^a	D^{Cor}
Rh13	Rh^A	—	Rh31	hr^B		
Rh14	Rh^B	—	Rh32	Product of $\frac{=}{R}N$		
Rh15	Rh^C	—	Rh33		R+Har	
Rh16	Rh^D	—	Rh34		Bas.	
Rh17	Hr_s	—	Rh35		FBC	(C)D(e)
Rh18	Hr	—				

* Designations written in between the "equivalent" columns do not belong to one or the other of the two older systems

22.7.2 Relationship of the Rh antigens to the erythrocyte membrane

Nicolson *et al.* (1971) used a technique for isolating RBC membrane surfaces from D+ cells to which had been bound anti-D and ferritin-labelled AHG. The preparations were studied in the electron microscope, and the number of sites on the membrane, as judged from the electron micrographs, correlated well with the estimates based on ^{125}I-labelled anti-D (which was used throughout the experiments). The electron micrographs also showed that the receptor sites were dispersed randomly in a two dimensional array. The evidence was further consistent with a generalized model of biological membrane structure called the fluid mosaic model (Singer and Nicolson, 1972). This model supposes that the membrane consists of various globular proteins embedded in a matrix of phospholipid. The phospholipid material is arranged as bilayer, and the globular proteins are arranged so that their polar groups are directed outward on either surface toward the aqueous exterior of the membrane, with apolar, hydrophobic groups buried in the interior of the membrane. The entire structure is fluid, analogous to a two-dimensionally oriented solution of integral proteins in a viscous phospholipid bilayer solvent. Singer and Nicolson (1972) discussed the evidence favoring their model. It may be necessary to think about the mechanism of proteolytic enzyme modification of cells, and the agglutination of protease-modified cells by incomplete antibodies, in terms of this membrane model. Knox (1977) carried out a re-analysis of the data of Nicolson *et al.* (1971) and said that it showed that the arrangement of antigen sites was orderly, rather than random.

Masouredis *et al.* (1977) carried out ^{125}I-labelled anti-D binding studies and electron microscope studies on ferritin-labelled anti-D sites with normal and protease modified D+ cells. Papain and neuraminidase treatment of cells brought about significant increases in the quantity of ferritin-anti-IgG bound to anti-D, although only papain induces agglutination of the cells by the IgG anti-D. The findings were interpreted to mean that the role of the proteolytic enzyme treatment in the enhancement of agglutination might have to do with alterations that are brought about in the biophysical structure of the membrane. The detailed studies of Margni *et al.* (1977) on the mechanism of agglutination of trypsin treated Rh+ cells by incomplete antibodies (and immunochemical fragments derived therefrom) do not appear to be inconsistent with the thinking of Singer and Nicolson, although they were not discussed in these terms. Morley (1978) conducted a study of the binding characteristics of two different anti-D antibodies to a series of cells of different phenotype. A positive correlation between site density and association constant of the antibody was demonstrated. It was suggested that there might be two "types" of antigenic sites on the cell-surface, recognizable only by affinity differences in the antibody. A higher affinity site would be capable of binding both arms of an IgG, whereas a low affinity site would bind only one arm.

22.7.3 Isolation and purification of the Rh antigens

Efforts to obtain "pure" preparations of Rh antigens, or substances, have not been nearly as successful as those directed toward ABH, Lewis or MNSs substances. The ABH and Lewis work was made easier by the existence of the secreted, soluble glycoproteins, and glycoproteins have generally been easier subjects for detailed biochemical study than have lipoproteins. Membrane-resident proteins are notoriously intractable in terms of their isolation in "pure" form with retention of the biological activity.

The earlier efforts reflect attempts to reason by analogy from what was being learned about ABH, Lewis and MNSs substances. There are, in effect, two approaches to the study of the nature of the antigenic determinant. In the direct approach, the biologically active molecule or substance is isolated and characterized. An indirect approach, which paid handsome dividends in the studies on ABH and Lewis substances (Section 19.9.2), was the use of inhibitor substances thought to be structurally related to the antigen. A small molecule which inhibits the binding of antibody to antigen may be doing so, the reasoning goes, because of its structural similarity to the immunodeterminant which thereby allows competition for the antibody binding site.

A number of studies were done using the inhibition approach, some of them with sugars or sialic acid because of the roles these appeared to have in the immunodominant end group structures of ABH, Lewis and MN substances. In 1958, Hackel *et al.* looked at the inhibition of agglutination by anti-Rh and anti-Lu sera by a large number of compounds (60 in all) representing various classes of biologically active molecules. Specific inhibition of the anti-C, anti-D, anti-E, anti-Lu^a and anti-Lu^b reactions was obtained with four compounds derived from RNA: cytidine sulfate, and adenylic, cytidylic and uridylic acids. These findings were interpreted to mean that the antigenic structures of the Rh and Lu determinants bore at least some resemblance to these nucleotides, a conviction that was further strengthened by the finding that the anti-c and anti-e reactions were also inhibited (Hackel and Spolyar, 1960) and that ribonuclease treated red cells showed significant decreases in titration scores with anti-Rh and anti-Lu antisera (Hackel and Smolker, 1960). Hackel extended his studies in 1964. Boyd *et al.* (1959) could not at first confirm Hackel's findings, but said later in a personal communication to Hackel that they had observed the effect. Boyd *et al.* (1959) got weak inhibition of Rh reactions with L-glucose, L-mannose and D-glucose, and speculated that L-sugars or less common D-sugars might be involved in the immunodeterminant structure. Chattoraj and Boyd (1965) found some inhibition with amino acids. In 1960, Dodd *et al.* reported inhibition of the anti-D reaction by crude and pure preparations of N-Ac-neuraminic acid. A sialic acid-containing brain ganglioside and a polysaccharide from a *Pseudomonas* showed inhibition as well. These studies were extended using other sialic acid compounds and a purified ganglioside (Dodd *et al.*, 1964). Johnson and McCluer (1961) were unable to confirm the NANA inhibition, using pure or crude preparations, and said further that neuraminidase treatment of D cells made them more, not less, agglutinable by anti-D. Prager and Lowry (1966) showed that enzymatic desialidation of D red cell membranes did not significantly decrease the activity with anti-D. Rule and Boyd (1964) saw some NANA inhibition of the anti-D reaction. Prager *et al.* (1963) observed weak inhibition of the anti-D reaction with cytidine sulfate and cytidylic acid, but other organic phosphates were found to be more potent inhibitors, α-tocopherol-phosphate being the strongest. These effects were considered to be nonspecific since the compounds did not affect the Coombs test, and acid and alkaline phosphatase, RNase and phosphodiesterase treatment of cells had no effect on Rh agglutination.

In 1950, Moskowitz *et al.* reported the preparation of a complicated fraction from red cell membranes which contained the ABH and Rh activity of the cells from which it was derived. The fraction was called "elinin". Several other fractions derived from membrane stroma contained no blood group substance activity (Moskowitz and Calvin, 1952). Wolf and Springer (1965) did not like the name "elinin", and called the fraction with blood group activity "AF" (active fraction). It was heat labile, sensitive to ethanol, inhibited by pH very far from 8, and inactivated by periodate and iodoacetamide, implicating carbohydrate and sulfhydryl groups as part of the immuno-active material. Proteases did not abolish the activity, and the fraction reacted with *Vicia graminea* lectin.

Various procedures involving butanol extraction of red cell stroma did not yield fractions possessing Rh activity (Rega *et al.*, 1967; Poulik and Lauf, 1965 and 1969). Green (1965) found that lyophilized red cell stroma derived from C or D containing cells had antigenic activity (would take up anti-C or anti-D). Reactivity was temperature and pH dependent, inhibited by high concentrations of sulfhydryl reagents, and abolished by $-SH$ binding reagents. High concentrations of sulfhydryl reagents afforded some protection against the abolition of activity by $-SH$ reagents. In 1967, Green showed that lyophilized membranes that had been solubilized and disaggregated by anionic detergents and SDS had lost detectable Rh activity, even following the removal of the detergent on an ion exchange resin. In 1968, Green showed that a butanol extractable component was required for Rh activity of membrane preparations, and that Rh antigenic activity could be reconstituted in butanol extracted membranes by adding back the contents of the butanol extract. Chloroform-methanol extracts would also restore activity to butanol extracted membranes. The extractable component, required for C or D activity was found to be a lecithin (phosphatidyl choline), and it was thought to be associated with a sulfhydryl protein in the membrane (Green, 1968a and 1968b). These findings helped to explain the earlier studies in which activity had not been found in solvent extracts of membranes. The evidence also suggested that the lecithin had to contain at least one unsaturated fatty acid residue. In 1972, Green showed that reconstitution of Rh antigenic activity to butanol extracted membrane fractions was correlated with the binding of lecithin to the membrane material. The evidence suggested that the phospholipid was associated with the membrane protein by hydrophobic forces.

In 1968, Weicker reported that he had obtained a small peptide, with a MW between 6000 and 12,000, by an exhaustive dialysis procedure, followed by gel filtration chromatography, which had D-activity as assessed by hemagglutination inhibition. The peptide contained 12 different amino acids, including Cys. With a refined purification pro-

cedure, Weicker and Metz (1971) isolated a peptide with MW about 10,000, with a small amount of bound phospholipid, and containing 14 amino acids. The antigenic activity was assessed by an anaphylactic shock procedure using isolated muscle, and was said to be established specifically as D-activity (Weicker and Roelke, 1971). Hemagglutination inhibition and gel precipitation were not thought to be satisfactory methods for detecting antigenic activity because the antigen was small and the freeze drying procedure affected reactivity. Jackson *et al.* (1972) could not confirm Weicker's findings using hemagglutination inhibition of the anti-D reaction with bromelin treated D cells as an assay procedure.

Loruso and Green (1975) reported obtaining D-active membrane fractions by solubilization with deoxycholate, followed by removal of the detergent and slow addition of Mg^{++} by dialysis. The "reaggregated vesicles" had D activity. In 1977, Loruso *et al.* extended these studies, using sodium dodecylsulfate in place of deoxycholate, and showing that Ca^{++} as well as Mg^{++} worked in the reconstitution stage. D activity was separable from A activity and did not reside on glycoprotein 1.

Beginning in 1974, Abraham and Bakerman have reported the solubilization and partial purification of Rh antigens D, C, E, c and e. The procedure was similar in each case. Hemoglobin free membranes (Limber *et al.*, 1970) were solubilized using EDTA and then NaCl solutions. The material was fractionated on ultrafilter membranes and further purified by isoelectric focusing in pH gradients. Immunological activity was tested by hemagglutination inhibition and by formation of specific antibodies after immunization of animals with the preparations. All the purified antigens migrated as single bands on disc electrophoresis. Antigen c had a MW in the 20,000–30,000 range (Abraham and Bakerman, 1974), antigen C in the 50,000–100,000 range (Abraham and Bakerman, 1975a), antigen D in the 10,000–20,000 range (Abraham and Bakerman, 1975b), antigen E in the 50,000–100,000 range (Abraham and Bakerman, 1976) and antigen e in the 20,000–30,000 range (Abraham and Bakerman, 1977), as judged by the exclusion limits of the ultrafilters. More recent studies on the isolated D antigen have been carried out to test the effect of various enzymes on the preparation (Litten *et al.*, 1978). A modification of the isolation procedure was introduced, and activity was measured by the ability of the preparation to inhibit hemagglutination. Because of the findings implicating phospholipid in the antigenic structure, the preparation was treated with phospholipases A and C, alkaline phosphatase, neuraminidase, leucine aminopeptidase and carboxypeptidases A and B. None of these affected hemagglutination inhibition, however. Activity was abolished by pronase, trypsin, chymotrypsin and papain, suggesting that protein was most important in the antigenic determinant structure in the solubilized preparation. Lipid molecules may play a role in maintaining the receptor in its structural conformation in the membrane, but is apparently not required for activity in the isolated preparation.

Plapp *et al.* (1979) partially purified D antigen by affinity chromatography of sodium deoxycholate-solubilized membrane material. Similar quantities of active material were obtained from Rh positive and Rh negative cells. This intriguing result suggests that Rh negative people make D antigen, but that it is somehow not exposed to the external cell surface. The authors said that their results would explain why neither d nor anti-d has ever been found. Interestingly too, the D antigen fraction contained the LW antigen. Toma *et al.* (1979) obtained what appear to be related results, using wholly different techniques. They used ^{99}Tc-pyrophosphate-labelled anti-D to label D^+, D^u and D^- cells, and observed binding in all cases. They said that d was not an allele of D, but rather the weakest in the antigen series $D > D^u > d$. They also employed uranyl-labelled anti-D for immunoelectron microscopy, and said that anti-D was bound to Rh negative red cell membranes. It seems reasonable to suppose that the results of Plapp *et al.* (1979) could provide a basis for the observations made by Toma *et al.* (1979).

Folkerd *et al.* (1977) estimated the *in situ* MW of D antigen as $174{,}000 \pm 10{,}000$ using an indirect radiation bombardment procedure.

22.7.4 Biochemical genetics of Rh

Before a detailed understanding of the biochemical genetics of Rh can be attained, more will have to be known about the exact nature of the antigens, and its relationship to red cell membrane structure. Knowledge of the antigen structure will enable a fuller investigation of the nature of the Rh gene products. Since there is not very much detailed information about the immunochemical structures involved, biochemical genetic schemes proposed for Rh are speculative. A number of models have been put forth, having to do with the order of activity of the genes in an Rh antigen synthetic pathway, with gene structure itself, and with structural complexity for both antigens and antibodies. Some references are Nijenhuis (1961), Lauer (1964), Boettcher (1964), Knox (1966), Edwards (1968, Hirschfeld (1973) and Rosenfield *et al.* (1973). The last mentioned is an effort to explain all the available serological and genetic data with a kind of Jacob-Monod operon model, which in certain up to date ways, retains some of Fisher's original notions. Some of the current thinking about Rh has recently been reviewed by Stroup (1977).

22.8 Medicolegal Applications of Rh

The Rh system has been in use for a long time in cases of disputed parentage. Wiener (1944a) mentioned that he was using Rh typing in some of his cases. The chances of excluding a true nonfather using Rh are better than for most other systems taken individually. Wiener (1950) reported on a large series of cases from New York, and Silveira (1967) reported on a case in which an exclusion was obtained in the Rh system, genotypes having been deduced from family studies.

The chances of excluding a falsely accused father with the Rh system, using the five common antisera, are about 18% for Blacks and about 27% for Whites (Chakraborty *et al.*, 1974; AABB, 1978). Ordinarily, Rh genotype cannot be determined from the phenotypes which represent multiple genotypes without family studies. Sometimes, special antisera (e.g. anti-ce, anti-Ce) can help to distinguish different genotypes within phenotypes. For example, anti-ce (anti-f) could distinguish between CDE/cde and CDe/cDE. The investigator must be aware of the complexities of the Rh system (section 22.5) in interpreting results in paternity cases, even though many of them are very rare. Tippett, in AABB (1978), has discussed the pitfalls in the use of the Rh system in parentage cases.

Rh typing in bloodstains did not come up in the literature until the late 1940's, and determination of Rh antigens in bloodstains on anything like a routine basis is still largely restricted to a few specialized laboratories.

In 1949, Closon carried out experiments on the detection of the "Rh antigen" (presumably $\mathbf{Rh_0}$ or D) in dried blood using an inhibition technique. The amounts of dried blood that were needed were large by present day standards, 100 mg being used with 0.6 mℓ antisera for the observation of unequivocal inhibition by Rh^+ dried blood. 100 mg dried blood corresponded to the residue from about 0.4 mℓ whole blood. Good results were obtained in dried blood up to 15 days old, but in samples older than this, activity was greatly diminished. Ruffié and Ducos refined the technique for the determination of Rh antigens in dried blood to a high degree. An inhibition method, modified from that of Holzer, was employed. Detection of C, D and E in bloodstains was reported in 1952, these being reliably detectable in dried blood up to 4 days old. In 1953 and 1954, c and C^w antigens were added to the list (Ruffié and Ducos, 1953; Ducos and Ruffié, 1954). Fairly large amounts of dried blood were needed for the tests, and Rh antigens were undectable in stains that were very old. Detailed methods were given, and the importance of selecting antisera of known specificity was stressed. Apparently, it was difficult to get anti-C which had no anti-D in it.

Ducos (1958) reviewed the status of antigen grouping in dried blood, and included the results of a survey of 27 laboratories in 18 countries, which had asked which antigens were grouped routinely. Two did D routinely, and four others on occasion, two did C and E routinely and one on occasion, and only one lab did C^w and c routinely.

The next step was taken when the elution method of Kind was introduced in 1960. The elution method is probably employed almost universally for Rh at present. In 1962, Nickolls and Pereira reported that the D antigen could be reliably determined in bloodstains by elution. In 1967, Bargagna and Pereira reported in detail on the determination of D, C, E, c, e and C^w in dried bloodstains by the elution method. Absorption was carried out at 37°, as was the washing step. Studies were done with saline agglutinating antisera as well as with incomplete antisera, using enzyme or AHG techniques. Both complete and incomplete antisera had certain advantages. Fixation of the stained material was not only unnecessary but deleterious to some of the antigens. There were some problems with anti-c, but properly diluted antisera always gave specific reactions by the AHG test. Some incomplete anti-e sera failed to react with e stains. The elution technique was believed to be very suitable for the Rh antigens in dried bloodstains, though it was said that some slight refinements were still needed. In 1968, Lincoln and Dodd put forth an elution procedure suitable for C, C^w, D, c, E and e (as well as S) (Lincoln and Dodd, 1968b). Certain antigens were detectable longer than others in experimental bloodstains, D, C, C^w and c being stable for 6 months, while E and e were stable for 4–6 months. Successful typing of still older stains has subsequently been reported (e.g. McDowall *et al.*, 1978b). Papain treated cells were used for the detection of all the antigens. It could be shown that the spurious anti-c reactions seen by Bargagna and Pereira did sometimes occur with bromelin-treated cells, but they were not observed if enzyme technique was carried out with papain. The most serious problem was the availability of suitable antisera. Reagents specific and suitable for cell grouping are not necessarily specific or suitable for stain typing by absorption-elution. Suitable anti-C were difficult to obtain. These sera often contain anti-D and anti-G. Depending upon the characteristics of the separate antibodies, the anti-D problem may be gotten around, but the anti-G problem is much more difficult. Anti-C reagents must be carefully tested with R_2R_2 or R_2r cells to insure specificity. Only a few suitable anti-E sera could be found. The careful selection and evaluation of specific and potent antisera was stressed. The matter was discussed in section 19.10.3.4. Goryanina (1973) discussed the selection of antisera for $\mathbf{Rh_0}$ determination in stains by absorption-elution. Denault *et al.* (1978) tested the survival of the Rh antigens in bloodstains on a number of substrata, using the elution procedure. D was detectable up to 26 weeks, but e up to only about two weeks, regardless of substratum or humidity. The antigens C, c and E were adversely affected by high humidity. Indications were that C was somewhat more stable at 20% relative humidity than were c or E. Antigens E and e could be missed in stains, and false positive C, c and E reactions were occasionally observed.

Martin (1977) presented a technique for determination of the Rh antigens C, c, D, E, e and C^w which required only 1 cm long stained threads. The technique consisted of affixing threads to a polycarbonate sheet for the absorption and washing stages, and then transferring them to tubes for elution and detection of the eluted antibodies. This represented an improvement over the older techniques which generally required larger amounts of material. Incomplete antisera were used, and papain-treated red cells were employed for detection of eluted antibody. Elution of Rh antibodies cannot be carried out directly into test cells, because the higher temperatures have a deleterious effect on the cellular antigens.

Autoanalyzer techniques have been applied to bloodstain grouping with successful results. The autoanalyzer tech-

niques were introduced in the 1960's, and are very helpful in large scale typing and screening of cells and of sera. There are many technical problems associated with blood grouping by autoanalyzer. Details will not be discussed here. A few references to autoanalyzer procedures are Rosenfield and Haber (1965), Sturgeon *et al.* (1965), Morton and Pickles (1965) and Kliman and Smith (1966). At the simplest level, the autoanalyzer serves as a device for mixing red cells with antisera and other necessary reagents, such as colloids, enzyme solutions, etc., and has a detection device for telling whether agglutination has occurred. Autoanalyzers are used for bloodstain analysis because they can detect very small amounts of antibody.

Douglas and Stavely (1969) reported successful results in grouping artificial bloodstains for D and c by autoanalyzer. In bloodstain grouping, absorption and elution are generally carried out manually, but the eluate is processed in the autoanalyzer. In 1970, Pereira (see in Culliford, 1971) described in some detail the autoanalyzer technique being used at the time for the Rh antigens. An advantage to the autoanalyzer, apart from the time saved and the objectivity introduced in the evaluation of agglutination results, was that D^u would ordinarily be missed if only a saline agglutinating anti-D were used in the test, and it is usually necessary to do an AHG test to check for the presence of D^u. The high sensitivity of the autoanalyzer in detecting antibodies is achieved by introducing colloidal media into the system which brings about rouleaux formation and allows for more efficient agglutination. The rouleaux are then dispersed by addition of saline, and the agglutinates remain. Lincoln (1973) carried out some collaborative experiments with Pereira, comparing the strength of the antigen-antibody reaction as measured by manual titration of the eluates with the peak area seen in the autoanalyzer for the same samples. The correlation between the results of the two methods was very close indeed. Refinements in autoanalyzer techniques for determination of the Rh antigens in stains, based on the use of low ionic strength media (see below), and so forth, have been discussed by Martin *et al.* (1975) and by Brewer *et al.* (1976). McDowall *et al.* (1978b) recently compared manual and autoanalyzer techniques for detecting antibodies eluted from bloodstains. Anti-D, anti-C, anti-E and anti-c were used in the experiments. The autoanalyzer system was a low ionic strength polybrene (LISP) system, described in Boorman *et al.* (1979). The autoanalyzer did not give significantly more sensitive detection than the manual technique. Furthermore, with stains older than about 4 weeks, the manual detection procedure actually gave more conclusive results. It is to be noted, however, that the manual procedure uses papain treated test cells whereas this particular autoanalyzer system does not.

Akaishi (1965) reported that $\mathbf{Rh_0}$ (D) could be grouped in stains by mixed agglutination if bromelin treated test cells were employed. Hasebe (1962a) said that D could be detected in red cell smears by the fluorescent antibody technique.

The Rh antigens do not survive aging in dried bloodstains as well as the ABH antigens generally speaking, but can often be determined in stains that are a number of months old depending upon the antisera, the technique, and the condition of the stain. The rate of disappearance of the different Rh antigens in dried blood is not necessarily the same, probably due in part to the different densities of sites on the red cells to begin with (Section 22.7.1). The interpretation of negative results is, therefore, something of a problem.

McDowall *et al.* (1978a) have recently shown that the enhancement of red cell antigen-antibody reactions by low ionic strength media can be productively exploited in bloodstain grouping. In 1964, Hughes-Jones *et al.* looked at the effect of several parameters, known to influence the equilibrium constant for antigen-antibody reactions, on the reaction between anti-D and D cells (Hughes-Jones *et al.*, 1964a). Using ^{131}I-labelled anti-D, it could be shown that the association constant was increased 1000-fold by reducing the ionic strength from 0.17 to 0.03. Atchley *et al.* (1964) obtained similar results using low ionic strength media made up with glycine or a number of nonelectrolytes such as sucrose. This effect could be shown to apply to a variety of antibodies, and is clearly a way of increasing the sensitivity of methods of detection of blood group antibodies. Low ionic strength solutions were useful in enhancing the titer of a number of antibodies if used in the first stage of an AHG test (Hughes-Jones *et al.*, 1964b; Elliot *et al.*, 1964). The effect was more pronounced with antibodies for factors other than ABO or Lewis, although there were exceptions. Low ionic strength media also leads, in some cases, to the nonspecific uptake of antibody. In 1974, Löw and Messeter indicated that a low ionic strength solution (LISS) consisting of 0.03M NaCl containing 0.24M sodium glycinate and 5 mM phosphate buffer, pH 6.7, gave the enhancement effect while virtually eliminating the nonspecific uptake of antibody. One of the main applications of LISS was to the first stage of the AHG test. By increasing both the rate and the amount of antibody uptake, the incubation times were shortened and antibodies present in small amounts, or those of low avidity, were more easily and quickly detected. The results were confirmed by Moore and Mollison (1976). The value of low ionic strength media in detecting antibodies, and the many variations in procedure, have now been studied extensively (Rosenfield *et al.*, 1979b; Jorgensen *et al.*, 1979a and 1979b; Fitzsimmons and Morel, 1979). Lincoln and Dodd (1978) demonstrated the applicability of LISS technique, in combination with the use of enzyme treated cells, to the detection of small amounts of blood group antibodies, and showed the technique to be especially useful for eluted antibodies. McDowell *et al.* (1978a) applied the technique to bloodstain grouping. The sensitivity of the elution technique of Lincoln and Dodd (1973) was increased markedly by the LISS-enzyme treated cell method of detecting Rh antigens and S. As with other serological procedures, the selection of appropriate antisera is an essential ingredient in obtaining good results.

The issue of interpretation of the results of Rh grouping

in bloodstains was raised recently by Martin (1977), although it is neither a new issue nor necessarily one which is restricted to the Rhesus system. The question which arises is two-fold: First, should negative results with a bloodstain be interpreted to mean that the antigen is in fact absent from the stain being examined, and second, should the results be reported in a way that states or implies a genotype or most probable genotype. The two are related, of course, and if one does not interpret negative findings to mean that the antigen is absent, the second part of the question never comes up. There are differences of opinion about the interpretation of negative results. As to the way results are reported, however, there is some agreement that only the results of the reactions with the antisera used in the tests should be reported. Thus in cases where a phenotype can be the result of a number of genotypes, no genotypical implications would be given in the report, according to this way of thinking. One can never give more than a *probable* genotype, if the phenotype contains more than one genotype without family studies in any case, even if fresh cells have been grouped. In cases where a phenotype is the result of a unique genotype, e.g. C^w-, C−,D−,E−,c+,e+, it would be up to the individual to decide whether this bloodstain was in fact rr or not. The possibility that a weak antigen was present (e.g. D^u) in the blood, or that one of the antigens originally present in the stain was not detected because of the age or condition of the stain, should not be overlooked. There is considerable value in reporting the positive and negative reactions because it is possible, then, to select informative results, and leave aside those results which would be meaningless or confusing, or which are unsatisfactory. An example would be the reporting of a D+ in a stain, but a D− result in the red cells of a suspect. This result is exclusionary, regardless of the results obtained with other antigens.

22.9 Frequency of Rh Phenotypes in U.S. Populations

Frequencies for Rh phenotypes in some U.S. populations are shown in Table 22.4. Phenotypes have been designated in Wiener nomenclature, as in column 1 of Table 22.2, and studies where only one or a few antigens were studied (e.g. D+ and D−) have been omitted.

Table 22.4 Rh Phenotypes in U.S. Populations

Population	Total	Frequency — Number (Percent) Rh_o	Rh_1rh	Rh_1Rh_1	Rh_2rh	Rh_2Rh_2	Rh_1Rh_2*	rh	Rare Phenotypes See Note:	Reference
CAUCASIAN										
New York, NY	2,390	69 (2.88)	798 (33.39)	489 (20.46)		349 (14.6)☆	330 (13.8)	321 (13.43)	1	Wiener and Gordon, 1951
University of Iowa	2,181	91 (4.17)	723 (33.15)	382 (17.51)	319 (14.63)		303 (13.89)	338 (15.5)	2	Buckwalter et al., 1962
Southeastern GA	331	9 (2.7)	112 (33.8)	65 (19.6)	31 (9.4)	13 (3.9)	44 (13.3)	46 (13.9)	3	Cooper et al., 1963
San Francisco, CA Bay Area Mothers Children	4,928	(2.68) (2.29)	(34.66) (34.80)	(19.66) (20.41)	(11.75) (11.85)	– –	(13.62) (12.56)	(13.62) (13.80)		Reed, 1967
San Francisco, CA Bay Area All Caucasians "Western European" Caucasians	8,982 5,056	206 (2.3) 99 (2.0)	3,086 (34.4) 1,746 (34.6)	1,728 (19.3) 912 (18.0)	1,033 (11.5) 594 (11.7)	212 (2.4) 109 (2.2)	1,169 (13.3) 661 (13.1)	1,365 (15.2) 853 (16.8)		Reed, 1968
New York, NY	500	11 (2.2)	162 (32.4)	111 (22.2)	47 (9.4)	9 (1.8)	80 (16.0)	60 (12.0)	4	Wiener, 1969
South Central WV	1,412	(2.1)	(33.4)	(17.4)	(12.8)	(3.9)	(14.4)	(14.3)	5	Juberg, 1970
Tecumseh, MI	8,963	188 (2.1)	3,051 (34.04)	1,452 (16.19)	1,124 (12.54)	208 (2.32)	1,124 (12.54)	1,412 (15.75)	6	Schreffler et al., 1971
Detroit, MI	505	(2.6)	(32.3)	(13.5)	(9.3)	(2.4)	(13.1)	(23.2)	20	Shaler, 1978 ◇
Miami/Dade Co., FL	370	10 (2.7)	138 (37.3)	64 (17.3)	41 (11.1)	12 (3.2)	43 (11.6)	51 (13.8)	15	Stuver, 1979 and see Shaler, 1978
Los Angeles, CA Case material	250	11 (4.4)	80 (32.0)	56 (22.4)	24 (9.6)	14 (5.6)	34 (13.6)	31 (12.4)	18	Sigler, 1979 and see Shaler, 1978
NEGRO										
New York, NY	200	(46.5)	(24.5)	(2.0)		(16)☆	(5)	(5.5)	7	Miller et al., 1951
Washington, D.C. Howard University Students	937	(44.5)	(24.6)		(17.3)		(5.1)	(7.3)	22	Moore, 1955
Baltimore, MD	580	(43.8)	(25.8)		(15.4)		(6.3)	(7.2)	8	Glass and Li, 1953
Southeastern GA	304	161 (53.5)	58 (19.3)	4 (1.3)	49 (16.3)	4 (1.3)	9 (3.0)	16 (5.3)	9	Cooper et al., 1963
San Francisco, CA Bay Area Mothers Children	1,453	(47.83) (48.04)	(22.85) (23.81)	(2.68) (2.13)	(14.11) (14.38)	– –	(3.72) (3.1)	(6.13) (5.71)		Reed, 1967
Birmingham, AL	613	320 (52.2)	132 (21.5)	2 (0.3)	77 (12.6)	13 (2.1)	18 (2.9)	40 (6.5)	10	Casey et al., 1968
San Francisco, CA	3,146	1,514 (48.1)	713 (2.7)	69 (2.2)	456 (14.6)	33 (1.0)	108 (3.4)	201 (6.4)		Reed, 1968
New York, NY	500	229 (45.8)	104 (20.8)	10 (2.0)	93 (18.6)	1 (0.2)	20 (4.0)	34 (6.8)	11	Wiener, 1969
South Central WV	133	(47)	(26)	(2)	(13)	(2)	(3)	(7)	12	Juberg, 1970
Detroit, MI	505	(50.3)	(20.8)	(1.6)	(13.5)	(1.6)	(3.6)	(7.3)	21	Shaler, 1978 ◇
Miami/Dade Co., FL	350	179 (51.1)	73 (20.9)	7 (2.0)	48 (13.7)	5 (1.4)	14 (4.0)	22 (6.3)	16	Stuver, 1979 and see Shaler, 1978
Los Angeles, CA Case material	125	63 (50.4)	26 (20.8)	5 (4.0)	12 (9.6)	3 (2.4)	10 (8.0)	6 (4.8)		Sigler, 1979 and see Shaler, 1978

Table 22.4 Cont'd.

Population	Total	Frequency — Number (Percent) Rh_o	Rh_1rh	Rh_1Rh_1	Rh_2rh	Rh_2Rh_2	Rh_1Rh_2 ★	rh	Rare Phenotypes See Note:	Reference
CHINESE										
New York, NY	103	(1)	(11.7)	(53.4)	(8.7) ☆		(24.2)	(1)		Miller et al., 1951
New York, NY	400	1 (0.3)	30 (7.5)	213 (53.3)	6 (1.5)	19 (4.8)	126 (31.5)		13	Wiener, 1969
New York, NY	946 ✪	3 (0.32)	70 (7.4)	506 (53.49)	21 (2.22)	42 (4.44)	284 (30.02)	2 (0.21)	14	Wiener, 1974
HISPANIC										
San Francisco, CA "Mexican"	335	8 (2.4)	103 (30.7)	81 (24.2)	33 (9.9)	13 (3.9)	59 (17.6)	20 (6.0)		Reed, 1968
Miami/Dade Co., FL	364	25 (6.9)	124 (34.1)	56 (15.4)	43 (11.8)	9 (2.5)	49 (13.5)	52 (14.3)	17	Stuver, 1979 and see Shafer, 1978
Los Angeles, CA Case material	111 □	1 (0.9)	33 (29.7)	26 (23.4)	12 (10.8)	7 (6.3)	29 (26.1)	3 (2.7)	19	Sigler, 1979 and see Shafer, 1978

★ Includes R_1R_2, R_1r'', R_2r', R_2r, R_2R_o and R_or_y

☆ Anti-hr'' (e) was not used — Rh_2rh and Rh_2Rh_2 indistinguishable

✪ Includes the 400 persons in the Wiener (1969) study

□ Primarily Mexican

◇ Data of Stolorow and collaborators

1. 22(0.92) were rh'rh, 11(0.46) were rh''rh'', and 1 was Rh_2Rh_1
2. 25(1.15) were rh'rh'' (or rh_yrh)
3. 7 were rh''rh, 2 were rh'rh, 1 was Rh_2Rh_2; 3 of the 9 Rh_o had D^u, 2 of the 112 Rh_1rh had C^u and 1 of the 85 Rh_1 was C^w (i.e. Rh^w_1)
4. 4 were rh'rh, 4 rh''rh, 1 rh_yrh, 5 $Rh^w_1Rh_1$, 5 Rh^w_1rh and 1 Rh_2Rh_1
5. 0.3% were rh'rh, 0.6% were rh''rh, 0.3 were CcD^uee, 0.1 were ccD^uEe, 0.2 were Rh_2Rh_1 and 0.3 were Rh_2Rh_2
6. 10(0.11) were Rh_2Rh_2, 1 was ccD^uEE, 5 were Rh_2Rh_1, 37(0.41) were $Rh^w_1Rh_2$, 15(0.17) were ccD^uEe, 79(0.88) were $Rh^w_1Rh_1$, 4 were CCD^uee, 109(1.22) were Rh^w_1rh, 33(0.37) were CcD^uee, 16(0.18) were ccD^uee, there was one each of rh''rh'', rh'rh' and rh'rh'' (or rh_yrh), 54(0.6) were rh''rh and 38(0.42) were rh'rh.
7. 0.5% were rh'rh'
8. 1.4% were rh'rh'
9. 3 were rh'rh
10. 1 was Rh_2Rh_2, 9 were rh'rh and 1 was ccD^uee
11. 5 were rh'rh, 3 were Rh_o and 1 was Rh^w_1rh
12. 1% were rh'rh
13. 4 were Rh_2Rh_1 and 1 was Rh_2Rh_2
14. 15(1.59) were Rh_2Rh_1, 2(0.21) were Rh_2Rh_2 and 1(0.1) was rh'rh
15. 2 were Rh_2Rh_1, 1 was Rh_2Rh_2, 3 were rh'rh, 3 were rh''rh, 1 was rh'rh' and 1 was rh_yrh''.
16. 2 were rh'rh
17. 2 were Rh_2Rh_1, 1 was Rh_2Rh_2 and 3 were rh'rh.
18. 2 were Rh_2Rh_2, 1 was Rh_2Rh_2, 1 was rh''rh and 2 were rh'rh.
19. 1 was Rh_2Rh_2, 2 were Rh_2Rh_2, 1 was rh''rh, and 2 were rh'rh.
20. 0.2% were Rh_2Rh_2, 0.4% were Rh_2Rh_2, 0.2% were Rh_2Rh_1, 0.6% were rh'rh', 1.2% were rh'rh, 1.0% were rh''rh and 0.2% were CcD^uee
21. 1.2% were rh'rh
22. 1.2% were rh'rh' or rh'rh

SECTION 23. THE KELL, DUFFY AND KIDD BLOOD GROUP SYSTEMS

23.1 Kell System

23.1.1 The K and k antigens

In 1946, Coombs *et al.* reported their results on investigations of 15 cases of children with hemolytic disease of the newborn, most of which had been caused by Rh incompatible pregnancies. In one case, the baby's cells gave a positive direct AHG test, and the mother's serum was capable of sensitizing the father's cells, but not those of 13 randomly selected persons representing all known Rh types. The antibody did not appear to belong to any known system. Race (1946) called the antigen "Kell", after the woman's surname, and said that anti-Kell serum reacted with about 7% of random blood samples. In 1947, Wiener and Sonn Gordon reported a hemolytic transfusion reaction in a woman that could not be accounted for on the basis of known antibodies. Her name was Singer, and they called the antibody "anti-Si". "Si" appeared to be inherited as a simple Mendelian dominant, and could be found in the cells of 19 out of 148 bloods from Caucasians. Some anti-Si was sent off to Dr. Mourant in England, and he showed that "anti-Si" and "anti-Kell" were identical. The name "Si" was abandoned, and the antigen was to be called "Kell", shortened to "K" (Wiener *et al.*, 1953a).

In 1949, Levine *et al.* found an antibody in a mother who had had a baby suffering from hemolytic disease. It reacted in saline at 37° and detected a very high incidence antigen (99.8% of 2,500 random blood samples reacted). The antibody was called "anti-Cellano" after the patient's name. Calculations on the expected percentage of occurrence of the allele of the Cellano gene suggested a possible association with Kell or Lutheran, and family studies using anti-Cellano and anti-K soon established that Kell and Cellano were determined by alleles. Cellano was thus called "k", and the Kell system consisted of two alleles, *K* and *k*, giving rise to the genotypes *KK*, *Kk* and *kk*. This simple situation persisted until 1956. The k antigen is found in fetuses aged 9 weeks or older in the same strength as in adults, and with the same frequency, while K may be found in 14–16 week old fetuses (Toivanen and Hirvonen, 1969b).

23.1.2 Complexities of the Kell system

In 1956, Allen found an antibody in the serum of a Mrs. Penney that agglutinated about 2% of randomly selected cells. Certain informative families revealed that 'Penney' was related to the Kell system, and the antigen was named "Kp^a". In another serum from a person called 'Rautenberg' was found an antibody which appeared to be detecting the antigen governed by the allele of Kp^a. Further studies were reported by Allen and Lewis in 1957. In 1958, Allen *et al.* showed that the 'Rautenberg' serum was indeed detecting Kp^b (Allen *et al.*, 1958a). Kp(a+) is quite rare (2% in 5,500 people). Allen *et al.* (1958a) said that, in theory, there could be four kinds of gene complexes: KKp^a, kKp^a, KKp^b and kKp^b, and these were denoted K^a, k^a, K^b and k^b, respectively, in shorthand. KKp^a had not been found. In 1960, Lewis *et al.* screened 14,611 bloods for K, and then examined the 1,221 K+ samples with anti-Kp^a in a search for KKp^a. 22 K+Kp(a+) samples were found, but family studies revealed that *K* and Kp^a were on different chromosomes in every case. In 1969, Dichupa *et al.* conducted another search for KKp^a, this time by screening for Kp(a+) first. 274 Kp(a+) bloods were found in 11,239 samples, of which 11 were also K+. 9 families were studied, but without finding KKp^a (K^a), which in this latter paper was denoted K^p.

In 1958, Giblett found an antibody in a White male cancer patient who had received transfusions of blood from Black donors. The antibody reacted with the cells of 33 out of 172 Black people, but not with any samples from 240 White people. Further investigation showed that the antibody did not appear to be part of any known system, and the antigen being detected was provisionally called Js. In 1959, Giblett and Chase studied the characteristic further, and called the antigen Js^a. It appeared to be part of a new blood group system, which was to be called 'Sutter', and it was supposed that an antibody detecting Js^b would be found. Js^a was inherited as a simple Mendelian dominant. A second example of anti-Js^a was found in a multiply transfused patient in Detroit by Jarkowski *et al.* (1962), who found that 34 of 244 examples of red cells from Black people reacted with it, but that none of the cells of 103 White people did so. In 1962, Greenwalt *et al.* found the expected anti-Js^b in the serum of a Black woman in Memphis, who was being treated for complications associated with her 11th pregnancy. Further studies were done by Walker *et al.* (1963). Js^b is of relatively high frequency, while Js^a is relatively low. In 1965, Stroup *et al.*, studying the Js^b characteristic, found that the cells of two people who lacked all Kell antigens, i.e., were K-k-Kp(a-b-) (see below), were also Js(a-b-). Studies on informative families established that the *Js* locus was closely, if not absolutely, linked to the Kell locus.

In 1957, Chown *et al.* found two sisters in a family of Polish extraction, the results of a marriage between second cousins, who lacked K, k, Kp^a and Kp^b. Their phenotype was written K-k-Kp(a-b-). Allen *et al.* (1958a) designated this condition as K^o. The serum of the proposita (Peltz) contained an antibody which reacted with cells of all Kell

phenotypes except K^o, and came to be known as anti-Ku. Using this serum, Kaita *et al.* (1959) found another K^o person (Kan.), also the result of a consanguineous marriage. In 1966, Nunn *et al.* found another K^o person with a second example of anti-Ku in her serum. Studies on this, and some other families indicated that K^o must be controlled by an allele at the Kell locus, rather than by an independent suppressor. If the Kell locus is thought of in terms of an operon model, the K^o gene could be regarded as an operator mutation. K^o is extremely rare, the population studies thus far indicating its occurrence on the order of 5 per 100,000. Its theoretical significance, however, far exceeds its frequency in the population. More will be said about K^o below.

In 1961, Allen *et al.* described a new Kell phenotype in a healthy man called McLeod. His red cells reacted very weakly with anti-k and anti-Kp^b, and very weakly with anti-Ku. They did not react with anti-K or anti-Kp^a. After Stroup *et al.* (1965) had shown that Sutter belonged to the Kell system, McLeod cells were found to be very weakly reactive with anti-Js^b. Thus, the McLeod phenotype, as it is usually called, is K-kwKp(a–bw)Js(a–bw), where "w" indicated "weak". McLeod's family was not informative as to the genetic background of this condition. In 1968, another case of depressed Kell antigens was found in a Dutch boy who was five years old in 1965 when the studies were done (van der Hart *et al.*, 1968). He had the McLeod phenotype, and his serum contained an antibody which reacted with every kind of cell tested. It did not react with his cells, though, and it did not react with McLeod's cells. This serum, "Claas", is now said to contain anti-KL. Anti-KL reacts with K^o cells, and may be a mixture of antibodies. By selective absorption and elution, the activity reacting with K^o cells can be separated from that which reacts with cells of the common Kell type. Marsh *et al.* (1975a) have shown the identical thing, and the antibody activity which is directed at the antigen present in large amounts on K^o cells was called anti-Kx. Marsh believes that the substance being detected by anti-Kx may be the precursor substance for all the Kell antigens. This point is returned to in section 23.1.4.

23.1.3 Numerical notation and nomenclature for the Kell system

In 1961, Allen and Rosenfield proposed a numerical notation for Kell antigens and antibodies. They thought that such a system should be put into practice at the time, since Kell was starting to reveal some of its complexities, before an unsystematic nomenclature, or several systems, became entrenched and it became difficult to make changes with general agreement, as had happened with Rhesus. At the time, there were five Kell antigens; there are now about 18. The numerical designations, along with their common name equivalents, are indicated in Table 23.1. Most of the antigens have been discussed in section 23.1. Those which have not: Ul^a was defined by an antibody found in a transfused Helsinki man in 1967. It is inherited as a dominant character, and occurs in about 2.6% of Helsinki donors, but is apparently quite rare in other populations. The exact relationship of Ul^a to the other genes in the complex is not completely clear. Wk^a is an antigen of low frequency controlled by a gene which has been shown to be allelic to a gene controlling the high frequency antigen Côté. Another high frequency antigen of the Kell system, K18, has been described by Barrasso *et al.* in 1975, this work being cited in Marsh *et al.* (1975a). In 1979, Sabo *et al.* reported on an antiserum to another Kell specificity, anti-K19. The antibody defined a high frequency antigen. Yamaguchi *et al.* (1979) found four Japanese sisters who were Kp(a–b–) but who had normal Kell antigens otherwise. The most likely explanation was a third allele, Kp^c. In 1946, Callender and Race had found three previously unknown antibodies in the serum of a multiply transfused woman. One was anti-C^w (section 22.5.2), a second was anti-Lu^a (section 24.2.1), and the third was called anti-Levay. The Levay antigen was named after the donor whose cells had elicited the antibody. Gavin *et al.* (1979) have now demonstrated that Levay belongs to Kell and is identical to the Kp^c seen in Japan by Yamaguchi *et al.* (1979).

23.1.4 Genetics of the Kell system

At present, there are four allelic loci belonging to Kell: Kk, $Kp^aKp^bKp^c$, Js^aJs^b, and K11 K17 (Wk^a Côté). Some antigens are on record whose association with the Kell system is not in doubt, but whose relationship to the other allelic loci is not clear. Race and Sanger (1975) refer to these latter as "para-Kell" antigens. Any theory of the biochemical genetics of the system must accommodate all the observations, and a fully acceptable understanding of Kell has not been attained. Interestingly, some of the clues about the biochemical genetics of the system have been gathered from studies of the very rare phenotypes.

In 1971, Giblett *et al.* noted that several boys from different places suffering from a rare, inherited disease called chronic granulomatous disease (CGD) all showed Kell system irregularities, having either K^o or the McLeod phenotypes. The occurrence of two very rare conditions in a number of patients is most improbable if the conditions are independent and simultaneous occurrence of the conditions is due to chance alone. There were also 13 cases of CGD people with no abnormality in Kell. CGD is an inherited defect of neutrophil metabolism, in which phagocytic activity is normal, but in which post-phagocytic bacterial destruction is impaired (see McKusick, 1975; catalogue number 30640). There is a body of evidence that CGD is an X-linked characteristic, but apparently the issue is not completely settled in every mind, as can be seen in reading the letters to the Editor of *The Lancet* (Windhorst, 1969). Marsh and his collaborators have taken up studies on the relationship between CGD and Kell antigens. As noted above, van der Hart and her colleagues had indicated that anti-KL serum appeared to have two different specificities which could be separated by selective absorption and elution procedures. Marsh *et al.* (1975a) confirmed these findings. Absorption of anti-KL (anti-K9) with cells of the common Kell phenotype, K−k+Kp(a−b+)Js(a−b+), removes the

Table 23.1 Kell System Numerical Nomenclature

Number	Common Name Equivalent	Number	Common Name Equivalent
K1	K	K10	Ul^a
K2	k	K11	Côté
K3	Kp^a	K12	Bøc
K4	Kp^b	K13	Sgro
K5	Ku	K14	San
K6	Js^a	K15	Kx
K7	Js^b	K16	—
K8	K^w	K17	Wk^a
K9	KL	K18	—

antibody directed at these, but leaves behind an anti-K^o activity. Overabsorption with common phenotype cells removes all activity. Conversely, absorption of anti-KL with K^o cells removes "anti-K^o", and leaves behind the antibody directed against the common type. The absorbed "anti-K^o" can be eluted, and this activity, directed against the antigen present in large amounts on K^o cells, is called anti-Kx (anti-K15). Marsh (1975a) and Marsh *et al.* (1975a) have shown that leucocytes, neutrophils in particular, have the Kell antigens, and that normal neutrophils absorb anti-Kx. In studying a number of CGD patients with Kell defects (K^o or McLeod phenotypes), they found that the neutrophils failed to absorb anti-Kx. It is postulated, therefore, that Kx is required for the expression of the Kell antigens on white cells or red cells. Marsh *et al.* (1975b) have tested the leucocytes of mothers of CGD patients and found that the Kx activity is intermediate between normal and absent. Further, these maternal leucocytes show mosaicism, i.e., there is a population of Kx deficient cells and a population of normal cells in equal mixture, just as would be predicted by the Lyon Hypothesis (section 1.2.4.4) if the gene governing the synthesis of Kx were X-linked. Marsh *et al.* believe, therefore, that the synthesis of Kx is under the control of an X-linked gene called X^1k. A genetic model for the Kell system is proposed in which the X^1k gene acts first, giving Kx in red cells and white cells. Kx is then acted upon by the products of the Kell locus genes (*K, k, Kp,* Ul^a, K^{17}, K^o, etc.) to give the Kell antigens, according to the individual genotype (Marsh *et al.*, 1976). In this view, the McLeod phenotype is due to a variant gene at the X^1k locus. Several variant alleles are postulated at this locus to explain the variations seen in Kell irregularities in red cells and/or white cells in people with and without CGD, and these are denoted X^2k, X^3k, etc. While the argument is put forth on the basis of the X^1k gene being X-linked because of the present evidence, the model does not stand or fall on the location of X^1k. The essence of the model is that Kx is the product of an allele not associated with the Kell locus, and that it serves as precursor substance for Kell locus gene products. If the X^1k locus were autosomal, and its product modified in some way by an X-linked gene product, or sex-modified in some other way, the data would still fit.

23.1.5 Kell antibodies

Anti-K is relatively common, while anti-k is much less so, because the KK genotype is relatively rare, and it is these people who can make anti-k. Anti-Kp^a may be relatively common, but Kp(a+) cells must be used to find it. Anti-Kp^b is comparatively rare because Kp^a homozygotes are comparatively rare. The rest of the Kell antibodies are fairly rare.

23.1.6 Medicolegal applications of Kell

The inheritance of the established allelic pairs of genes in the Kell system is well established, and the system can be used in disputed affiliation cases. Because of the distribution of phenotypes, however, few exclusions of nonfathers are to be expected on the basis of Kell alone. The probability

of excluding a true nonfather with anti-K and anti-k is about 3% for Whites and about 0.5% for Blacks (Chakraborty *et al.*, 1974; AABB, 1978). The probability for Whites, using anti-Kp^a and anti-Kp^b, is about 1%, while that for Blacks, using anti-Js^a and anti-Js^b, is about 6%. In the case of Kell, as with the other blood group systems, the rare peculiarities must be kept in mind in using the system for paternity testing.

There is little in the literature on Kell grouping in bloodstains. There is no doubt that K can be determined in bloodstains by inhibition or by elution techniques. Perhaps because anti-k is relatively difficult to obtain, and in part because the determination of k is not especially informative in most cases, no reports on the determination of k in bloodstains were found. If one were to do such determinations at all, they would probably be done to show that k is active when K is negative, or occasionally on K+ stains to determine zygosity.

At around the same time, and independently, Jones and Diamond (1955) in this country and Ducos in France (Planques and Ducos, 1957; and see Ducos, 1958) reported determination of K in bloodstains by inhibition techniques. Jones and Diamond (1955) carried out the determination on exhibits in a homicide case, and were able to distinguish the victim from the assailant. The amounts of material required for the inhibition tests were relatively large by today's standards. In 1969, Douglas and Stavely showed that K could be determined in bloodstains by an absorption-elution technique, the eluates being tested in an autoanalyzer. Amounts of material as small as 0.5 cm long threads from 27–34 day old bloodstains could be grouped correctly. In 1975, Lincoln and Dodd described a microelution procedure which needed a 2 mm square fragment of bloodstained material for each antigen to be tested, and was applicable to K. Incomplete anti-K was used, with incubation at 37°, washing at 4°, elution for 10 min at 60°, and detection by the AHG method. Stains up to 10 months old could be correctly grouped for K. Selection of appropriate, specific, high titer anti-K was important. Detection of K was successful in bloodstained fragments that gave negative results with the inhibition technique (Lincoln and Dodd, 1975a and 1975b). McDowall *et al.* (1978b) recently compared manual and autoanalyzer techniques for the detection of anti-K eluted from bloodstains. The manual technique gave more reliable results and was preferred.

Denault *et al.* (1978) could detect K in bloodstains by elution-AHG procedure only up to about 2 weeks of aging. And K could be missed in a one week old bloodstain on cotton. Burke and Tumosa (1978) could detect K in a 4 year old bloodstain using the microelution procedure of Lincoln and Dodd (1975b).

23.2 Duffy System

23.2.1 Discovery and development

In 1950, Cutbush *et al.* found an antibody in the serum of a 43 year old hemophiliac, who had received a number of transfusions, which reacted with about 2/3 of randomly selected red cells from English donors. By its reactions, the antibody could be shown to be defining a new antigen. With the patient's permission, the system was named Duffy, and the antigen being detected by his serum, Fy^a. Further studies (Cutbush and Mollison, 1950) showed that 64.9% of 205 English blood samples reacted with anti-Fy^a. Such cells were designated Fy(a+) by analogy to the nomenclature for Lutheran and Lewis (see in section 24.2.1). The antigen was inherited in a straightforward manner, was fully developed in embryos, and no Fy^a could be found in saliva. The predicted hypothetical allele was called Fy^b. Race *et al.* (1951a) and Race and Sanger (1952) carried out family and population investigations on Duffy, using anti-Fy^a, in a number of unselected families, and the data were in agreement with the postulation that Fy(a+) people could be Fy^aFy^a or Fy^aFy^b the Fy^b being hypothetical at the time. Ikin *et al.* (1951) and Blumenthal and Pettenkofer (1952) found in the serum of a Mrs. Hahn in Berlin the anticipated anti-Fy^b, and it gave the reactions to be expected if it were detecting the product of the Fy^b allele. Tests with anti-Fy^a and anti-Fy^b revealed that English blood donors were distributed approximately as 20% Fy(a+b−), 48% Fy (a+b+) and 32% Fy(a−b+).

In 1955, Levine *et al.* found a second example of anti-Fy^b in a Mrs. MF (Levine *et al.*, 1955c). Some of the serum was provided to Sanger *et al.* (1955) who used it, along with anti-Fy^a, to test a series of 125 bloods from Black donors from New York City. To everyone's astonishment, 68% of the cells did not react with *either* serum, i.e., they were Fy(a−b−). The remainder of the sample was 8.8% Fy(a+b−), 1.6% Fy(a+b+) and 27% Fy(a−b+). The Fy(a−b−) reactions could not be understood on the basis of the two alleles Fy^a and Fy^b. A third allelomorphic gene was postulated, and called *Fy*. This allowed Fy(a−b+) to be either Fy^bFy^b or Fy^bFy and Fy(a+b−) to be either Fy^aFy^a or Fy^aFy, genotypically, while Fy(a−b−) represented the *FyFy* genotype. Some support for this view was generated using a particular anti-Fy^a serum which showed a dosage effect, and whose reactions indicated that most of the Fy(a+) cells from the Black donors had a single dose of Fy^a. If *Fy* turned out to be coding for another antigen—which would only be known if and when an anti-Fy was found—it would then be promoted to Fy^c. The anti-Fy would be expected to be found in a Caucasian who had been transfused with blood from Black donors, but would have to be looked for using test cells from Black donors who had the *Fy* gene.

In 1965, Chown *et al.* found that the explanation for the rare Fy(a−b−) phenotype in Caucasians was not the same as it is in Black people (Chown *et al.*, 1965a). A new Duffy locus allele, Fy^x, was found to be the usual reason for the condition in Whites. Fy^x is not qualitatively different from Fy^b, but it is different quantitatively. With carefully selected anti-Fy^b and AHG sera, a small amount of Fy^b can be found in Fy^x people. The gene is quite rare, and unless special testing is done with selected anti-Fy^b, Fy^aFy^x people will routinely be classified as Fy(a+b−), when in fact they are Fy(a+bw). Lewis *et al.* (1972) found a person who was

probably Fy^xFy^x. The gene *Fy* does occur in Caucasians but is extremely rare. Libich *et al.* (1978) described a family of gypsy origin in Czechoslovakia which had 5 Fy(a−b−) members due to homozygosity for the *Fy* allele. The red cells of these people were Fy(−3) (see in section 23.2.2) as well. The *Fy* gene may reach appreciable frequencies in the gypsies, the authors said.

23.2.2 Extension of the Duffy system

In 1971, Albrey *et al.* found an antibody in an Fy(a−b−) Australian White woman which agglutinated the cells of every kind of blood except Fy(a−b−). Her cells did not give the Fy^x reactions. The age of numerical notation having arrived (see sections 22.6.2 and 23.1.3), the antibody was called anti-Fy3. It reacted with Fy(a+b−), Fy(a−b+) and Fy(a+b+) cells from White or Black donors. But for its behavior with papain treated cells, anti-Fy3 could have been regarded as anti-Fy^aFy^b. Treatment of Fy(a+) or Fy(b+) cells with papain abolishes their reactivity with anti-Fy^a and anti-Fy^b, whereas it enhanced their reactivity with anti-Fy3. The antibody may be detecting some precursor substance for Fy^a and Fy^b. Anti-Fy4 was found in a 12 year old Black sickle cell anemia patient who was Fy(a+b+) by Behzad *et al.* (1973). The antibody was weak, but reacted with all Fy(a−b−) cells, and with most Fy(a+b−) and Fy(a−b+) cells from Black donors. It appeared in general to be the long sought for anti-Fy^c of Sanger *et al.* (1955). Its reactions, however, were enhanced by papain treatment of cells, as are those of anti-Fy3. Anti-Fy5 was found in a Black Fy(a−b−) donor by Colledge *et al.* (1973). The antibody reacted very much like anti-Fy3, except that it reacted with the cells of the person in whom anti-Fy3 had first been found. It did not react with Fy(a−b−) cells from Black donors, and curiously, it did not react with Rh_{null} phenotype cells which had ordinary Duffy antigens. The last observation led to speculation that anti-Fy5 might be an antibody to some combination of Rh and Duffy gene products.

23.2.3 Other aspects of the Duffy system

The great majority of anti-Fy^a and anti-Fy^b are incomplete antibodies, best detected by the AHG technique and having a 37° temperature optimum. Both anti-Fy^a and anti-Fy^b are inactive with enzyme-treated cells. Ficin, papain and bromelin markedly reduce or abolish the reactivity of the Duffy receptors. This characteristic can be exploited in identifying Duffy and non-Duffy antibodies in a human serum which contains a mixture of antibodies of differing blood group specificity. Anti-Fy^a is quite a bit more common than anti-Fy^b. Fy^a is, by one measure, about 40 times less immunogenic than the Kell antigen, and Fy^b is probably still less so, as only about 1 example of anti-Fy^b is found for every 20 examples of anti-Fy^a (Marsh, 1975b). Duffy antigens are well developed at birth, and can be found in 6–7 week old fetuses (Toivanen and Hirvonen, 1969b and 1973).

There have recently been suggestions that Fy^a or Fy^b receptors on red cells may also be receptors for one type of organism that causes malaria. *FyFy* erythrocytes could be shown to be resistant to infection by one species of *Plasmodium*, and it is possible that malaria infection may be an important selective force in the maintenance of the *Fy* gene in parts of West Africa (Miller *et al.*, 1975; Gelpi and King, 1976).

The Duffy system has recently been extensively and well reviewed by Marsh (1975b).

23.2.4 Medicolegal applications

The Duffy system is well established as a genetic marker in parentage investigations. Using anti-Fy^a and anti-Fy^b, the probability of excluding a falsely accused father is about 18% for Whites and about 4% in Blacks. Great caution must be used in the interpretation of results involving Black people because of the very high frequency of the silent allele *Fy*. Ordinarily, Fy^aFy and Fy^aFy^a people will be indistinguishable, as will Fy^bFy and Fy^bFy^b people. Morel, in AABB (1978) has noted that exclusions of the second order have no meaning when Black people are involved in the case. Even in non-Black populations, *Fy* can occur rarely, suggesting that caution should be exercised in general with second order exclusions in the Duffy system (Libich *et al.*, 1978).

In 1957, Ruffié and Ducos reported successful determination of Fy^a in dessicated blood by an inhibition technique. The anti-Fy^a they had available was incomplete, and its reactions best detected by AHG technique. Following absorption of 0.3 mℓ by 40 mg dried blood specimen for 36 hrs at 37°, the absorbed serum was titrated with Fy(a+) cells using AHG to bring about agglutination. Anti-Fy^a was used at a titer of about 1:8 and Fy^a-containing dried blood up to six days old gave an inhibition of about three dilutions. Planques and Ducos (1957) mentioned Fy^a in the list of antigens then being determined in dried blood. Ducos (1958) reported that the results of his survey of a number of laboratories (see in Section 22.8) indicated that only one laboratory was carrying out determination of Fy^a in dried blood.

In 1975, Lincoln and Dodd (1975a and 1975b) reported successful determination of Fy^a and Fy^b in small amounts of bloodstained material using an absorption-elution technique. The antisera were incomplete, and the reactions were detected using AHG. No difficulty was experienced in the determination of both antigens in relatively fresh bloodstains. The technique was very similar to that employed by these investigators for s (Section 21.9.5) and for Kell (Section 23.1.6). Denault *et al.* (1978) could detect Fy^a in 1–2 week old bloodstains by elution-AHG technique, whether the stains were on cotton or on wool. Burke and Tumosa (1978) detected Fy^a in a 4 year old bloodstain, although they said that the eluate gave a weak reaction. They used the microelution procedure of Lincoln and Dodd (1975b).

23.3 Kidd System

23.3.1 Discovery and development

In 1951, Allen *et al.* found an antibody in the serum of a recently delivered mother, Mrs. Kidd, whose baby was suffering from hemolytic disease of the newborn. She had had

five previous unremarkable pregnancies, and no known transfusions. Her serum contained anti-K in addition to the new antibody. The new antibody was active in saline at 37°, but gave a higher titer by AHG technique. The saline reacting antibodies disappeared upon storage of the antiserum. The antigen being detected by this antibody was called Jk^a after Mrs. Kidd's son. People having the antigen were denoted Jk(a+), those lacking it, Jk(a−). Race *et al.* (1951b) found that 76–77% of people in Boston and London were Jk(a+) and studies on 51 families indicated that the antigen was governed by a Mendelian dominant gene which could be present in the homozygous or heterozygous condition. In the same year, another example of anti-Jk^a was found in the serum of a Mrs. McGimpsey. She had been recently delivered of her first child, who was healthy, and she had no history of transfusions (Hunter *et al.*, 1951). The third example was found in a Scottish mother, whose serum also contained anti-E and a cold reacting anti-P (Milne *et al.*, 1953). Rosenfield *et al.* (1953a) reported three additional examples of anti-Jk^a, and van der Hart and van Loghem (1953) found another one. Many examples have since been found.

In 1953, Plaut *et al.* found the expected anti-Jk^b in the serum of a mother of two children, who had had two miscarriages, and previous transfusions. None of her children had been affected by hemolytic disease. Her serum contained an anti-Fy^a in addition to the anti-Jk^b. In 1951, Unger had found that the sensitivity of the AHG test could be substantially improved by carrying out the test with trypsin-treated red cells. This study was carried out primarily on different examples of incomplete anti-$\mathbf{Rh_0}$, and the technique was recommended for the detection of antibodies present in very low titer in serum. Plaut *et al.* (1953) found that the technique was very helpful in the detection of the new anti-Jk^b. A second example of anti-Jk^b was identified by Sanger *et al.* (1953) in the serum of a volunteer who had been immunized to produce anti-C, anti-D and anti-E. In addition, he produced anti-s and anti-Jk^b. Van Loghem *et al.* (1953) reported a third example of anti-Jk^b in a serum which also contained anti-C and anti-M. The titer was improved by carrying out the AHG test with trypsin or papain treated cells.

A large number of family studies have established beyond much doubt that the inheritance of Kidd can be explained most easily on the basis of two alleles Jk^a and Jk^b. Race and Sanger (1968), in the 5th edition of *Blood Groups in Man*, analyzed 1197 matings with 3116 children, and the data fit very well. They noted that their data show an excess of Jk^a Jk^b heterozygotes, and they said that there may be something more to the anti-Jk^b reagent than is presently obvious. Chown *et al.* (1965b) did not observe any excess of heterozygotes in their data. Kidd antigens are well developed at birth. Jk^a has been reported in fetuses 10–11 weeks old and Jk^b in 6–7 week old ones (Toivanen and Hirvonen, 1973).

23.3.2 The Jk(a−b−) phenotype

In 1959, a Phillipine woman of some Spanish and Chinese ancestry was found to have an antibody in her serum that reacted with all cells tested except her own. She had given birth to two children, neither of whom showed any sign of hemolytic disease. Investigation showed that her cells were Jk(a−b−), and that her serum contained an antibody which was a kind of anti-Jk^aJk^b, with some separable anti-Jk^b (Pinkerton *et al.*, 1959). The condition could be accounted for on the basis of a third allele, *Jk*, or of a modifying gene. In 1965, Day *et al.* reported the Jk(a−b−) phenotype in a Chinese mother of six, and her serum contained the cross-reacting anti-Jk^aJk^b with a separable anti-Jk^a. Day *et al.* mentioned that seven examples of Jk(a−b−) had been described in addition to the original case and the one being reported in their paper. Some further examples of the phenotype were found in a family in Hawaii by Yokoyama *et al.* (1967), and another family with three Jk(a−b−) members was studied by Arcara *et al.* (1969). Most Jk(a−b−) people have anti-Jk^aJk^b in their sera, sometimes with separable anti-Jk^a or anti-Jk^b, but not always. The Jk(a−b−) sister of the propositus in the family studied by Arcara *et al.* (1969) had no Kidd antibodies, although she had been pregnant seven times. All the Jk(a−b−) people described thus far have been of Asian ancestry. The phenotype can occur in Europeans, however. Crawford *et al.* (1961) described a family in which a Jk(a−b+) father and Jk(a+b−) mother had three Jk(a+b−) children. This could be explained most easily by the presence of a segregating *Jk* allele. The case was complicated by the fact that the rare Lutheran phenotype Lu(a−b−) was segregating in this family as well (section 24.2.2). Race and Sanger (1975) mentioned that a few other European families had been tested in which *Jk* appeared to be present. It is not clear whether the genetic background of the European and Asian kind of *Jk* are the same or not.

23.3.3 Kidd antibodies

There is a tendency for Kidd antibodies to become inactive both *in vivo* and *in vitro*. Sometimes, the antisera work both in saline and by AHG technique, and only the saline reactivity decreases. It is helpful in many cases to carry out the AHG test with trypsin treated cells (Unger, 1951) or papain treated red cells. Complement can be involved in the anti-Jk^a reaction. Stratton (1956) found that refrigerated, and even frozen, anti-Jk^a becomes less active with time, but that there is some restoration of activity if fresh serum is added. Restoration of activity by addition of fresh serum is indeed the result of adding complement. Freeze dried sera appeared to be stable. Polley and Mollison (1961) examined the role of complement in the reactions of Kidd and several other antisera. Anti-Jk^a can be the cause of hemolytic disease of the newborn on rare occasions (see, for example, Greenwalt *et al.*, 1956), and so can anti-Jk^b (Kornstad and Halvorsen, 1958).

The anti-Jk^aJk^b antibodies made by Jk(a−b−) people appears to be more than a mixture of anti-Jk^a and anti-Jk^b, or an antibody cross-reacting with both antigens. Marsh *et al.* (1974) have found that neutrophils will absorb anti-Jk^aJk^b (anti-Jk3) made by Jk(a−b−) people, but that they do not absorb either anti-Jk^a or anti-Jk^b. These data suggest

that "Jk^aJk^b" is a distinct and separate antigen, which should perhaps be called Jk3, and Marsh *et al.* (1974) did not find it on lymphocytes nor on platelets.

23.3.4 Medicolegal applications

Anti-Jk^a and anti-Jk^b can be used in disputed affiliation cases, although the antisera are relatively scarce, and the matings which can show exclusions are relatively infrequent. The probabilities of exclusion for a true nonfather are about 18% for Whites, and about 15% for Blacks (Chakraborty *et al.*, 1974; AABB, 1978). Anti-Jk^b is very scarce, however, and may not be available. The probability of exclusion is obviously less if only anti-Jk^a is used. In Whites, for example, the value is about 3%. The possible occurrence of the rare, silent allele, *Jk*, should not be forgotten in interpreting exclusions with Kidd, even when both antisera can be used (Morel, in AABB, 1978).

There does not appear to have been any effort to determine Kidd antigens in bloodstains until the work of Lincoln and Dodd (1975a and 1975b). They showed that Jk^a could be determined in experimental bloodstains a few days old using an AHG reacting anti-Jk^a in a micro-elution procedure. There does not appear to be any reason why Jk^b could not be determined as well, except that, as these authors noted, suitable examples of anti-Jk^b are quite rare and difficult to obtain. Denault *et al.* (1978) detected Jk^a in week-old bloodstains on cotton or on denim by an elution-AHG procedure.

23.4 Distribution of Kell, Duffy and Kidd Phenotypes in U.S. Populations

The distribution of Kell phenotypes in some U.S. populations is given in Table 23.2 and that for Duffy and Kidd phenotypes in Table 23.3.

Table 23.2 Kell Phenotypes in U.S. Populations

Population	Total	Frequency — Number (Percent) KK	Kk	kk	Kp(a+b−)	Kp(a−b+)	Kp(a+b+)	Js(a+)	Js(b+)	Reference
CAUCASIAN										
Boston, MA	210 ★		21 (10.0)	189 (90.0)						Allen et al., 1951
Minnesota	300 ★		(11)	(89)						Matson et al., 1954
Boston, MA	1,925	(0.3)	(9.5)	(90.2)	(2.0)					Allen and Lewis, 1957
Seattle, WA	240 ○								240	Giblett, 1958
Boston, MA	2,709 ●									Allen et al., 1958a
Seattle, WA	500 ○								500	Giblett and Chase, 1959
Boston, MA	6,830					*6,830 (100)*				Walker et al., 1961
Southeastern GA	333 ★		28 (8.4)	305 (91.6)						Cooper et al., 1963
Eastern San Francisco Bay Area, CA										
Mothers	4,928 ★		(8.26)	(91.74)						Reed, 1967
Babies			(8.56)	(91.44)						
NC and VA										
Mongoloids	585 ★		44 (7.5)	541 (92.5)						
Controls	585 ★		36 (6.2)	549 (93.8)						Goodman and Thomas, 1968
Donors	253 ★		19 (7.51)							
San Francisco Bay Area, CA										
All Caucasians	8,962 ★		768 (8.6)	8,194 (91.4)						
Caucasians of "Western European" origin	5,056		452 (8.9)	4,604 (91.1)						Reed, 1968
New York, NY	500 ★		42 (8.4)	458 (91.6)						Wiener, 1969
South Central WV	1,412	(0.4)	(8.1)	(91.4)						Juberg, 1970
Tecumseh, MI	8,443 ☆	6 (.07)	594 (7.04)	7,842 (92.88)		8,312 (98.45)	130 (1.54)			Schreffler et al., 1971

Table 23.2 Cont'd

Population	Total	Frequency — Number (Percent) KK	Kk	kk	Kp(a+b−)	Kp(a−b+)	Kp(a+b+)	Js(a+)	Js(b+)	Reference
NEGRO										
New York, NY	200 ★		(3.5)	(96.5)						Miller et al., 1951
New York, NY	126 ★		2 (1.6)	124 (98.4)						*Race and Sanger, 1955* cited by Mourant et al., 1976
Seattle, WA	172 ○							33 (19.2)	139 (80.8)	*Giblett, 1958*
Seattle, WA	440 ○							86 (19.5)	354 (80.5)	Giblett and Chase, 1959
Southeastern GA	303 ★		3 (1.0)	300 (99.0)						Cooper et al., 1963
Philadelphia, PA								18 (0.9)	2,048 (99.1)	Lovett and Crawford, 1967
Eastern San Francisco Bay Area, CA										
Mothers	1,453 ★		(1.58)	(98.42)						Reed, 1967
Babies			(2.13)	(97.87)						
San Francisco Bay Area, CA	3,146 ★		52 (1.7)	3,094 (98.3)						Reed, 1968
New York, NY	500 ★		5 (1.0)	495 (99.0)						Wiener, 1969
South Central WV	133	(0)	(1.0)	(99.0)						Juberg, 1970
CHINESE										
New York, NY	103 ★		(0)	(100)						Miller et al., 1951
New York, NY	160 ★		0	160						Sussman, 1956
New York, NY	946 ★		2 (0.11)	944 (99.89)						Wiener, 1974
HISPANIC										
San Francisco, CA										
"Mexican"	335 ★		14 (4.2)	321 (95.8)						*Reed, 1968*

★ Tests done with anti-K

☆ Of 8,312 Kp(a−b+), 7,717 were kk, 589 Kk and 6 KK; Of 130 Kp(a+b+), 125 were kk and 5 were Kk; 1 person was kk Kp(a+b+)

✪ Approximate frequencies were: kKp^a 0.011, KKp^b 0.048, kKp^b 0.936

○ Tests done with anti-Js^a

Table 23.3 Duffy and Kidd Phenotypes in U.S. Populations

Population	Total	Fy(a+b−)	Fy(a+b+)	Fy(a−b+)	Fy(a−b−)	Jk(a+b−)	Jk(a+b+)	Jk(a−b+)	Reference
CAUCASIAN									
Boston, MA	189 ★☆					146 (77.2)		43 (22.8)	Allen et al., 1951
New York, NY	726 ☆					557 (76.72)		169 (23.28)	Rosenfield et al., 1953a
Minnesota	100 ○		68 (68)		32 (32)				Matson et al., 1954
Southeastern GA	333 ○		221 (66.4)		112 (33.6)	92 (27.6)	173 (52.0)	68 (20.4)	Cooper et al., 1963
Eastern San Francisco Bay Area, CA									
Mothers	4,928 ○		(66.52)		(33.48)				Reed, 1967
Babies			(65.87)		(34.13)				
San Francisco Bay Area, CA									
All Caucasians	8,962 ○		6,007 (67.0)		2,955 (33.0)				Reed, 1968
"Western European" Caucasians	5,056 ○		3,405 (67.4)		1,651 (32.6)				
South Central WV	1,016 ☆	(15.4)	(46.2)	(38.3)			(77.4)		Juberg, 1970
Tecumseh, MI	8,946 ○		6,009 (67.17)		2,937 (32.83)				Schreffler et al., 1971
	6,546 for Jk^a and 1,892 for Jk^b								
NEGRO									
New York, NY	305 ☆					283 (92.79)		22 (7.21)	Rosenfield et al., 1953a
New York, NY	125	11 (8.8)	2 (1.6)	27 (21.6)	85 (68)				Sanger et al., 1955
New York, NY	179	19 (10.6)	9 (5.0)	43 (24)	108 (60.4)				Race and Sanger, 1968
New York, NY	67					38 (57)	23 (34)	6 (9)	Race and Sanger, 1968
Southeastern GA	304 ○		27 (8.9)		277 (91.1)	166 (54.8)	118 (38.9)	19 (6.3)	Cooper et al., 1963

Table 23.3 Cont'd.

Population	Total	Fy(a+b−)	Fy(a+b+)	Fy(a−b+)	Fy(a−b−)	Jk(a+b−)	Jk(a+b+)	Jk(a−b+)	Reference
San Francisco Bay Area,CA									
Mothers	1,453		(17.41)		(82.59)				Reed, 1967
Babies			(17.14)		(82.86)				
San Francisco Bay Area, CA	3,146 ○		564 (17.9)		2,582 (82.1)				Reed, 1968
Detroit, MI	404 ○		85 (21.04)		319 (78.96)				Gershowitz, 1968, cited by Reed, 1968
South Central WV	103	(17)		(5)	(78)		(86)	(14)	Juberg, 1970
CHINESE									
New York, NY	103 ☆					54 (52.43)		49 (47.57)	Rosenfield et al., 1953a
HISPANIC									
San Francisco, CA									
"Mexicans"	335 ○		268 (80)		67 (20)				Reed, 1968

★ All subjects were K−

☆ Only anti-Jk^a used in tests

○ Only anti-Fy^a used in tests

● 4,974 (75.99) were Jk(a+) and 1,572 (24.01) were Jk(a−); 1,398 were Jk(b+) and 494 (26.11) were Jk(b−)

SECTION 24. THE P AND LUTHERAN SYSTEMS

Although the P and Lutheran systems have become somewhat more complicated than they first appeared, they will be described fairly briefly here. They have not yet been applied to any appreciable extent to the analysis of antigens in dried blood.

24.1 P System

24.1.1 Discovery and development

Landsteiner and Levine (1927b) first described the antigen which they called P, based on reactions of an immune rabbit serum they had prepared against human red cells. These were the same experiments that resulted in the discovery of the M and N antigens, as noted in Section 21.1. Anti-P divided bloods into two categories, P+ and P−. The characteristic appeared to be inherited as a Mendelian dominant, but because some bloods reacted weakly with the original serum, it was difficult to establish the frequency of P with certainty. It was clear that the frequencies were not the same in Black and White people (Landsteiner and Levine, 1929). An anti-P was soon found in a human serum (Landsteiner and Levine, 1930), and normal, non-immune sera from some horses, cattle, pigs and rabbits contained it as well. Landsteiner and Levine (1931) suggested the symbols P_i, P_n and P_a to denote the P being detected on human cells by the rabbit immune serum, the naturally occurring human agglutinin, and the naturally occurring animal serum agglutinins, respectively. These distinctions have not persisted, because the conception of P is somewhat different today than it was at the time. The antigen that Landsteiner and Levine first described is now called P_1, and its inheritance as a Mendelian dominant characteristic has been widely confirmed.

In the first communication, Landsteiner and Levine (1927b) mentioned that there appeared to be two kinds of reactions of cells with anti-P, strong and weak. In 1929, Landsteiner and Levine said that the reactions of different examples of red cells with anti-P could be divided into four categories, based upon the strength of reaction. They regarded the division of cells into categories on this basis, however, as arbitrary. The question of the strength of P_1 reactions has been investigated by a number of workers. Henningsen (1949a), who used a titration technique to study P_1 in a large number of people, did not think that the antigen could be divided up into distinct categories, based upon the strength of its reaction. The results showed a continuous normal distribution. Grosjean (1952), however, interpreted the results of his tests on 1,000 people to mean that the differences were discontinuous, and controlled by several different alleles. Cazal and Mathieu (1950) carried out quantitative studies as well, but the results were somewhat complicated by the problem of the Q blood factor (see below). Henningsen (1949b) carried out extensive studies on the inheritance of P_1 and on the strength of the receptor in the cells of a number of people. The data indicated to him that antigen strength might well be inherited. Fisher (1953) did a statistical genetic analysis of Henningsen's (1949b) data, and concluded that homozygosity could provide a solid basis for the variation in reaction strength, and that it would be premature to invoke other genetic origins as a foundation for the differences. The inheritance of P was studied in a considerable body of family material from Germany by Dahr (1940 and 1942a) and from Scandinavia by Henningsen (1949a and 1949b).

24.1.2 Extension of the P system

In 1951, Levine *et al.* found an antibody in the serum of a woman, whose blood was being cross matched in preparation for some surgery, which detected a very high incidence antigen (Levine *et al.*, 1951a). Her name was Mrs. Jay, and the Jay antigen being detected by her serum was denoted Tj^a. The gene responsible for it was called Tj^a, and the antibody, anti-Tj^a. Mrs. Jay's sister was also Tj(a−), and it was supposed that Tj^a had an allele, Tj^b, for which these sibs were homozygous. In 1952, Zontendyk and Levine found a second example of anti-Tj^a in a South African woman 37 years old. She had been pregnant four times, but never transfused. The South African antibody was an agglutinin when found, while the original Jay antibody was hemolytic when fresh, but behaved as an agglutinin when inactivated. In 1952, Hirszfeld and Grabowska found anti-Tj^a in a person called Fran Z., and they called it anti-Z^a at first. In 1954, Levine *et al.* found the antibody in 2 of 3 sibs in another family (Levine *et al.*, 1954a). In the same year, anti-Tj^a was identified in the serum of a 22 year old Australian woman with no history of pregnancy or transfusion. Her 19 year old sister had the antibody as well (Walsh and Kooptzoff, 1954). The rare (Tj(a−) people were assumed to be Tj^bTj^b, and their parents Tj^aTj^b. By 1955, 14 Tj(a−) people had been identified, and Sanger (1955) noticed that three unrelated examples of Tj(a−) cells were P−, an improbable result, since about 1 in 5 people in Europe is P−. A look at the literature revealed that three other cases of Tj(a−) cells had been found to be P−, and Sanger said that the probability of six unrelated Tj(a−) people being P− by chance alone was too great to be considered reasonable. It was then found that absorption of anti-Tj^a with P− cells left an anti-P antibody in serum. Absorption of anti-Tj^a with P+ cells removed all the antibody. These observations were readily understandable, she said, if Jay were part of P, and if the P reactions were thought of by analogy to the A_1A_2O reactions. If P_1 designated "old" P+ and P_2 designated "old" P−, then anti-Tj^a could be regarded as an anti-PP_1, like

anti-AA_1. Just as absorption of anti-AA_1 by A_2 cells leaves anti-A_1 behind, absorption of anti-PP_1 with P_2 cells left anti-P_1 behind, while absorption of anti-PP_1 with P_1 cells removed all activity, just as absorption of anti-AA_1 with A_1 cells does. The designation "p" was applied to the rare cells which did not react with anti-PP_1, i.e., to Tj(a−) cells. At the least, the analogy is useful for remembering the relationships within P. Salmon *et al.* (1979) have reported on the segregation of the *pp* genotype in two generations of a highly inbred Tunisian family.

In 1959, an antibody was found in Mrs. Mys. in Minnesota by Matson *et al.* which was first thought to be anti-PP_1, because it did not agglutinate p cells. She would have thus been *pp*. But her cells were agglutinated by anti-PP_1 and by anti-P_1, so the simple explanation was not workable. It had to be supposed that the cells of this proposita (and her sister) possessed a new antigen, which was called P^k, and that antisera previously regarded as being anti-PP_1 and anti-P_1 were also anti-P^k. A second family having P^k was described by Kortekangas *et al.* (1959). P^k was envisaged as being controlled by an allele P^k, but this allele was not straightforwardly inherited. It is expressed only when homozygous, or in combination with *p*, but not in the presence of P_1 or P_2. P^k people have the P^k antigen, but lack P. Most have P_1 (phenotype $P_1{}^k$) but some do not (phenotype $P_2{}^k$). Anti-P regularly occurs in the serum of P^k subjects. Extensive studies on P^k were carried out by Kortekangas *et al.* (1965). The relationships of P_1, P_2, P^k and p are given in Table 24.1, as adapted from Race and Sanger (1975). Anti-P^k can be left behind in the sera of some *pp* people after absorption with P_1 cells. Kato *et al.* (1978) have recently studied the anti-P and anti-P^k antibodies in p sera, and the anti-P in P^k sera, by complement fixation reactions using the purified antigen-active glycosphingolipids (see in section 21.1.5). Naiki and Kato (1979) have shown that P^k can be detected on P_2 cells if a p serum absorbed with P glycolipid to remove anti-P_1 is used. This fact had not been previously appreciated because P_1 cells (which contain trihexosyl ceramide, now known to be P^k) had always been used to absorb the P sera. Anti-P^k with different affinities for trihexosyl ceramide and its derivatives could also be prepared by partial absorption of p sera with P_1 cells, and elution.

Wiener (1968) did not agree with the explanation of P^k on the basis of a separate allele P^k. He said that the failure of anti-PP_1P^k serum, after absorption with P_1 cells, to agglutinate P_1 cells, and retention of its ability to agglutinate P^k cells, could be explained in other ways. His explanation was based on a different conception of the P system, which was proposed in the paper. In his view, four allelic genes and two antisera were involved. The genes *p, p', P* and P_1 give rise to the corresponding agglutinogens p, p', P and P_1, and these have associated with them the blood factors **p'** and **P** as follows: p' has **p**; P has **P**; P_1 has **p'** and **P**; and p has none. There are two antisera, anti-**p'** and anti-**P**. The relationships are summarized in Table 24.2. In this scheme, P^k corresponds to the phenotype p', and anti-PP_1P^k to anti-

Table 24.1 P System Relationships

Phenotype	Anti-P_1	Anti-PP_1P^k	Anti-P	Anti-P^k
P_1	+	+	+	−
P_2	−	+	+	−
p	−	−	−★	−
P_1^k	+	+	−	+
P_2^k	−	+	−	+

Anti-P_1 is found in P_2 persons and some animal sera. Anti-PP_1P^k (anti-Tj^a) is found in pp persons. Anti-P is found in P^k people. Anti-P^k is prepared by absorbing certain anti-PP_1P^k with P_1 cells.

★ Some examples of anti-P react weakly.

Table 24.2 P System Relationships According to Wiener (1968)

Phenotype	Reaction With Anti-P	Reaction With Anti-p′	Genotypes	Isoantibodies that may be present in serum
p	−	−	*pp*	Anti-P, Anti-p′
p′	−	+	*p′p′, p′p*	Anti-P
P	+	−	*pp, Pp*	Anti-p′
P_1	+	+	*P^1P^1,P^1P,P^1p,P^1p',Pp'*	None

p′ + anti-P. Absorption of this serum with P_1 cells could leave behind anti-p′ which would be reactive. Wiener admitted, however, that the data of Kortekangas *et al.* (1965) did support the notion of an additional specificity, except that he wanted to regard it as being associated with his p′ phenotype, and to call it p^k.

24.1.3 Blood factor Q

In 1935, Imamura and Furuhata found an antibody in the serum of pigs which agglutinated the red cells of some people, but not of others. The factor being detected was called "Q", and was shown to be inherited. The papers are written in Japanese, and references may be found in Prokop and Uhlenbruck (1969) and in Race and Sanger (1975). There was a suspicion that Q might be P, and the Japanese investigators apparently obtained some anti-P from Dr. Landsteiner, but did not find the anti-P and anti-Q reactions to be identical. Cazal and Mathieu (1950) arrived at the same conclusions. Furuhata and Hasebe (1955) reported on population and family studies on the Q factor. In 1955, Henningsen and Jacobsen compared an anti-Q from Japan with a series of anti-P reagents, and said that P and Q were identical. Race and Sanger (1975) and Wiener (1943c) regarded this problem as solved, but Prokop and Uhlenbruck (1969), while admitting that P may very well be Q, were less certain.

21.1.4 Additional notes about the P system

Another complication of the P system came to light in 1965, when Tippett *et al.* found a very peculiar agglutinin in the serum of Mr. Luke P. in Oklahoma City. He had Hodgkins disease, and had died in 1960. The agglutinin gave the following reactions with red cells that were tested: most samples were Luke(+) or Luke (w); a few rare ones were Luke(−), including p and P^k people's cells. Of the remaining P(−) people, the type was commoner in P_2 than in P_1 people, and commoner in A_1 and A_1B than in other ABO types. Other examples of the antibody have been found.

It is known that P_1 is not necessarily developed in fetuses or in children, and this circumstance can, of course, lead to serious errors if children are being tested. Ikin *et al.* (1961) found the antigen to be present in a higher proportion of younger than of older fetuses, and the reactions were stronger, but the numbers were still much lower than those for adults. Heiken (1966) said that the antigen could be undeveloped in children several years old, and he did not think that P_1 grouping in children less than one year old should be considered reliable.

There is a rare inhibitor gene called *In(Lu)* which gives rise to the dominant kind of Lu(a−b−) phenotype of the Lutheran system (Section 24.2.2), but which also inhibits the antigens Au^a (Section 25.5) and P_1 (Crawford *et al.*, 1974). Because of the P_1 inhibition, apparent contradictions to P inheritance can occur in Lu(a−b−) families, $P_2 \times P_2$ matings giving rise to P_1 offspring (Contreras and Tippett, 1974).

24.1.5 Biochemical studies on the P system

The P substances, whose occurrence is not restricted to the red cell membrane, have turned out to have carbohydrates as their immunochemical determinant groups. Considerable progress has been made in understanding the structure of the P substances, although the biochemical

genetic pathways do not as yet appear to have been completely worked out.

In 1957, Cameron and Staveley noticed that two patients being treated for hydatid cyst disease had developed potent anti-P_1 in their sera. The finding led them to search for P_1 or P_1-like substances in the cyst fluid. Hydatid cysts are fluid filled cysts which form in the livers of human beings and of ruminants due to the presence of the larvae of *Echinococcus* tapeworms. It was found that cyst fluid from sheep liver cysts contained an anti-P_1 inhibiting substance, which varied in inhibitive titer, and which was present only in those cysts which contained scolices. The substance was stable to boiling for 10 min. Staveley and Cameron (1958) went on to show that the fluid substance partially inhibited anti-P + P_1 sera. The presence of P_1 or P_1-like substance in these cyst fluids enabled Watkins and Morgan to initiate structural and immunochemical studies on the material (Watkins and Morgan, 1962; Morgan and Watkins, 1962). Partial purification of the hydatid cyst fluid substance gave three glycoprotein fractions. These materials could be precipitated by a number of examples of anti-P_1 reagent, a mixture of glycosidases from *T. fetus* destroyed the activity, and the reaction between P substance and anti-P_1 was inhibited by compounds having terminal α-D-galactosyl residues. If the substances were combined with a conjugated protein from *Shigella shigae*, and injected into rabbits, powerful anti-P_1 precipitin and agglutinin sera could be obtained.

Prokop and Schlesinger (1965a and 1965b) have shown that saline extracts of the bodies of certain worms possess P_1 substance (as well as ABH substances in some cases). P_1 substance or P_1-like substance could be isolated from *Lumbricus terrestris* and from *Ascaris suum*, and the authors speculated that immunization of people who have anti-P_1 in their serum might have resulted from infection by these or similar parasitic worms.

In 1974, Cory *et al.* purified a P_1-active glycoprotein material from sheep hydatid cyst fluid, and found that the terminal carbohydrate structure was D-Gal-α-(1→4)D-Gal-β-(1→4)NAc-D-GlcNH_2. The next steps have been taken primarily by Marcus and his collaborators. Their studies concentrated on purification and structure determination of P-active glycosphingolipids from the red cell membrane. A host of glycosphingolipids can be isolated from the erythrocyte membrane by various solvent extraction procedures, and purification of the extracts on silicic acid columns and thin-layer chromatographic plates commonly follows (see Vance and Sweeley, 1967). Marcus first isolated a P_1-active fraction from lyophilized red cell stroma in 1971, and it contained two glycosphingolipids. In the discussion which follows, it will be helpful to refer to Table 24.3, which gives the specialized terminology for the glycosphingolipids used by the workers in this field, taken from Schwarting *et al.* (1977).

Ando and Yamakawa (1973) and Ando *et al.* (1973) isolated and structurally characterized three glycosphingolipids from erythrocyte stroma, which they called A-30-III, A-60-III and G_{M3}. These had the structures of the paragloboside, sialosylparagloboside, and hematoside shown in Table 24.3, respectively. Siddiqui and Hakomori (1973) likewise isolated and characterized the first two of these compounds. In 1974, Naiki and Marcus identified the P antigen as globoside and the P^k antigen as trihexosyl ceramide. It was suggested that P^k is a precursor of P_1, and that P^k people lack the enzyme required to add the needed NAc-galactosyl residue. P_1-active glycosphingolipid was isolated and characterized by Naiki *et al.* (1975) and found to have the structure shown in Table 24.3. Treatment of the material with α-galactosidase produced galactose and paragloboside. Neither P nor P^k were therefore thought to be precursors of P_1. It will be noted that the terminal trisaccharide structure of P_1 glycosphingolipid is identical to that of the P_1 glycoprotein isolated by Morgan and Watkins and their collaborators (Cory *et al.*, 1974) above. This work was extended and further discussed by Marcus *et al.* (1976). A biosynthetic scheme (Fig. 24.1) could be proposed based upon this work in which lactosyl ceramide is converted to P^k by a transferase controlled by the P^k gene, and P^k in turn converted to P by a transferase under the control of the P_2 gene. P_1 is made on a different pathway from paragloboside by the intervention of an α-galactosyl transferase. If the α-galactosyl transferases converting lactosyl ceramide to P^k on the one hand, and paragloboside to P_1 on the other, are identical, and the product of the P^k gene, then it had to be supposed that P_1 gene makes a product which somehow modifies this enzyme's action in P_1 cells. Otherwise, the fact that P_2 people make P^k but not P_1 could not be understood. In this view, the primary biochemical feature of p cells is the absence of the α-galactosyl transferase which converts lactosyl ceramide to P^k and paragloboside to P_1.

Fellous *et al.* (1974) studied the P, P_1 and P^k antigens in cultured somatic cells, and found that P^k, which is so rare on red cells, was almost universally present in fibroblasts. Its absence from red cells was suggested to be the result of the control of P^k expression in red cells by an independent genetic locus, called F/f. P^k would be expressed in red cells only in people of *ff* genotype. The data also indicated that the *P* locus genes (P_1 and P_2) act before the P^k gene, and a scheme was proposed incorporating these ideas. If the structures of the P substances are correct, however, the scheme shown in Fig. 24.1, or one similar to it, would make better sense biochemically.

There have been two reports of an anti-p. The first, by Engelfriet *et al.* (1971) described a biphasic hemolysin in the serum of a 72 year old woman who was P_1. The serum also contained an agglutinin which acted optimally at lower temperatures and on *pp* cells. The characteristics of the antibody changed over the course of time, and activity eventually disappeared. Recently, Metaxas, Metaxas-Bühler and Tippett (cited by Schwarting *et al.*, 1977) found a serum called "Föl." which reacted strongly with p cells, and to a lesser extent with P_2, P_1 and P_1^k cells. Schwarting *et al.* (1977) studied the specificity of this serum and found it to be specifically inhibited by sialosylparagloboside. This observation supports the suggestion above that p cells lack the

Table 24.3 Names and Structures of Glycosphingolipids of Erythrocytes, after Schwarting et al., 1977

Lactosylceramide	Gal- β (1—4)-Glc-Cer
Trihexosyl ceramide	Gal- α -(1—4)Gal- β (1—4)-Glc-Cer
Globoside	GalNAc- β -(1—3)-Gal- α -(1—4)Gal- β (1—4)-Glc-Cer
Sialosylparagloboside	NANA- α -(2—3)-Gal- β -(1—4)-GlcNAc- β -(1—3)-Gal- β -(1—4)-Glc-Cer
Paragloboside	Gal- β -(1—4)-GlcNAc- β -(1—3)-Gal- β -(1—4)-Glc-Cer
P_1	Gal- α -(1—4)-Gal- β -(1—4)-GlcNAc- β -(1—3)-Gal- β -(1—4)-Glc-Cer
Hematoside	NANA- α -(2—3)-Gal- β -(1—4)-Glc-Cer
G_{Dla}	NANA- α -(2—3)-Gal- β -(1—3)-GalNAc- β -(1—4)-Gal- β -(1—4)-Glc-Cer ↑ α -(2—3) NANA

Abbreviations: Gal — D-galactose; Glc — D-glucose; GalNAc — N-acetyl-D-galatosamine; GlcNAc — N-acetyl-D-glucosamine; NANA — N-acetylneuraminic acid; Cer — ceramide (N-acylsphingosine)

α-galatosyltransferase(s), and accounts for the accumulation of paragloboside and its sialosyl derivative in these cells. While the reactivity of *pp* cells with Föl. serum is thus explained, the fact that this serum reacts more strongly with P_2 than with P_1 cells is not. The relationships as outlined by Naiki and Marcus (1975) and by Schwarting *et al.* (1977) are shown in Fig. 24.1. Kundu *et al.* (1978) described a normal Chinese man, whose cells were weakly reactive with anti-P_1PP^k serum. His red cell glycosphingolipids were analyzed, and his P antigen and P^k antigen content were found to be way less than normal. His ganglioside and sialosylparagloboside content were many fold above normal. The combination of features suggested that he represented a new phenotype, probably due to homozygosity for an allele of P^k that produces defective, or abnormally small amounts of α-galactosyl transferase.

Some further discussion of the genetic model, based on measurements of the glycosphingolipid content of red cell membranes, is given by Fletcher *et al.* (1979).

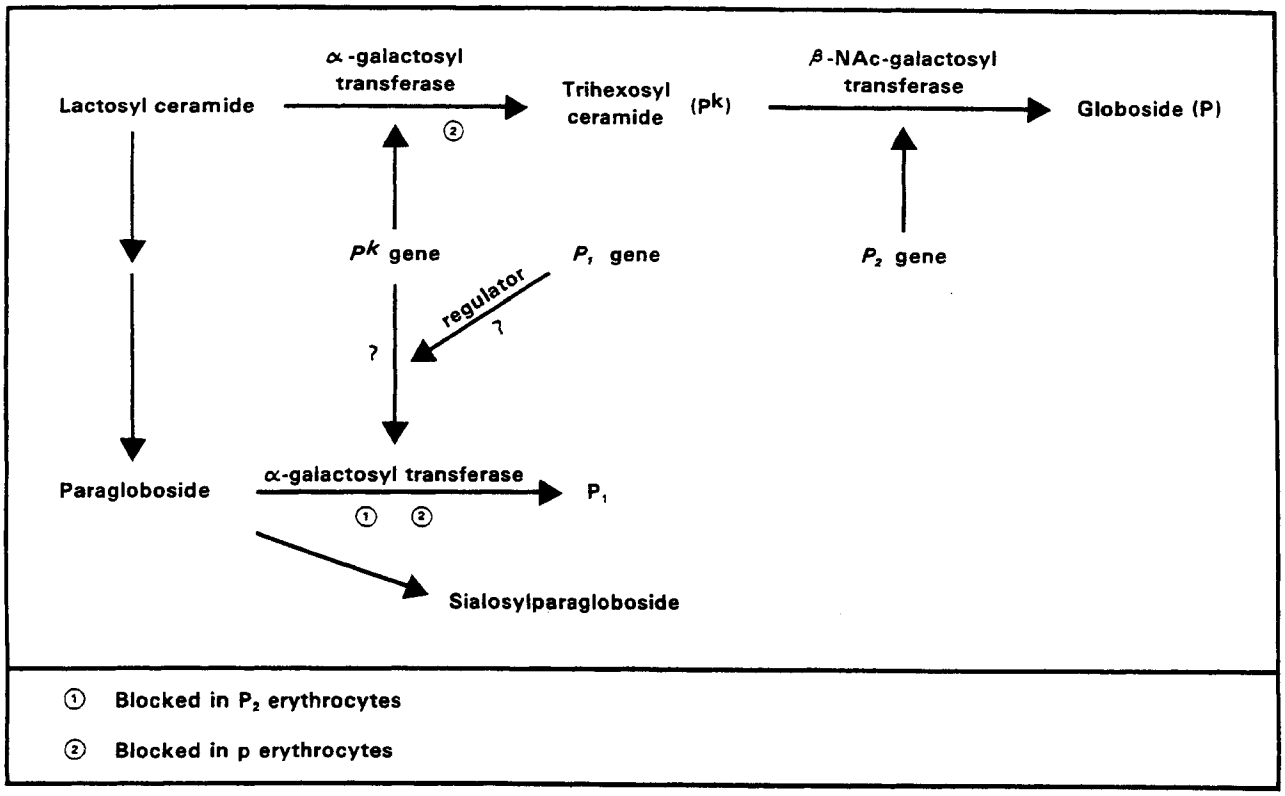
Watkins (1978) reviewed the biochemistry of the P system.

24.1.6 Medicolegal applications

The P system has not been applied to medicolegal problems to the same extent as have a number of the other systems. It can be a problem in disputed parentage cases because of the slow development of P_1 in children. The P system is useful in paternity cases only when a P_1+ child is seen as the result of an alleged $P_1- \times P_1-$ mating, and the situation does not arise very often. The chances of excluding a falsely accused father are about 2% in Whites, and less than 1% in Blacks. Difficulty can also be experienced because of the variability of anti-P_1 sera (Chakraborty *et al.*, 1974; Morel, in AABB, 1978).

The first efforts to detect P_1 in dried blood were apparently those of Roesgen in his Inaugural Dissertation of 1942 (cited by Schnug, 1952). The antigen could be detected in 2 day old bloodstains on linen, but not on wool or silk, by an inhibition technique. Schnug (1952) used a pig serum anti-P for determination of P in dried blood by inhibition. From 40 to 60 mg dried blood was incubated with 0.15 mℓ antiserum for 24 hrs at room temperature. Strong P_1 test cells were used, and the antigen could be detected in some samples of several month old bloodstains on a number of different substrata. Certain substrata interfered with the determination. Weaker P antigen was less reliably detectable and decayed faster over time. The test was said to have been used in casework. Mishakova (1961) reported that P could be determined in dried blood. Pettenkofer and Bickerich (1955) tested the P content of various organs and did not find it in muscle or kidney tissue, except that heart muscle from human or guinea pig sources gave some inhibition of anti-P.

Figure 24.1 Biosynthetic Scheme for the P System Antigens



24.2 Lutheran System

24.2.1 Discovery and development

In 1945, Callender *et al.* reported that a young woman, diagnosed as having lupus erythematosus diffusus, had made a remarkable series of antibodies in response to a series of transfusions. Among them were antibodies defining three antigens not previously encountered, and these antigens were named after the blood donors whose cells had apparently elicited the response. The antigen called Willis later became C^w (section 22.5.2), and a rare antigen called Levay, now known to be Kp^c (section 23.1.3) was detected. The third new antigen was Lutheran. A full report by Callender and Race followed in 1946. The symbols *L* and *l* were originally used to denote the genes for Lutheran(+) and Lutheran(−). It will be recalled the symbols *L* and *l* had been used to designate the first Lewis antigen too, however (Section 20.2). The ambiguity was cleared up at a conference of a group of interested people (Andresen *et al.*, 1949). It was decided to assign the symbol *Le* to Lewis, and *Lu* to Lutheran. The Lewis genes would be denoted Le^a and Le^b, the genotypes Le^aLe^a, Le^bLe^b, etc., the phenotypes Le(a+b−), Le(a−b+), etc., and the antisera, anti-Le^a and anti-Le^b. Lutheran would be handled similarly, the genes being Lu^a and Lu^b, genotypes Lu^aLu^a, Lu^aLu^b, etc., and phenotypes, Lu(a+b−), Lu(a+b+), etc. This convenient notation has been applied to most of the systems discovered since the conference. Dr. Ford suggested that the first antigen discovered be given the superscript "a", e.g., Lu^a, but that the product of the hypothetical allele not be called "b" until the antibody defining it was actually found. Thus, the hypothetical allele of Lu^a would be called *Lu* until an anti-Lu^b was found, and so forth for any system.

The antigen "Lutheran", defined by the serum of the patient discussed above, became Lu^a. The gene controlling this antigen was inherited as a simple Mendelian dominant character. This fact was established in the original work, and confirmed by a number of subsequent studies. About 7.7% of British people were Lu(a+) (Mainwaring and Pickles, 1948; Lawler, 1950; Bertinshaw *et al.*, 1950). In 1956, Cutbush and Chanarin found the expected anti-Lu^b in the serum of a Mrs. R. It gave the expected reactions, and a second example was soon reported by Greenwalt and Sasaki (1957). Tests on families and populations showed that the Lu^a and Lu^b antigens were indeed governed by an allelic pair of genes, giving rise to the phenotypes Lu(a+b+), Lu(a+b−) and Lu(a−b+). Lu(b−) people are fairly uncommon. In the United States, a few studies have been done which give an idea of the phenotypic frequencies. Dublin *et al.* (1964) found 82 Lu(a+b+), 1,116 Lu(a−b+) and 3 Lu(a+b−) in 1,201 Boston Caucasians. This population consisted of control and rheumatic people in a study designed to test for correlations between rheumatic heart disease and blood groups. Juberg (1970) found 25

Lu(a+b+), 291 Lu(a−b+) and 2 Lu(a+b−) in 318 Caucasians in South Central West Virginia. Molthan and Crawford (1966) found 27 Lu(a+b+) and 529 Lu(a−b+) in 556 Black donors in Philadelphia. Juberg (1970) reported 6 Lu(a+b+) and 29 Lu(a−b+) in 35 Black people in the West Virginia study.

24.2.2 The Lu(a−b−) phenotype

In 1961, Crawford *et al.* reported finding six people in three generations of a Caucasian family who were Lu(a−b−), the proposita in this case being Mary Crawford herself. This minus-minus phenotype, unlike those of any other known system, behaved as a dominant characteristic. Lu^b was obviously inhibited in this phenotype, and it was reasonable to suppose that Lu^a would be as well. Additional examples of Lu(a−b−) cells were found (e.g. Stanbury and Francis, 1967). It seemed likely that this phenotype was controlled by a gene coding for the absence of some important precursor substance. It became clear from the studies of Tippett (1971) and Taliano *et al.* (1973) that Lu^a was inhibited in this phenotype as well as was Lu^b, and that the inhibitor locus was different from the Lutheran locus. Taliano *et al.* (1973) suggested the symbol *In(Lu)* for the dominant inhibitor gene at the inhibitor locus, and *in(Lu)* for the normal, recessive allele. The symbol may be something of a misnomer, as it turns out, since it is now clear that *In(Lu)* affects more than the Lutheran alleles. There are disturbances in the expression of P_1, of i in the Ii system (Section 25.2) and of Au^a (Section 25.5) as well (Tippett, 1963; Contreras and Tippett, 1974; Crawford *et al.*, 1974).

In 1963, Darnborough *et al.* found a patient with Lu(a−b−) red cells who had come to their attention because of an anti-Lu^aLu^b in the serum. This antibody was the first example of its kind, and the anti-Lu^a and anti-Lu^b activities were inseparable, all activity being removed by absorption with either Lu(a−b+) or Lu(a+b−) cells. This serum was used to screen the cells of 18,000 additional people, and one more Lu(a−b−) blood was found. There was a suspicion that this Lu(a−b−) was not the same as the dominant kind described above, and this proved to be the case when informative families were found (Brown *et al.*, 1974). This recessively inherited kind of Lu(a−b−) appears to be best explained by the presence of an allele *Lu* at the Lutheran locus which is either silent, or coding for an as yet undiscovered antigen.

24.2.3 Extension of the Lutheran system

In recent years, a number of antibodies have been found which detect antigens related to Lutheran. Most of them are of high frequency, but a few are not. Numerical designations have been used in naming these new antigens. The designation anti-Lu3 has been applied to the anti-Lu^aLu^b first found in the Lu(a−b−) person by Darnborough *et al.* in 1963 (Bove *et al.*, 1971). Bove *et al.* (1971) described anti-Lu4. Anti-Lu5, anti-Lu6 and anti-Lu7 were reported by Marsh (1972). All define high incidence antigens present on almost all Lu(a+b+), Lu(a−b+) and Lu(a+b−) cells, but absent in both the dominant and recessive kinds of Lu(a−b−) cells. Lu4 is inherited, but not controlled by an allele of Lu^a and Lu^b (Bove *et al.*, 1971). It is also clear that Lu5 (Bowen *et al.*, 1972) as well as Lu6 and Lu7 (Marsh, 1972) are inherited. Lu8 was described by MacIlroy *et al.* (1972) and it, too, is inherited. Lu9 is a low incidence antigen, and has been shown to be controlled by a gene allelic to that controlling Lu6 (Wrobel *et al.*, 1972; Molthan *et al.*, 1973a). Dybkjaer *et al.* (1974) have, however, described a family in which the proposita is Lu:−6, and, therefore, presumably Lu^9Lu^9, but her cells reacted only weakly with anti-Lu9. The sister of this proposita was also Lu:−6 and reacted as expected (strongly) with anti-Lu9. Anti-Lu10 is, according to Race and Sanger (1975), mentioned occasionally, but no full description of it has been published. There is apparently a suspicion that Lu^{10} may be an allele of Lu^5. Lu11 is a high incidence antigen described by Gralnik *et al.* (1974). The designation Lu12 has been applied to the antigen defined by the "Much." serum, described by Sinclair *et al.* (1973). The authors agreed to the suggestion that it be called anti-Lu12, although they do not do so in the paper. The Much. serum reacts negatively with Lu(a−b−) cells of either type, and with some but not all Lu(a−bw) cells. The evidence suggested that this antigen is not controlled by a gene in the Lutheran complex locus. Lu14 was described by Judd *et al.* (1977) and their data indicated that Lu^{14} is allelic to Lu^8. It appears, therefore, that there are three allelic pairs at the Lutheran complex locus, Lu^aLu^b, Lu^6Lu^9,, and Lu^8Lu^{14}. The relationships of the other Lutheran antigens are not as yet clear.

24.2.4 Development of Lutheran antigens

Greenwalt *et al.* (1967) found that Lu^a is weakly expressed in Lu(a+b+) newborns, and increases in strength for many years, to about age 15. Lu(a+) cells can be detected, however, in fetal blood. Lu^a was detected in a 12 week fetus by Race and Sanger (1975), and in a 14 week fetus by Toivanen and Hirvonen (1973). Lu^b is weaker in fetal cells than in adult ones (Greenwalt *et al.*, 1967) and Toivanen and Hirvonen (1969b and 1973) could detect it in one 10 week fetus but not in one 9 week old one.

24.2.5 Medicolegal applications

The Lutheran system can be used in disputed affiliation cases, although its power of discrimination is fairly low. There is about a 3% probability of excluding a falsely accused father, whether he be Black or White, using both antisera. Anti-Lu^a and anti-Lu^b are quite scarce, and the system is not commonly included in paternity tests for this reason (Chakraborty *et al.*, 1974; Morel, in AABB, 1978). If the system is employed, the possible occurrence of the dominant *In(Lu)* gene which produces Lu(a−b−) as well as of the silent *Lu* allele must be kept in mind, even though both are very rare (AABB, 1978).

Perhaps because the antisera are rare, no papers on the determination of Lutheran antigens in bloodstains were found in the literature.

SECTION 25. SOME OTHER BLOOD GROUP SYSTEMS

25.1 Introduction

There are a number of other blood groups besides those discussed thus far in Unit V. There has been little, if any, application of these to the individualization of bloodstains. Some of them will be discussed briefly in this section for the sake of completeness.

25.2 The Ii System

What has come to be known as the Ii system derived from observations on the "cold" type of agglutinins found in the sera of patients suffering from acquired hemolytic anemia. The "system" no longer appears to be simple, and is related in some way to ABH and Lewis.

In 1956, Wiener *et al.* gave the name anti-I to a high titer cold autoagglutinin in the serum of a patient suffering from hemolytic anemia. The antigen I, defined by this serum, was of extremely high incidence, but rare I-negative people (denoted "i") could be found. Only five people of the i phenotype were found in the first 22,000 bloods examined. There was variability in the anti-I reaction with different examples of cells, and it was suggested that I might consist of a number of subgroups, I_1, I_2, etc. Jenkins *et al.* (1960) studied the original serum along with another anti-I serum called "Steg.", and a great range of I strength was noted, which could be fitted to a normal distribution curve. The I antigen was feeble in the cells of newborns and in cord blood cells. Tippett *et al.* (1960) extended these studies. A few more i people were found, and they had anti-I in the serum. Not all the anti-I found were quite the same. In 1960, Marsh and Jenkins found an anti-i in the serum of a patient. It reacted weakly with the patient's and most other red cells, but strongly with i cells and cord cells. Like its partner, anti-i was a cold reacting antibody. Marsh (1961) extended these studies, and suggested that five phenotypic categories could be distinguished, based on the strength of the I antigen and the presence of any anti-I in the serum. Most adults, it was said, develop I from i, since all infants have i, and the transition was suggested to be under genetic control. Infants have a strong i antigen, and very little I, but over the course of about 18 months, the relationship reverses itself. Dzierzkowa-Boradej and Voak (1979) have suggested that four subtypes of i can be distinguished based upon a complete array of evidence. Joshi and Bhatia (1979) found several Asian Indian blood donors who were I-i- in a large survey.

There is evidence of more than one kind of serologically distinguishable I (Marsh *et al.*, 1971; McGinnis *et al.*, 1974). As mentioned, anti-I is often the culprit in cases of acquired hemolytic anemia. Anti-i may sometimes be involved as well (Bell *et al.*, 1967).

It has been clear for some time that I is in some way associated with ABO, and it appears that this relationship as well as the complicated serological characteristics of the system may be clarified by the immunochemical studies on the antigens. Marcus *et al.* (1963) studied the effect of treating red cells with β-galactosidase from *Clostridium tertium* on anti-I reactivity. The data suggested the involvement of β-galactose and NAc-neuraminic acid in the immunodeterminant structure of the I receptor. Gardas and Koscielak (1974) isolated an I-active material from erythrocyte stroma and found it to be immunochemically indistinguishable from ABH-active substance. This finding is in conformity with the immunochemical studies of Feizi *et al.* (1971a and 1971b). They studied a partially purified I-active substance, isolated from milk. It was active only with selected examples of anti-I, and these were used to carry out quantitative precipitation studies. Different examples of anti-I were found to contain different antibody specificities against the milk-derived I substance, Le^a substance, precursors of ABHLe substances, and molecules resulting from various stages of chemical degradation of ABH substances. The most striking finding was the apparent heterogeneity of both different anti-I sera and of the I determinants. Mild degradation of ABH substances appeared to enhance I-activity generally, and suggested that I determinants are concealed in the interior of the ABHLe molecules, and of various biosynthetic stages of them. At the least, these findings suggest an immunochemical basis for the observed serological variability of I reactivity. These studies have been refined and extended more recently (Feizi *et al.*, 1978; Watanabe *et al.*, 1979). It may be mentioned that wheat germ agglutinin reacts with I substance purified from papain treated red cells (Oppenheim *et al.*, 1977). Maniatis *et al.* (1977) said that the red cells of sickle cell trait and sickle cell anemia patients show higher Ii reactivity than cells with normal hemoglobin.

Ducos *et al.* (1969b) said that I and i antigens could be diagnosed in bloodstains by an inhibition procedure with appropriate antisera, and that this fact could be useful in investigating bloodstains thought to be of infant or fetal origin. Tumosa (1977b) demonstrated that cold agglutinins of the anti-I type would readily absorb onto, and elute from bloodstains. There is no doubt that this fact would cause false reactions if there were cold agglutinin contamination of routine grouping reagents used for bloodstains. No such contamination was noted with several commercial ABH grouping antisera.

25.3 Diego

Diego was first mentioned by Levine *et al.* (1954b), who had tested a number of bloods from Caucasians with anti-Diego, and found them to be uniformly negative. The anti-

gen was, therefore, described as a low incidence antigen. The antibody was found in a mother in Caracas, whose baby suffered from hemolytic disease of the newborn, and further investigation showed that the antigen being detected occurred in appreciable frequencies in certain South American Indian populations (Layrisse *et al.*, 1955; Levine *et al.*, 1956). The antigen was denoted Di^a, and the gene responsible for it, Di^a, behaved as a Mendelian dominant characteristic (Layrisse *et al.*, 1955). Layrisse and Arends (1956) indicated that Di^a occurs with reasonable frequency in Chinese and Japanese populations, in addition to the South American Indian populations that have it, but it is extremely rare in Caucasians and in Negroes. An extensive study of Venezuelan Indian populations by Layrisse and Wilbert (1966) included Di^a frequencies in the various populations. Family studies suggest that Diego is probably an independent system. In 1967, Thompson *et al.* reported the first two examples of anti-Di^b in the sera of women of Mexican Indian ancestry. Other examples have since been found, but anti-Di^b is rarer than anti-Di^a. The Diego system appears to consist, therefore, of two alleles, Di^a and Di^b, which can give rise to three phenotypes. As noted above, however, the system is not significantly polymorphic in Caucasian and Black populations.

25.4 Yt

In 1956, Eaton *et al.* reported an antibody in the serum of a young woman who had had several pregnancies and had been transfused. She had died of cancer in 1955. The antigen being detected was called Yt^a, and was of very high incidence in Europeans. The antibody reacted weakly, and was best detected by a Coombs test using trypsinized cells. Papain appeared to destroy the receptor. It appeared that the gene could be present in single or double dose. In 1964, Giles and Metaxas found the expected anti-Yt^b. The system is understandable on the basis of two codominant alleles, Yt^a and Yt^b. Evidence thus far indicates that Yt is independent of other systems. Yt(b+) occurs in about 8% of Europeans. Giles *et al.* (1967) indicated that the phenotypic frequencies in 1030 people in London were: 946 Yt(a+b−), 82 Yt(a+b+) and 2 Yt(a−b+). In addition, they tested 69 U.S. Black people and found only one to be Yt(b+). Wurzel and Haesler (1968) tested 714 Blacks in the Philadelphia area, however, and found 60 to be Yt(b+). The system makes fairly useful distinctions, but antisera are not common, and in some cases the antibodies have been found in sera containing other antibodies, making it more difficult to obtain workable antibody preparations.

25.5 Auberger

The antibody defining Au^a was found in a multiply transfused woman in Paris by Salmon *et al.* (1961). A number of other antibodies were present in her serum as well. Another example of the antibody was found 10 years later. Both sera were AHG-reactive with papain treated cells. Europeans appear to be about 82% Au(a+). Au^a is inherited as a dominant characteristic, and is not thus far associated with any established system. Au^a is affected by the *In(Lu)* gene responsible for the dominant kind of Lu(a−b−) (see in section 24.2.2).

25.6 Dombrock

In 1965, Swanson *et al.* found the antibody defining Do^a in the serum of Mrs. Dombrock. The antibody reacted by the AHG test using papain treated cells, but AHG sera varied in their ability to demonstrate the reaction. The serum reacted with about 64% of cells from Europeans, and family studies indicated that the Do^a gene was dominant, and independent of a number of other systems. Polesky and Swanson (1966) extended the population studies, and found that fewer Blacks than Whites were Do(a+), about 55% of 161 as against 64% of 814, respectively. Tippett (1967) and Tippett *et al.* (1972) carried out family and population studies on Dombrock and confirmed its dominant mode of inheritance as well as establishing its independence from most other blood group systems. In 1973, anti-Do^b was found in the serum of a Mrs. Pam. by Molthan *et al.* (1973b). She was the mother of monozygotic twins and had been transfused a number of times. The antibody reacted best by the AHG test using enzyme treated cells. Dombrock consists, therefore, of the alleles Do^a and Do^b. If antisera become more available, Dombrock can be quite useful since it makes very good distinctions in populations.

25.7 Colton

In 1967, three examples of sera containing an antibody to a high incidence antigen were reported by Heisto *et al.* The antigen being detected was named after the first patient in whom the antibody was found, and was called Co^a. Family studies indicated a dominant pattern of inheritance, and dosage effects with the antiserum indicated that single or double doses of the Co^a gene could be present. Co(a−) people are very rare in Caucasian and Negro populations, something of the order of 1 or 2 per 1,000. Giles *et al.* (1970) found anti-Co^b in a multiply transfused patient, whose serum contained a number of other antibodies. Colton appeared to consist of the two alleles, Co^a and Co^b. Moulds *et al.* (1974) briefly reported a family, however, containing Co(a−) members whose reactions with anti-Co^b were weaker than expected, and the best explanation for which was a silent allele, *Co*, segregating in three generations. In addition, three Co(a−b−) people have been found by Rogers *et al.* (1974). The serum of one of them contained an inseparable anti-Co^aCo^b (anti-Co3, perhaps).

25.8 Sid and Cad

In 1967, Renton *et al.* reported an antibody which agglutinated most of the cells of Europeans but with widely varying strength. The antigen, named after the first strong reactor, was called Sd^a, and the system was called "Sid". About 91% of Europeans were Sd(a+) but the range of reactivity was striking, and the antibody was not easy to work with. Macvie *et al.* (1967) reported on Sd^a simultaneously. The AHG test appeared to be the best way of detecting reactions

with this serum. *Sd*^{a} was inherited as a dominant characteristic, and its allele was, for the time being, called *Sd*. Sd^a is found in the saliva of people whose red cells are Sd(a+), but the amounts present show wide variation. Macvie *et al.* (1967) said that it was not always easy to distinguish Sd^a "secretors" because of this variability. Morton *et al.* (1970) showed that Sd^a was present in most human secretions, urine being the richest source. The saliva of newborns has more Sd^a than that of adults. About half the people whose cells are Sd(a−) secrete some Sd^a in urine. Further, the occurrence of Sd^a is not limited to human beings, the antigen being found in guinea pig kidneys and urine, and in the urine of a number of other animals as well.

In 1968, Cazal *et al.* found an antigen of very low occurrence in a family of Indian extraction from Mauritius Island. The cells of certain members of this family were agglutinated by every example of human serum tested, i.e., were polyagglutinable. The polyagglutinability was not related to T nor to Tn. Extracts of *Dolichos biflorus*, previously thought to have only anti-A_1 specificity, agglutinated these cells, too, even though they were not of group A. The cells were said to have the "Cad" antigen after the name of the family. Cad was inherited, but very rare. This was the first example ever of an apparently inherited kind of polyagglutinability. Cazal *et al.* (1968) said that 250,000 examples of cells from Europeans were found to be Cad(−)! Cazal *et al.* (1971) extended their studies and found that certain other anti-A agglutinins from snail sources agglutinate non-A Cad(+) cells too, e.g. the anti-A from *Helix pomatia* or *Helix aspersa*. Sanger *et al.* (1971) found several *Dolichos* reacting non-A members of a Danish family, and noticed that they reacted very strongly with anti-Sd^a. Other members of the family whose cells did not react with *Dolichos* lectin showed weak or negative reactions with anti-Sd^a. The strength of Sd^a in Cad(+) cells was shown to be the highest ever observed, and the link between Sid and Cad was thus established. The conclusion was that Cad amounted to Sd^a in the extreme. Race and Sanger (1975) used the designation Sd(a++) to denote Cad(+), and Cad may be called "super Sid". Uhlenbruck *et al.* (1971) have shown that several anti-A agglutinins from snails, including *Helix pomatia*, *Helix aspersa* and *Cepea nemoralis*, react with Cad(+) cells whether they are of group A or not, but the *Phaseolus* (lima bean) agglutinin does not. Bird and Wingham (1971) found the very same thing. That *Phaseolus* lectin fails to react is a puzzle, since it is thought that it, like *Dolichos* lectin, is specific for terminal α-NAc-galactosaminyl residues. *Salvia farinacea* and *Salvia horminium* seed extracts contain an anti-Cad activity, separable from their anti-Tn activity (Bird and Wingham, 1974). Myllylä *et al.* (1971) found that the electrophoretic mobility of Cad(+) cells was not much reduced compared to normal ones, in contrast to T and Tn red cells. Race and Sanger (1975) noted that they had suggested previously that the polyagglutinability of Sd(a++) cells is not polyagglutinability in the usual sense, but is probably due to the presence of anti-Sd^a in the great majority of sera. The evidence indicates that the Cad agglutinating power of a serum is in approximately inverse proportion to the strength of the Sd^a antigen of the serum donor's cells. Some studies on the biochemical properties of a partially purified Sd^a substance from urine were reported by Morton and Terry (1970). The material behaved as if its serological specificity depended on a terminal oligosaccharide structure. Prokop *et al.* (1976) have recently reviewed Sid and Cad.

25.9 Some General Considerations on Blood Groups

There are many antigens known besides those which have been discussed in Unit V. Some of them belong to what are already, or are close to being established as independent blood group systems. Examples are Sm and Bu^a which are controlled by allelic genes, and make up the Scianna system, and Wright. Many other antigens exist whose relationship to one another and to established systems is not yet clear. The antigen Xg^a is of interest because it is the only blood group antigen coded for by an X-linked gene. This antigen has its own chapter in Race and Sanger (1975).

There are many antigens which are either of very high incidence or very low incidence. Some workers call these "public" and "private" antigens, respectively. Such antigens do not make useful distinctions in the population very often and are not, therefore, usually included in medicolegal tests. Antisera for these antigens are usually very scarce as well. If one happens to have antisera for rare antigens, tests might be done in an occasional case, since if a rare phenotype is found, it gives a very strong indication of exclusion or of inclusion. Thus, while the rare types are seldom encountered, they can be very valuable when they do occur in a case. It should be kept in mind that an antigen can be fairly well distributed in certain populations, and not in others. The Diego groups (Section 25.3) are a good example of this behavior.

The number of known antigens is now quite large. There are at least nine established systems: ABH, Rh, MNSs, P, Lutheran, Kell, Ii, Duffy and Kidd. Diego, Yt, Dombrock, Colton, Auberger, Scianna, Sid, Wright and Xg may sooner or later be added to the list. Issitt and Issitt (1975) list almost 400 antigens in their book. Some of these may be identical. Establishing the independence of a new antigen is not easy. Antisera to the public antigens are very rare, while cells having the private antigens are equally rare. Only a few laboratories have very extensive collections of these materials, and testing new cells or sera against dozens and dozens of sera or cells is very tedious work indeed.

The list of blood group antigens which have been successfully determined in dried blood continues to grow. The subject was excellently reviewed by Dodd in 1972. Theoretically, any antigen which is known to be inherited, and which makes a useful distinction in the population, could be useful in individualizing a bloodstain, provided that a technique is available for its reliable determination. In many cases, there are practical reasons for the inability to make use of a particular antigen or system in bloodstain analysis on a large

scale. The availability of antisera is sometimes a problem. In addition, the interpretation of negative results is not a trivial problem when grouping bloodstains. At the present time, the ABO, Rh and MNSs antigens along with Kell, Fy^a, Fy^b and Jk^a are well established as bloodstain markers. Leaving aside the problems with MN determinations in bloodstains, it appears that an informative discrimination could be obtained in most cases with anti-A, anti-B, anti-H, anti-D, anti-C, anti-E, anti-c, anti-e, anti-M, anti-N, anti-S, anti-s, anti-K, anti-Fy^a, anti-Fy^b and anti-Jk^a. Using very rough estimates of the phenotypic frequencies in U.S. populations, a White person having all the most common types, e.g. O, R_1r, MNs or MNSs, K-, Fy(a+b+), Jk(a+) would occur once in 85 people, while one having all uncommon types, e.g. AB, R_oR_o, MNS or NSs, K+, Fy(a+b−), Jk(a−), would occur only once in about 5 million people. A Black person with the groups O, R_oR_o, MNs, K-, Fy(a−b−), Jk(a+) would be expected about once in 25 people, while one with the groups AB, R_1R_1, MS or NSs, K+, Fy(a+b+), Jk(a−) would be seen only once in about 200 million people. Clearly, in most cases the discrimination will fall somewhere between the extremes. Good discrimination can also be obtained in many cases if only some of the antigens mentioned are used.

There are many excellent reference works in blood group serology. Race and Sanger (1975), Prokop and Uhlenbruck (1969) and Giblett (1969) make up an encyclopedic reference set, the latter two also containing considerable information on the material of Units VI and VII as well. Issitt and Issitt (1975) is a very good review, in addition to being a detailed reference to whole blood grouping techniques. Boorman, Dodd and Lincoln (1977) provide an excellent review of all the systems, and give a variety of detailed procedures which have been thoroughly checked in their own laboratory. The book has the additional advantage of containing substantial information about the medicolegal applications of blood grouping, based upon the authors' own considerable contributions and experience.

REFERENCES FOR UNIT V

AABB [American Association of Blood Banks]. 1978. *Paternity Testing*, Seminar presented by Committee on Technical Workshops of AABB, New Orleans, H. Silver (ed.), Washington, D.C.

Abraham, C. V. and S. Bakerman. 1974. Isolation and purification of the Rh(c) antigen. *Biochim. Biophys. Acta* **365**: 445–449

Abraham, C. V. and S. Bakerman. 1975a. Isolation and purification of the Rh(C) antigen. *Clin. Chim. Acta* **59**: 129–132

Abraham, C. V. and S. Bakerman. 1975b. Isolation and purification of the Rh(D) blood group receptor component from human erythrocyte membrane. *Clin. Chim. Acta* **60**: 33–43

Abraham, C. V. and S. Bakerman. 1976. Isolation and purification of the Rh(E) antigen. *Biochim. Biophys. Acta* **420**: 221–224

Abraham, C. V. and S. Bakerman. 1977. Isolation and purification of Rh(e) antigen. *Clin. Chim. Acta* **76**: 177–181

Ackerman, D. R. 1969. The absorption of anti-A and anti-B antibodies on human spermatozoa. *Fertil. Steril.* **20**: 324–334

Adamo, M. 1957. Metodi e techniche nuove in ematologia forense. Risultati e prospettive. *Med. Leg. Assicuraz.* **5**: 453–466

Akaishi, S. 1965. Studies on the group specific double combination method. *Jpn. J. Leg. Med.* **19**: 177–187 §'

Albrey, J. A., E. E. R. Vincent, J. Hutchinson, W. L. Marsh, F. H. Allen, Jr., J. Gavin and R. Sanger, 1971. *Vox Sang.* **20**: 29–35

Aldershoff, H. 1927. De benaming der bloedgroepen. *Nederl. Tijdschr. Geneeskd.* **71**, 2 Hlft B: 1365–1367

Alfultis, H. M. 1965. A microtitration method for grouping dried bloodstains. *J. Forensic Sci.* **10**: 319–334

Aliyev, G. K. 1927. Opredeleniye gruppovoy prinadlezhnosti krovi putem absorbtsii agglytininov. *Russkaya Klinika* **3**: 55–67

Allen, F. H., Jr. 1956. A new antigen in the Kell blood group system, *in*: Proc. 6th Congr. Int. Soc. Blood Transf., Boston, Sept., 1956, *Bibliotheca Haematol.*, Fasc. **7**: 106–109, S. Karger, Basel and N.Y. (1958)

Allen, F. H., Jr. and S. J. Lewis. 1957. Kp^a (Penney), a new antigen in the Kell blood group system. *Vox Sang.* **2**: 81–87

Allen, F. H., Jr. and R. E. Rosenfield. 1961. Notation for the Kell blood group system. *Transfusion* (Phila.) **1**: 305–307

Allen, F. H., Jr. and R. E. Rosenfield. 1972. Review of Rh serology. Eight new antigens in nine years. *Haematologia* **6**: 113–120

Allen, F. H., Jr. and P. A. Tippett. 1958. A new Rh blood type which reveals the Rh antigen G. *Vox Sang.* **3**: 321–330

Allen, F. H., Jr., S.M. Beiser, W.C. Boyd, I.W. Brown, Jr., R. Ceppellini, H. Chaplin, Jr., B. Chown, W.H. Crosby, W. Dameshek, I. Davidsohn, L.K. Diamond, E.R. Giblett, T.J. Greenwalt, M. Grove-Rasmussen, K.H. Gunson, S. Haberman, J.M. Hill, C. Howe, E.A. Kabat, J.R. Krevans, P. Levine, C. McNeill, J.F. Mohn, S. Raffel, A. Richardson-Jones, R. E. Rosenfield, J.B. Ross, J. Scudder, A.G. Steinberg, P. Sturgeon and M.M. Wintrobe. 1958c. [Letter. No formal title.] *Science* **127**: 1255–1256

Allen, F.H., Jr., P.A. Corcoran and F.R. Ellis. 1960. Some new observations on the MN system. *Vox Sang.* **5**: 224–231

Allen, F.H., Jr., P.A. Corcoran, H.B. Kenton and N. Breare. 1958b. M^g, a new blood group antigen in the MNSs system. *Vox Sang.* **3**: 81–91

Allen, F.H., Jr., L.K. Diamond and B. Niedziela. 1951. A new blood group antigen. *Nature* **167**: 482

Allen, F.H., Jr., S.M.R. Krabbe and P.A. Corcoran. 1961. A new phenotype (McLeod) in the Kell blood-group system. *Vox Sang.* **6**: 555–560

Allen, F.H., Jr., S.J. Lewis and H. Fudenberg. 1958a. Studies of anti-Kp^b, a new antibody in the Kell blood group system. *Vox Sang* **3**: 1–13

Allen, F.H., Jr., H. J. Madden and R.W. King. 1963. The MN gene *MU*, which produces M and U but no N, S or s. *Vox Sang.* **8**: 549–556

Alter, A.A., A.G. Gelb, B. Chown, R.E. Rosenfield and T.E. Cleghorn. 1967. Gonzales (Go^a), a new blood group character. *Transfusion* (Phila.) **7**: 88–91

Alter, A.A., A.G. Gelb and St. T. Lee. 1962. Hemolytic disease of the newborn caused by a new antibody (anti-Go^a), *in*: Proc. 9th Congr. Int. Soc. Blood Transf., Mexico City, *Bibliotheca Haematol.*, Fasc. **19**: 341–343 (1964)

Andersen, A. 1951. *Om H-receptorens forekomst og dens betydning for Retsmedicinen*, Dansk Videnskabs Forlag, Copenhagen

Andersen, A. 1952. Investigations in the inheritance of the characters secretor and non-secretor. *Acta Path. Microbiol. Scand.* **31**: 448–461

Andersen, J. 1958. Modifying influence of the secretor gene on the development of the ABH substance. A contribution to the conception of the Lewis group system. *Vox Sang.* **3**: 251–261

Andersen, J. 1969. The ABO, secretor and Lewis systems. Some aspects of the action of the secretor gene. *Series Haematol.* **2**(1): 34–82

Ando, S., K. Kon, M. Isobe and T. Yamakawa. 1973. Structural study on tetraglycosyl ceramide and gangliosides isolated from human red blood cells. *J. Biochem.* (Tokyo) **73**:: 893–895

Ando, S. and T. Yamakawa. 1973. Separation of polar glycolipids from human red blood cells with special reference to blood group-A activity. *J. Biochem.* (Tokyo) **73**: 387–396

Andresen, P.H. 1947. Blood group with characteristic phenotypical aspects.*Acta Pathol. Microbiol. Scand.* **24**: 616–618

Andresen, P.H. 1948. The blood group system L. A new blood group L_2, A case of epistasis within the blood groups. *Acta Pathol. Microbiol. Scand.* **25**: 728–731

Andresen, P.H. 1961. Relations between the ABO, secretor/non-secretor, and Lewis systems with particular reference to the Lewis system. *Am. J. Hum. Genet.* **13**: 396–412

Andresen, P. 1972. Demonstration of Le^x substances in the saliva of ABH non-secretors. *Vox Sang.* **23**: 262–269

Andresen, P.H., A. Andersen, K. Jordal and K. Henningsen. 1950. Corrélation entre le système Lewis et le système sécréteur-non-sécréteur. (Recherches sur 71 familles). *Rev. Hematol.* **5**: 305–314

Andresen, P.H., S.T. Callender, R.A. Fisher, R. Grubb, W.T.J. Morgan, A.E. Mourant, M.M. Pickles and R.R. Race 1949. A notation for the Lewis and Lutheran blood group systems. *Nature* **163**: 580–581

Andresen, P. and K. Henningsen. 1951. The Lewis blood group system and the X-system in 5 tables. *Acta Haematol.* **5**: 123–127

Andresen, P.H. and K. Jordal. 1949. An incomplete agglutinin related to the L-(Lewis) system. *Acta Pathol. Microbiol. Scand.* **26**: 636–638

Anstee, D.J., W.J. Mawby and M.J.A. Tanner. 1979. Abnormal blood-group-Ss-active sialoglycoproteins in the membrane of Miltenberger class III, IV and V human erythrocytes. *Biochem. J.* **183**: 193–203

Anstee, D.J. and M.J.A. Tanner. 1978. Genetic variants involving the major membrane sialoglycoproteins of human erythrocytes. Studies on erythrocytes of type M^k, Miltenberger class V and M^g. *Biochem. J.* **175**: 149–157

Arcara, P.C., M.A. O'Connor and R.M. Dimmette. 1969. A family with three Jk(a–b–) members. *Transfusion* (Phila.) **9**: 282 [Abstr.]

Arcilla, M.B. and P. Sturgeon. 1973. Studies on the secretion of blood group substances. II. Observations on the red cell phenotype Le(a–b–x–). *Vox Sang.* **25**: 72–87

Argall, B.A., J.M. Ball and E. Trentelman. 1953. Presence of anti-D antibody in the serum of a D^u patient. *J. Lab. Clin. Med.* **41**: 895–898

Asano, M., K. Minakata and H. Hattori. 1980a. General formulas of the estimated likelihood ratio Y/X in the diagnosis of paternity of a deceased putative father. *Z. Rechtsmed.* **84**: 125–133

Asano, M., K., Minakata and H. Hattori. 1980b. Diagnosis of paternity for cases without mother and without both mother and putative father

based on blood group findings from the relatives. *Z. Rechtsmed.* **84:** 135–144

Atchley, W.A., N.V. Bhagavan and S.P. Masouredis, 1964. Influence of ionic strength on the reaction between anti-D and D positive red cells. *J. Immunol.* **93:** 701–712

Austin, R.J. and G. Riches. 1978. Inherited lack of Ss antigens with weak expression of U in a Caucasian family. *Vox Sang.* **34:** 343–346

Badet, J., C. Ropars, J.P. Cartron, C. Doinel and C. Salmon. 1976. Groups of α-D-galactosyltransferase activity in sera of individuals with normal B-phenotype. II. Relationship between transferase activity and red cell agglutinability. *Vox Sang.* **30:** 105–113

Badet, J., C. Ropars and C. Salmon. 1978. α-D-galactosyltransferase activities in sera of *cis* AB blood group individuals. *J. Immunogenet.* **5:** 221–231

Baecchi, B. 1910. Sulla diagnosi individuale di sangue umano. *Arch. Antropol. Crim. Psichiatr. Med. Leg.* **31** (4 ser. **2**): 433–462

Baecchi, B. 1912. Ueber die individuelle Diagnose des menschilichen Blutes. *Friedreich's Blätter Gerichtl. Med. Sanitätspolizei* **63**: 97–126

Baecchi, B. 1913. Ancora sulla diagnosi individuale sangue umano. *Cesalpino* **9**: 469–479

Baecchi, B. 1920. Isoagglutinazione e individualita del sangue. *Riv. Med. Leg. Giurisprud. Med.* **10:** 138–147

Baer, H., H.W. Kloepfer and U. Rasmussen. 1961. The immunochemistry and genetics of blood group O. II. A study of the secretion of blood group A, B and O(H) substances in the saliva of family groups using precipitating antibody prepared in chickens. *J. Immunol.* **87:** 342–350

Baer, H., I. Naylor, N. Gibbel and R.E. Rosenfield. 1959. The production of precipitating antibody in chickens to a substance present in the fluids of nonsecretors of blood groups A, B and O. *J. Immunol.* **82:** 183–189

Balgairies, E. and L. Christiaens. 1937. Recherches expérimentales sur la détection des isoagglutinines dans les taches de sang. (2^{e} note). *Ann. Med. Leg. Criminol. Police Scientif.* **17:** 215–218

Bali, J.P., R. Magous, C. Lecou and M. Mousseron-Canet. 1976. Presence of blood group H antigen on a carcinoembryonic antign, and its enzymatic modification into blood group A and B specificities. *Cancer Res.* **36:** 2124–2129

Baranowski, T., E. Lisowska, A. Morawiecki, E. Romanowska and K. Strozecka. 1959. Studies on blood group antigens M and N. III. Chemical composition of purified antigens. *Arch. Immunol. Terap. Doswiad.* **7:** 15–27

Bargagna, M. and M. Pereira. 1967. A study of absorption-elution as a method of identification of Rhesus antigens in dried bloodstains. *J. Forensic Sci. Soc.* **7:** 123–130

Bar-Shany, S., A. Ben-David, J. Nusbacher, I. Szymanski, S. Kochwa and R.E. Rosenfield. 1970. The action of A^1 and A^2 genes on salivary blood-group substances. *Ann. N.Y. Acad. Sci.* **169:** 217–224

Bashinski, J.S. and J.E. Davis. 1973. A simple procedure for ABO typing of dried bloodstains on fibers by the absorption-elution technique. *J. Forensic Sci. Soc.* **13:** 217–222

Bauer, K.H. 1928. Zur Lösung des Problems der Blutgruppenvererbung. *Klin. Wochenschr.* **7**(34): 1588–1592

Bauer, K.H. 1929. Zur Genetik der menschlichen Blutgruppen. *Z. Indukt. Abstamm.-Verebungslehre* **50:** 3–62

Behzad, O., C.L. Lee, J. Gavin and W.L. Marsh. 1973. A new antierythrocyte antibody in the Duffy system: anti-Fy4. *Vox Sang.* **24:** 337–342

Bell, C.A., H. Zwicker and H.J. Sacks. 1967. Anti-i: identification of the "non-specific" cold agglutinin. *Vox Sang.* **13:** 4–7

Bell, C.E., Jr. and H.P. Fortwengler. 1971. Salivary anti-A and anti-B activity of group O males. *Vox Sang.* **21:** 493–508

Benciolini, P. and P. Cortivo. 1977. Problems in determining A_2 B group specific properties in bloodstains. *Z. Rechtsmed.* **79:** 285–288

Berger, K-H. 1933. Blutgruppenbestimmung an verunreinigten Blutflecken. *Z. Rassenphysiol.* **6:** 3–15

Bernstein, F. 1924. Ergebnisse einer biostatistischen zusammenfassenden Betrachtung über die erblichen Blutstrukturen des Menschen. *Klin. Wochenschr.* **3:** 1495–1497 and see Camp *et al.*, 1974.

Bernstein, F. 1925. Zusammenfassende Betrachtungen über die erblichen Blutstrukturen des Menschen. *Z. Indukt. Abstamm.-Vererbungslehre* **37:** 237–270 and see Camp *et al.*, 1974

Bernstein, F. 1930a. Über die Erblichkeit der Blutgruppen. *Z. Indukt. Abstamm.-Vererbungslehre* **54:** 400–426 and see Camp *et al.*, 1974

Bernstein, F. 1930b. Fortgesetzte Untersuchungen aus der Theorie der Blutgruppen. *Z. Indukt. Abstamm.-Vererbungslehre* **56:** 233–273 and see Camp *et al.*, 1974

Bertinshaw, D., S. D. Lawler, H.A. Holt, B.H. Kirman and R.R. Race. 1950. The combination of blood groups in a sample of 475 people in a London hospital. *Ann. Eugenics* **15:** 234–242

Bhalla, V. and S. Bhasin. 1976. A study of blood group antigens in saliva. *Indian J. Med. Res.* **64:** 1245–1249

Bhaskar, K.R. and J.M. Creeth. 1974. The macromolecular properties of blood-group-specific glycoproteins: characterization of a series of fractions obtained by density gradient ultracentrifugation. *Biochem. J.* **143:** 669–679

Bhatia, H.M. and K.J. Randeria. 1970. Studies on blood group antigens in saliva: incidence and type of aberrant secretors. *Indian J. Med. Res.* **58:** 194–201

Bhatnagar, R.K., S.K. Bhandari, J.S. Sijher and P.P. Srivastava. 1974. Bewahrungseffekte bei forensischen ABO-Bestimmungen anhand der Analyse von Ergebnissen bei Beweismitteln in Kriminalfällen. *Arch. Kriminol.* **154:** 91–99

Bhende, Y.M., C.K. Deshpande, H.M. Bhatia, R. Sanger, R.R. Race, W.T.J. Morgan and W.M. Watkins. 1952. A "new" blood group character related to the ABO system. *Lancet* **1:** 903–904

Biffi, U. 1903. Des hémagglutinines du sang humaine et de l'hématologie de la maladie de Carrion. *Bol. Acad. Nacion. Med.* (Lima) **3**(2), abstracted in *Sem. Med.* **23**(52): 424–425

Bird, G.W.G. 1951. Specific agglutinating activity for human red blood corpuscles in extracts of Dolichos biflorus. *Curr. Sci.* **20**(11): 298–299

Bird, G.W.G. 1952. Relationship of the blood sub-groups A_1, A_2 and A_1B, A_2B to haemagglutinins present in the seeds of *Dolichos biflorus*. *Nature* **170:** 674

Bird, G.W.G. 1953. Observations on haemagglutinin "linkage" in relation to iso-agglutinins and auto-agglutinins. *Br. J. Exp. Pathol.* **34:** 131–137

Bird, G.W.G. 1954. The hypothetical factor C of the ABO system of blood groups. *Vox. Sang.* **4**(O.S.): 66–68 §[2]

Bird, G.W.G. 1959. Haemagglutinins in seeds. *Br. Med. Bull.* **15**(2): 165–168

Bird, G.W.G. 1964. A-intermediates in Maharastrian blood donors. *Vox Sang.* **9:** 629–630

Bird, G.W.G. and J. Wingham. 1974. Haemagglutinins from *Salvia*. *Vox Sang.* **26:** 163–166

Bird, G.W.G. and J. Wingham. 1971. Some serological properties of the Cad receptor. *Vox Sang.* **20:** 55–61

Blumenfeld, O.O. and A.M. Adamany. 1978. Structural polymorphism within the amino-terminal region of MM, NN and MN glycoproteins (glycophorins) of the human erythrocyte membrane. *Proc. Nat. Acad. Sci. USA* **75:** 2727–2731

Blumenfeld, O. and K.V. Puglia. 1979. Preparation of cyanogen bromide fragments of MM, NN and MN glycoproteins (glycophorins) from human erythrocyte membranes of single donors. *Biochim. Biophys. Acta* **579:** 95–106

Blumenthal, G. and H.J. Pettenkofer. 1952. Über das neuentdecke Anti-$Duffy^b$ (Fy^b). *Z. Immunitaetsforsch Exp. Ther.* **109:** 267–273

Boettcher, B. 1964. The Rh 'delection' phenotypes and the information they provide about the Rh genes. *Vox Sang.* **9:** 641–652

Boettcher, B. 1966. Modification of Bernstein's multiple allele theory for the inheritance of the ABO blood groups in the light of modern genetical concepts. *Vox Sang.* **11:** 129–136

Boettcher, B. 1967a. Precipitation of a substance in salivas from A_1 and A_2 secretors. *Aust. J. Exp. Biol. Med. Sci.* **45:** 485–493

Boettcher, B. 1967b. Correlations between inhibition titres of blood group substances in salivas from A_1, A_2, and B secretors. *Aust. J. Exp. Biol. Med. Sci.* **45:** 495–506

Boettcher, B. 1967c. ABO blood group agglutinins in saliva. *Acta Haematol.* **38:** 351–360

Boettcher, B. 1968. Correlation between human ABO blood group antigens in seminal plasma and on seminal spermatozoa. *J. Reprod. Fertil.* **16:** 49–54

Boettcher, B. and D.J. Kay. 1973. ABO blood grouping of human hair using radioactively-labelled antibodies. *Vox Sang.* **25**: 420–425

Boettcher, B. and R. Kenny. 1971. A quantitative study of Le^a, A and H antigens in salivas of Australian Caucasians and Aborigines. *Human Hered.* **21**: 334–345

Boettcher, B. and S. Watts. 1978. Studies on blood from the original Rh_{null} proposita and relatives. *Vox Sang.* **34**: 339–342

Bohne. 1913. Ueber Isoagglutinine. *Vierteljahrschr. Gerichtl. Med. Oeff. Sanitaetswes.* **45** (3F) I Suppl: 254–261

Bond, C.J. 1919. The physical state of the blood serum in relation to its agglutinin and antibody content: the effect of friction and pressure. *Br. Med. J.* **1**: 729–733

Boorman, K.E. and B.E. Dodd. 1970. *An Introduction to Blood Group Serology,* 4th ed., J & A Churchill, London

Boorman, K.E., B.E. Dodd and B.E. Gilbey. 1948. A serum which demonstrates the co-dominance of the blood-group gene *O* with *A* and *B*. *Ann. Eugenics* **14**: 201–208

Boorman, K.E., B.E. Dodd and P.J. Lincoln. 1977. *Blood Group Serology, Theory, Techniques, Practical Applications.* 5th ed., Churchill Livingstone, Edinburgh, London and N.Y.

Booth, P.B. 1971. Anti-N^A. An antibody sub-dividing Melanesian N. *Vox Sang.* **21**: 522–530

Bove, J.R., F.H. Allen, Jr., P. Chiewsilp, W.L. Marsh and T.E. Cleghorn. 1971. Anti-Lu4: a new antibody related to the Lutheran blood group system. *Vox Sang.* **21**: 302–310

Bove, J.R., A.M. Holburn and P.L. Mollison. 1973. Non-specific binding of IgG to antibody-coated red cells (The "Matuhasi-Ogata Phenomenon"). *Immunology* **25**: 793–801

Bowen, A.B., A.L. Haist, L.L. Talley, M.E. Reid and W.L. Marsh. 1972. Further examples of the Lutheran Lu(-5) blood type. *Vox Sang.* **23**: 201–204

Boyd, W.C. 1939. Blood groups. *Tabulae Biol.* **17**: 113–240

Boyd, W.C. and L.G. Boyd. 1937. Blood grouping in forensic medicine. *J. Immunol.* **33**: 159–172

Boyd, W.C., M.H. McMaster and E. Waszczenko-Zacharczenko. 1959. Specific inhibition of anti-Rh serum by 'unnatural' sugars. *Nature* **184**: 989–990

Brahn, B. and F. Schiff. 1929. Das chemische Verhalten der serologischen Gruppenstoffe A and B, ihr Vorkommen and ihr Nachweis in Körperflüssigkeiten. *Klin. Wochenscher.* **3**(33): 1523–1525

Brahn, B., F. Schiff and F. Weinmann. 1932. Über die chemische Natur der gruppen-Substanz A. *Klin. Wochenscher.* **11**(38): 1592

Brain, P. 1966. Subgroups of A in the South African Bantu. *Vox Sang.* **11**: 686–698

Bray, H.G., H. Henry and M. Stacey. 1946. Chemistry of tissues. 2. Polysaccharides showing blood group A-specificity and the nature of the constituent units of the stable carbohydrate residue of the A substance from pepsin. *Biochem. J.* **40**: 124–130

Brendemoen, O.J. 1949. Studies of agglutination and inhibition in two Lewis antibodies. *J. Lab. Clin. Med.* **34**: 538–542

Brendemoen, O.J. 1950. Further studies o)f agglutination and inhibition in the Le^a-Le^b system. *J. Lab. Clin. Med.* **36**: 335–341

Brewer, C.A., P.L. Cropp and L.E. Sharman. 1976. A low ionic strength, hemagglutinating, AutoAnalyzer for Rhesus typing of dried bloodstains. *J. Forensic Sci.* **21**: 811–815

Brocteur, J. 1968. L'importance médico-légale du facteur de groupe sanguin M^g. *Med. Leg. Domm. Corpor.* **1**: 56–63

Broman, B., A. Heiken, P.A. Tippett and C.M. Giles. 1963. The D(C)(e) gene complex revealed in the Swedish population. *Vox Sang.* **8**: 588–593

Brown, F., S. Simpson, S. Cornwall, B.P.L. Moore, R. Øyen and W.L. Marsh. 1974. The recessive Lu(a–b–) phenotype. A family study. *Vox Sang.* **26**: 259–264

Brown, P.C., L.E. Glynn and E.J. Holborow. 1959. Lewis ^{a} substance in saliva. A quantitative difference between secretors and non-secretors. *Vox Sang.* **4**: 1–12

Brunner, L. 1927. Die Isoagglutination im eingetrockneten Blute und das Agglutininanreicherungsverfahren von M.A. Müller. Inaugural-Dissert., Univ. Zurich Faculty of Medicine

Buchanan, J.A. 1922a. Medicolegal application of the blood groups. *J. Am. Med. Assoc.* **78**: 89–92

Buchanan, J.A. 1922b. Medicolegal application of the blood groups. *J. Am. Med. Assoc.* **79**: 180–181

Buckwalter, J.A. and L.A. Knowler. 1958. Blood donor controls for blood group disease research. *Am. J. Hum. Genet.* **10**: 165–174

Buckwalter, J.A., G.S. Naifeh and J.E. Auer. 1962. Rheumatic fever and the blood groups. *Br. Med. J.* **2**: 1023–1027

Buckwalter, J.A., R.T. Tidrick, L.A. Knowler, E.B. Wohlwend, D.C. Colter, J.H. Turner, L. Raterman, H.H. Gamber, G.J. Roller, C.B. Pollock and V.K. Naifeh. 1958. The Iowa blood type disease research project. *J. Iowa State Med. Assoc.* **48**: 76–81

Budvári, R. 1963. Über eine neue Methode Blutgruppenuntersuchungen in Blutflecken mittels Agglutininelution. *Dtsch. Z. Gesamte Gerichtl. Med.* **54**: 24–26

Bunker, H.A. and S. Meyers. 1927. Blood groups in general paralysis. A study of the agglutinin-agglutinogen formula in ninety-one cases. *J. Lab. Clin. Med.* **12**: 415–426.

Burke, R. and C.S. Tumosa. 1978. The persistence of K and Fy^a antigens in bloodstains. *Forensic Serol. News* **4**(3) §[a]

Burnet, M. 1959. *The Clonal Selection Theory of Acquired Immunity,* Cambridge Univ. Press, Cambridge, and Vanderbilt Univ. Press, Nashville

Butts, D.C.A. 1955. Blood groups of the Bush Negroes of Surinam. *Doc. Med. Geogr. Trop.* **7**: 43–49 cited by Mourant *et al.* (1976)

Cahan, A., J.A. Jack, J. Scudder, M. Sargent, R. Sanger and R.R. Race. 1957. A family in which A_x is transmitted through a person of the blood group A_2B. *Vox Sang.* **2**: 8–15

Callender, S.T. and R.R. Race. 1946. A serological and genetical study of multiple antibodies formed in response to blood transfusion by a patient with lupus erythematosus diffusus. *Ann. Eugenics* **13**: 102–117

Callender, S. R.R. Race and Z.V. Paykoc, 1945. Hypersensitivity to transfused blood. *Br. Med. J.* **2**: 83–84

Cameron, C., F. Graham, I. Dunsford, G. Sickles, C.R. MacPherson, A. Cahan, R. Sanger and R.R. Race. 1959. Acquisition of a B-like antigen by red blood cells. *Br. Med. J.* **2**: 29–32

Cameron, G.L. and J.M. Staveley. 1957. Blood group P substance in hydatid cyst fluid. *Nature* **179**: 147–148

Cameron, J.W. and L.K. Diamond. 1945. Chemical, clinical and immunological studies on the products of human plasma fractionation. XXIX. Serum albumin as a diluent for Rh typing reagents. *J. Clin. Invest.* **24**: 793–801

Camp, F.R., Jr., F.R. Ellis and N.F. Conte. 1974. *Selected Contributions to the Literature of Blood Groups and Immunology,* 2nd ed., The Translation and Reproduction of Scientific Treatises in Blood Group Immunology 1963–1974, U.S. Army Medical Research Laboratory, Blood Bank Center, Ft. Knox, KY in 5 volumes with two monographs:

I. ABO System. Dunsford Memorial
II. Secretion of Blood Group Substances and Lewis System
III. M, N and P Systems
IV. Anthropologic and Other Applications of Blood Grouping Data
V. Landsteiner Centennial
Arne Gammelgarrd Thesis
Grethe Hartmann Monograph

Cappell, D.F. 1944. The significance of the Rh factor in medicine and obstetrics. *Glasgow Med. J.* **142** (7 ser. **24**): 125–155

Carne, L.R. and W.M. Watkins. 1977. Human blood group *B* gene-specified α-3-galactosyltransferase; purification of the enzyme in serum by biospecific absorption onto blood group O erythrocyte membranes. *Biochem. Biophys. Res. Comm.* **77**: 700–707

Cartron, J.P., A. Gerbal, N.C. Hughes-Jones and C. Salmon. 1974. 'Weak A' phenotypes. Relationship between red cell agglutinability and antigen site density. *Immunology* **27**: 723–727

Casey, A.E., C. Phillips, K. Hale, B.R. Kynerd and E. Downey. 1968. ABO, MN and Rh blood groups among north Alabama Negroes. *Alabama J. Med. Sci.* **5**: 209–212

Cazal, P. and M. Lalurie. 1952. Recherches sur quelques phytoagglutinines spécifiques des groupes sanguins ABO. *Acta Haematol.* **8**: 73–80

Cazal, P. and M. Mathieu. 1950. Recherches sur les groupes sanguines du système PQ. *Sang* **21:** 717–727

Cazal, P., M. Monis and M. Bizot. 1971. Les antigènes Cad et leurs rapports avec les antigènes A. *Rev. Fr. Transf.* **14:** 321–334

Cazal, P., M. Monis, J. Caubel and J. Brives. 1968. Polyagglutinabilité héréditaire dominante: antigène privé (Cad) correspondant à un anticorps public et à une lectine de *Dolichos biflorus. Rev. Fr. Transf.* **11:** 209–211

Cegla, Z. and B. Popielski. 1976. Erfahrungen mit ABO Blutgruppenbestimmung in menschlichen Haaren. *Forensic Sci.* **7:** 185 [Abstr.]

Ceppellini, R. 1959. Physiological genetics of human blood factors, *in*: Ciba Foundation Symp. on *Biochemistry of Human Genetics,* 242–263, Little, Brown & Co., Boston

Ceppellini, R., L.C. Dunn and F. Innella. 1959. Immunogenetica. II. Analisi genetica formale dei carrateri Lewis con particolare riguardo alla natura epistatica della specificita serologica Le^b. *Folia Hered. Pathol.* **8:** 261–296

Cepellini, R., E.W. Ikin and A.E. Mourant. 1950. A new allele of the Rh gene E. *Boll. Ist. Sieroterap. Milano* **29:** 123–124

Cepellini, R., S. Nasso and F. Tecilazich. 1952. *La Malattia Emolitica del Neonato,* Istituto Sieroterapico Milanese Serafino Belfanti, Milano

Ceppellini, R. and M. Siniscalco. 1955. Una nuova ipotesi genetica per il sistema Lewis secretore e suoi riflessi nei riguardi di alcune evidenze di linkage con altri loci. *Riv. Ist. Sieroterap. Ital.* **30:** 431–455 and see Camp *et al.,* 1974

Chakraborty, R., M. Shaw and W.J. Schull. 1974. Exclusion of Paternity: the current state of the art. *Am. J. Hum. Genet.* **26:** 477–488

Chan-Shu, S.A. and O. Blair. 1979. A new method of antibody elution from red blood cells. *Transfusion* (Phila) **19:** 182–185

Charney, M. 1969. ABO blood groups and asthma - a suspected correlation. Thesis. Univ. of Colorado. cited by Mourant *et al.* (1976)

Chatterji, P.K. 1977. A simplified mixed agglutination technique for ABO grouping of dried bloodstains using cellulose acetate sheets. *J. Forensic Sci. Soc.* **17:** 143–144

Chattoraj, A. and W.C. Boyd. 1965. Inhibition of hemagglutinins by amino acids. *Vox Sang.* **10:** 700–707

Chisum, W.J. 1971. A rapid method for grouping dried bloodstains. *J. Forensic Sci. Soc.* **11:** 205–206

Chown, B., M. Lewis and K. Kaita. 1957. A 'new' Kell blood-group phenotype. *Nature* **180:** 711

Chown, B., M. Lewis and K. Kaita. 1965a. The Duffy blood group system in Caucasians: evidence for a new allele. *Am. J. Hum. Genet.* **17:** 384–389

Chown, B., M. Lewis and K. Kaita. 1965b. The Kidd blood group system in Caucasians. *Transfusion* (Phila) **5:** 506–507

Chown, B., M. Lewis and K. Kaita. 1971. The Rh system. An anomaly of inheritance, probably due to mutation. *Vox Sang.* **21:** 385–396

Clark, G. 1973 (ed) *Staining Procedures Used By the Biological Stain Commission,* 3rd ed., Williams and Wilkins, Baltimore

Clarke, C.A., R.B. McConnell and P.M. Sheppard. 1960. A genetical study of the variations in ABH secretion. *Ann. Hum. Genet.* **24:** 295–301

Clausen, Johs. 1933. Über die serologischen Eigenschaften M und N und ihre Bedeutung in der Gerichtsmedizin. *Z. Rassenphysiol.* **6:** 49–65

Cleghorn, T.E. 1966. A memorandum on the Miltenberger blood groups. *Vox Sang.* **11:** 219–222

Closon, J. 1949. L'antigène Rh dans le sang désséché. *Acta Med. Leg. Social.* **2:** 227–230

Coca, A.F. and H. Klein. 1923a. A hitherto undescribed pair of isoagglutination elements in human beings. *Proc. Soc. Exp. Biol. Med.* **20:** 466–468

Coca, A.F. and H. Klein. 1923b. A hitherto undescribed pair of isoagglutination elements in human beings. *J. Immunol.* **8:** 477–485

Cohen, E., S.C. Roberts, S. Nordling and G. Uhlenbruck. 1972. Specificity of *Limulus polyphemus* agglutinins for erythrocyte receptor sites common to M and N antigenic determinants. *Vox Sang.* **23:** 300–307

Cohen, F. and W.W. Zuelzer. 1965. Interrelationship of the various subgroups of the blood group A: study with immunofluorescence. *Transfusion* (Phila) **5:** 223–229

Committee on Medicolegal Problems, American Medical Association. 1952. Medicolegal application of blood grouping tests. *J. Am. Med. Assoc.* **149:** 699–706

Committee on Medicolegal Problems, American Medical Association. 1956. Medicolegal applications of blood grouping tests. Final Report. *J. Am. Med. Assoc.* **164:** 2036–2044

Colledge, K.I., M. Pezzulich and W.L. Marsh. 1973. Anti-Fy5, an antibody disclosing a probable association between the Rhesus and Duffy blood group genes. *Vox Sang.* **24:** 193–199

Contreras, M., B. Stebbing, M. Blessing and J. Gavin. 1978. The Rh antigen Evans. *Vox Sang.* **34:** 208–211

Contreras, M. and P. Tippett. 1974. The Lu(a-b-) syndrome and an apparent upset of P_1 inheritance. *Vox Sang.* **27:** 369–371

Coombs, R.R.A. and D. Bedford. 1955. The A and B antigens on human platelets demonstrated by means of mixed erythrocyte-platelet agglutination. *Vox Sang.* **5:** (O.S.): 111–115 §²

Coombs, R.R.A., D. Bedford and L.M. Rouillard. 1956. A and B bloodgroup antigens on human epidermal cells demonstrated by mixed agglutination. *Lancet* **1:** 461–463

Cooms, R.R.A. and B. Dodd. 1961. Possible application of the principle of mixed agglutination in the identification of blood stains. *Med. Sci. Law* **1:** 359–377

Coombs, R.R.A., A.E. Mourant and R.R. Race 1945a. Detection of weak and "incomplete" Rh agglutinins: a new test. *Lancet* **2:** 15–16

Coombs, R.R.A., A.E. Mourant and R.R. Race. 1945b. A new test for the detection of weak and "incomplete" Rh agglutinins. *Br. J. Exp. Pathol.* **26:** 255–266

Coombs, R.R.A., A.E. Mourant and R.R. Race. 1946. In-vivo isosensitization of red cells in babies with haemolytic disease. *Lancet* **1:** 264–266

Cooper, A.J., B.S. Blumberg, P.L. Workman and J.R. McDonough. 1963. Biochemical polymorphic traits in a U.S. White and Negro population. *Am. J. Hum. Genet.* **15:** 420–428

Cory, H.T., A.D. Yates, A.S.R. Donald, W.M. Watkins and W.T.J. Morgan. 1974. The nature of the human blood group P_1 determinant,. *Biochem. Biophys. Res. Comm.* **61:** 1289–1296

Crawford, M.N., T.J. Greenwalt, T. Sasaki, P. Tippett, R. Sanger and R.R. Race. 1961. The phenotype Lu(a–b–) together with unconventional Kidd groups in one family. *Transfusion* (Phila.) **1:** 228–232

Crawford, M.N., P. Tippett and R. Sanger. 1974. Antigens Au^a, i and P_1 of cells of the dominant type of Lu(a-b-). *Vox Sang.* **26:** 283–287

Crome, W. 1935. Über Blutgruppenfragen: Mutter M. Kind N. *Dtsch. Z. Gesamte Gerichtl. Med.* **24:** 167–175 and see Camp *et al.,* 1974

Culliford, B.J. 1971 *The Examination and Typing of Bloodstains in the Crime Laboratory,* U.S. Government Printing Office, Washington, D.C.

Cutbush, M. and I. Chanarin. 1956. The expected blood-group antibody anti-Lu^b. *Nature* **178:** 855–856

Cutbush, M., E.R. Giblett and P.L. Mollison. 1956. Demonstration of the phenotype Le(a+b+) in infants and in adults. *Br. J. Haematol.* **2:** 210–220

Cutbush, M. and P.L. Mollison. 1950. The *Duffy* blood group system. *Heredity* **4:** 383–389

Cutbush, M., P.L. Mollison and D.M. Parkin. 1950. A new human blood group. *Nature* **165:** 188–189

Dahr, P. 1938. Untersuchungen über die Moglichkeit einer serologischen Unterscheidung homozygoter und heterozygoter A- und B-Menschen. *Z. Immunitaetsforsch. Exp. Ther.* **92:** 180–220

Dahr, P. 1940. Erblichkeitsuntersuchungen über den Blutfaktor P in Familien and Zwillingen. *Z. Immunitaetsforsch. Exp. Ther.* **97:** 168–188 and see Camp *et al.,* 1974

Dahr, P. 1942a. Über die bisher im Kölner Hygienischen Institut gewonnen Untersuchungsergebnisse über das Blutmerkmal P. *Z. Immunitaetsforsch. Exp. Ther.* **101:** 346–355 and see Camp *et al.,* 1974

Dahr, P. 1942b. Zur Serologie der Blut-Untergruppen A_1 und A_2. *Z. Immunitaetsforsch. Exp. Ther.* **102:** 98–112

Dahr, P. 1953. *Technik der Blutgruppen- und Blutfaktoren Bestimmung,* 6th Aufl., Georg Thieme Verlag, Stuttgart

Dahr, W., P. Issitt, J. Moulds and B. Pavone. 1978. Further studies on the

membrane glycoprotein defects of S-s- and En(a-)-erythrocytes. *Hoppe-Seyler's Z. Physiol. Chem.* **359:** 1217–1224

Dahr, W. and G. Uhlenbruck. 1978. Structural properties of the human MN blood group antigen receptor sites. *Hoppe-Seyler's Z. Physiol. Chem.* **359:** 835–843

Dahr, W., G. Uhlenbruck and G.W.G. Bird. 1975b. Influence of free amino and carboxyl groups on the specificity of plant anti-N. *Vox Sang.* **28:** 389–391

Dahr, W., G. Uhlenbruck, P.D. Issitt and F.H. Allen, Jr. 1975c. SDS-polyacrylamide gel electrophoretic analysis of the membrane glycoproteins from S-s-U- erythrocytes. *J. Immunogenet.* **2:** 249–251

Dahr, W., G. Uhlenbruck, E. Janssen and R. Schmalisch. 1977. Different N-terminal amino acids in the MN-glycoprotein from MM and NN erythrocytes. *Human Genet* **35:** 335–343

Dahr, W., G. Uhlenbruck and H. Knott. 1975a. Immunochemical aspects of the MNSs-blood group system. *J. Immunogenet.* **2:** 87–100

Darnborough, J., R. Firth, C.M. Giles, K.L.G. Goldsmith and M.N. Crawford, 1963. A 'new' antibody anti-Lu^aLu^b and two further examples of the genotype Lu(a-b-). *Nature* **198:** 796

Davidsohn, I. 1972. Early immunologic diagnosis and prognosis of carcinoma. *Am. J. Clin. Pathol.* **57:** 715–730

Davidsohn, I., K. Stern, E.R. Strauser and W. Spurrier. 1953. Be, a new "private" blood factor. *Blood* **8:** 747–754

Davie, M.J. 1979. Lewis grouping on microtitre plates. *HOCRE Rept.* No. 320, Home Office Central Res. Establ., Aldermaston, Reading, Berks., England

Davie, M.J., M.J. Dorrill and P.H. Whitehead. 1979. A quantitative survey of ABH blood group substances in semen. *HOCRE Rept.* No. 318, Home Office Central Res. Establ., Aldermaston, Reading, Berks., England

Day, D., H.A. Perkins and B. Sams. 1965. The minum-minus phenotype in the Kidd system. *Transfusion* (Phila.) **5:** 315–319

Dell'Erba, A. and L. Ambrosi. 1957. Il metodo elettroforetico in ematologia forense. II. La diagnosi individuale su macchie di sangue. *G. Med. Leg. Infortun. Tossicol.* **3:** 212–235

Dell'Erba, A. and L. Ambrosi. 1960. A proposito della diagnosi individuale su macchie di sangue mediante elettroforesi su carte. *Minerva Medicoleg.* **80:** 164–165

DeNatale, A., A. Cahan, J.A. Jack, R.R. Race and R. Sanger. 1955. V, a "new" Rh antigen, common in Negroes, rare in White people. *J. Am. Med. Assoc.* **159:** 247–250

Denault, G.C., H.H. Takimoto, Q.Y. Kwan and A. Pallos. 1978. *Detectability of Selected Genetic Markers in Dried Blood Upon Aging,* National Inst. Law Enforcement Crim. Just., LEAA, U.S. Dept. of Justice, Washington, D.C and see Denault *et al.,* 1980

Denault, G.C., H.H. Takimoto, Q.Y. Kwan and A. Pallos. 1980. Detectability of selected genetic markers in dried blood on aging. *J. Forensic Sci.* **25:** 479–498

DeRen, G., G. Petiet, F. Streiff and B. Genetet. 1970. Difficultés rencontrées dans la determination des antigènes de groupes sanguins des taches de sang sec. *Med. Leg. Domm. Corpor.* **3:** 71–73

Dervieux, (F.). 1921. Procédé de diagnostic individuel du sang et du sperme. *C. R. Acad. Sci.* **172:** 1385–1386

Dervieux, (F.). 1923. Notes sur un nouveau sérum précipitant préparee en vue de l'individualisation du sang et du sperme. *Ann. Med. Leg. Criminol. Police Scientif.* **3:** 454–458

Diamond, L.K. and N.M. Abelson. 1945. The demonstration of anti-Rh agglutinins—an accurate and rapid slide test. *J. Lab. Clin. Med.* **30:** 204–212

Diamond, L.K. and R.L. Denton. 1945. Rh agglutination in various media with particular reference to the value of albumin. *J. Lab. Clin. Med.* **30:** 821–830

Dichupa, P.J., C. Anderson and B. Chown. 1969. A further search for hypothetic K^p of the Kell-system. *Vox Sang.* **17:** 1–4

Dillon, S.I. 1971. A, B and H group specific substances in cerumen. *J. Crim. Law Criminol. Police Sci.* **61:** 573–575

Dodd, B.E. 1952. Linked anti-A and anti-B antibodies from group O sera. *Br. J. Exp. Pathol.* **33:** 1–18

Dodd, B.E. 1957. Further studies of antibody molecules having both A and B specificity, *in: Trans. 6th Congr. Europ. Soc. Haematol.,* Copenhagen, Part 2: 733–739, S. Karger, Basel and N.Y.

Dodd, B.E. 1972. Some recent advances in forensic serology. *Med. Sci. Law* **12:** 195–199

Dodd, B.E. 1980. When blood is their argument. *Med. Sci. Law* **20:** 231–238

Dodd, B.E. and D. Hunter. 1963. Saliva stains—A comparison between the inhibition and mixed agglutination techniques for the detection of A, B and H, *in: Forensic Immunology, Medicine, Pathology and Toxicology,* Excerpta Medica Int. Cong. Ser. No. 80, Excerpta Medica Foundation, Amsterdam, New York, etc.

Dodd, B.E. and P.J. Lincoln. 1978. An analysis of 1,556 cases of doubtful parentage submitted for blood group investiation. *Med. Sci. Law* **18:** 185–190

Dodd, B.E. and P.J. Lincoln. 1979. The application of blood groups and other genetic polymorphisms to problems of parentage. *Biotest Bull.,* No. 5 (D. Tills, ed.), pp. 1–10, Biotest Serum Inst., GmbH, Frankfurt a. M.

Dodd, B.E., P.J. Lincoln and K.E. Boorman. 1967. The cross-reacting antibodies of group O sera; immunological studies and possible explanation of the observed facts. *Immunology* **12:** 39–52

Dodd, M.C., N.J. Bigley and V.B. Geyer. 1960. Specific inhibition of Rh_o (D) antibody by sialic acids. *Science* **132:** 1389–1399

Dodd, M.C., N.J. Bigley, G.A. Johnson and R.H. McCluer. 1964. Chemical aspects of inhibitors of Rh_o (D) antibody. *Nature* **204:** 549–552

Dorrill, M.J. and P.H. Whitehead. 1979. A quantitative study of ABH blood group substances in saliva and semen. *Forensic Sci. Int.* **14:** 11 [Abstr.]

Douglas, R. and J.M. Staveley. 1969. Rh and Kell typings of dried bloodstains. *J. Forensic Sci.* **14:** 255–262

Driesen, H.H. and M. Keller. 1973. Beitrag zur Erfassung der Antigene M und N in angetrockneten Blutspuren. *Arch. Kriminol.* **151:** 180–183

Dublin, T.D., A.D. Bernanke, E.L. Pitt, B.F. Massell, F.H. Allen, Jr., and F. Amezcua. 1964. Red blood cell groups and ABH secretor system as genetic indicators of susceptibility to rheumatic fever and rheumatic heart disease. *Br. Med. J.* **2:** 775–779

Ducos, J. 1958. La détermination des antigènes de groupes sanguins dans les taches de sang sec. *Vox Sang.* **3:** 385–392

Ducos, J. 1960. Utilisation d'hématies papainisées pour la detection des anticorps anti-A et anti-B dans les taches de sang sec. *Ann. Med. Leg.* **40:** 519–521

Ducos, J., Y. Marty and Ph. Lefevre-Withier. 1969a. Mise en évidence de l'antigène V^w dans les taches de sang sec. *Rev. Fr. Transf.* **12,** Suppl to No. 1: 189–193

Ducos, J., Y. Marty and Ph. Lefevre-Withier. 1969b. Mise en évidence des facteurs I et i dans les taches de sang sec. *Rev. Fr. Transf.* **12,** Suppl. to No. 1: 195–201

Ducos, J., Y. Marty, J. Ruffie, J. Gavin, P. Teesdale and P. Tippett. 1974. Cited as "in preparation" by Race and Sanger, 1975

Ducos, J. and J. Ruffié. 1954. Recherches médico-légales des antigènes sanguins du type Rhésus dans les taches de sang sec. *Acta Med. Leg. Social.* **7:** 111–119

Dunsford, I. 1952. Group A blood weaker in reaction than A_1. *Bull. Cent. Lab. Bloedtransf. Nederlandse Rode Kruis* **2**(4): 209–219 §²

Dunsford, I. 1959. A critical review of the ABO subgroups, in: Proc. 7th Congr. Int. Soc. Blood Transf., Rome, 1958, *Bibliotheca Haematol.,* Fasc. **10:** 685–691

Dunsford, I. and P. Aspinall. 1952. An A_x cD^ue/cde blood in an English family. *Ann. Eugenics* **17:** 30–34

Dupont, M. 1934. Contribution a l'étude des antigènes des globules rouges. *Arch. Int. Med. Exp.* **9:** 133–167

Dybkjaer, E., K. Lylloff and P. Tippett. 1974. Weak Lu^9 antigen in one Lu:-6 member of a family. *Vox Sang.* **26:** 94–96

Dyke, S.C. 1922a. On the medico-legal importance of the blood groups. *Lancet* **203** (2 for 1922): 1271–1272

Dyke, S.C. 1922b. On isohaemagglutination. *Br. J. Exp. Pathol.* **3:** 146–150

Dyke, S.C. 1930. The significance of blood groups. Part II. *Lancet* **218**(1 for 1930): 1029–1030

Dzierzkowa-Borodej, W. and D. Voak. 1979. Subtypes of i demonstrated by the use of a typical Ii cell types and inhibition studies. *Br. J. Haematol.* **41:** 105–113

Eaton, B.R., J.A. Morton, M.M. Pickles and K.E. White. 1956. A new antibody, anti-Yt^a, characterising a blood-group antigen of high incidence. *Br. J. Haematol.* **2:** 333–341

Economidou, J., N.C. Hughes-Jones and B. Gardner. 1967. Quantitative measurements concerning A and B antigen sites. *Vox Sang* **12:** 321–328

Editorial. 1976. An independent witness required. *J. Forensic Sci. Soc.* **16:** 180

Edwards, J.H. 1968. The Rhesus locus. *Vox Sang.* **15:** 392–395

Eisele, R. 1973. Über den Einfluss von Fremdsubstanzen auf die ABO-Bestimmung. *Beitr. Gerichtl. Med.* **31:** 283–286

Eisler, M. 1930. ueber ein gemeinsames Antigen in den Zellen des Menschen und in Schigabazillen. *Z. Immunitaetsforsch. Exp. Ther.* **67:** 38–48

Eisler, M. 1931. Weitere Untersuchungen über des Menschenblutantigen in Shigabakterien und seine Verhältnis zu deren Forssman Antigen. *Z. Immunitaetsforsch. Exp. Ther.* **70:** 48–57

Elliot, M., E. Bossom, M.E. Dupuy and S.P. Masouredis. 1964. Effect of ionic strength on the serologic behavior of red cell isoantibodies. *Vox Sang.* **9:** 396–414

Ellis, F.R. and L.P. Cawley. 1958. A third instance of agglutinogen A_0. Report of a family study, *in*: Proc. 6th Congr. Int. Soc. Blood Transf., Boston, 1956, *Bibliotheca Haematol.*, Fasc. **7:** 135

Engelfriet, C.P., D. Beckers, A.E.G. Kr. von dem Borne, E. Reynierse and J.J. van Loghem. 1971. Haemolysins probably recognizing the antigen p. *Vox Sang.* **23:** 176–181

Epstein, A.A. and R. Ottenberg. 1908. A simple method of performing serum reactions. *Proc. N.Y. Pathol. Soc.* **8**(N.S.): 117–123

Estola, E. and J. Elo. 1952. Occurrence of an exceedingly weak "A" blood group property in a family. *Ann. Med. Exp. Biol. Fenn.* **30:** 79–87

Etzler, M.E. and E.A. Kabat. 1970. Purification and characterization of a lectin (plant hemagglutinin) with blood group A specificity from *Dolichos biflorus. Biochemistry* **9:** 869–877

Fairbanks, G., T.L. Steck and D.F.H. Wallach. 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. *Biochemistry* **10:** 2606–2617

Faraone, G. 1941. Sulle cause della restituzione aspecifica delle isoagglutinine nella dimostrazione della sostanza isoagglutinabile delle macchie di sangue. *Zacchia* **5** (2 ser.): 1–20

Faraone, G. 1942. Su di tecnica di micro-estrazione a caldo delle agglutinine nelle macchie di sangue. *Zacchia* **6** (2 ser.): 166–178

Feizi, T., R.A. Childs, S-I. Hakomori and M.E. Powell. 1978. Blood-group-Ii-active gangliosides of human erythrocyte membranes. *Biochem. J.* **173:** 245–254

Feizi, T., E.A. Kabat, G. Vicari, B. Anderson and W.L. Marsh. 1971a. Immunochemical studies on blood groups. XLVII. The I antigen complex—precursors in the A, B, H, Le^a and Le^b blood group system-hemagglutination inhibition studies. *J. Exp. Med.* **133:** 39–52

Feizi, T., E.A. Kabat, G. Vicari, B. Anderson and W.L. Marsh. 1971b. Immunochemical studies on blood groups. XLIX. The I antigen complex: specificity differences among anti-I sera revealed by quantitative precipitin studies; partial structure of the I determinant specific for one anti-I serum. *J. Immunol.* **103:** 1578–1592

Fellous, M., A. Gerbal, C. Tessier, J. Frezal, J. Dausset and C. Salmon. 1974. Studies on the biosynthetic pathway of human P erythrocyte antigens using somatic cells in culture. *Vox Sang.* **26:** 518–536

Fernandez-Collazo, E. and E. Thierer. 1972. Action of ABO antisera on human spermatozoa. *Fertil. Steril.* **23:** 376–382

Fillitti-Wurmser, S., G. Aubel-Lesure and R. Wurmser. 1953a. Constantes de sedimentation des isoagglutinines $\beta(A_1O)$, $\beta(OO)$ et $\beta(A_1A_1)$. *J. Chim. Phys.* **50:** 236–239

Filitti-Wurmser, S., G. Aubel-Lesure and R.,Wurmser. 1953b. Étude des mélanges d'isoagglutinines humaines anti-B. *J. Chim. Phys.* **50:** 317–320

Filitti-Wurmser, S. and Y. Jacquot-Armand. 1947. Étude quantitative de l'isohémagglutination. Reversibilité de l'isohémagglutination. *Arch. Sci. Physiol.* **1:** 151–163

Filitti-Wurmser, S., Y. Jacquot-Armand, G. Aubel-Lesure and R. Wurmser. 1954. Physico-chemical study of human isohemagglutination. *Ann. Eugenics* **18:** 183–202

Filitti-Wurmser, S., Y. Jacquot-Armand and R. Wurmser. 1950. Sur la combinaison des isohémagglutinines avec les groupes agglutinogènes. *J. Chim. Phys.* **47:** 419–431

Filitti-Wurmser, S., Y. Jacquot-Armand and R. Wurmser. 1952. Sur l'agglutinogene B et les isoagglutinines $\beta(A_1O)$ et $\beta(OO)$. *J. Chim. Phys.* **49:** 550–556

Filitti-Wurmser, S., Y. Jacquot-Armand and R. Wurmser. 1953c. Sur l'isoagglutinine $\beta(A_1A_1)$. *J. Chim. Phys.* **50:** 240–242

Filitti-Wurmser, S., Y. Jacquot-Armand and R. Wurmser. 1960. Sur les isohémagglutinines naturelles du système A,B,O (Réponse à des critiques parues un livre de E.A. Kabat). *Rev. Hematol.* **15:** 201–216

Fiori, A. 1961. L'agglutinazione mista nell'identificazione degli antigeni ABO in traccie di sangue. *Med. Leg. Assicuraz.* **9:** 205–228

Fiori, A. 1963. Systematic procedure for micro-analysis of bloodstains, *in: Forensic Immunology, Medicine, Pathology and Toxicology,* Excerpta Medica Int. Congr. Ser. No. 80: 46–47, Excerpta Medica Foundation, Amsterdam, N.Y., etc.

Fiori, A. 1976. Technical remarks on the detection of ABH polymorphism in human body fluids. *Forensic Sci.* **7:** 91–94

Fiori, A. and P. Benciolini. 1972. The ABO grouping of stains from body fluids. *Z. Rechtsmed* **70:** 214–222

Fiori, A., G.V. Giusti and G. Panari. 1969. Mise en évidence de différents types moleculaires ABH dans la salive des sécréteurs. *Med. Leg. Domm. Corpor.* **2:** 364–366

Fiori, A., G.V. Giusti and G. Panari. 1971b. Gel filtration of ABH blood group substances. II. Individual gel chromatographic patterns of ABH substances in the saliva of secretors and nonsecretors. III. ABH gel filtration patterns of solubilised red cell stroma. *J. Chromatogr.* **55:** 351–363 and 365–375

Fiori, A., G.V. Giusti, G. Panari and G. Porcelli. 1971a. Gel filtration of ABH blood group substances. I. Fractionation of ABH substances of human saliva. *J. Chromatogr.* **55:** 337–349

Fiori, A., M. Marigo and P. Benciolini. 1963. Modified absorption-elution method of Siracusa for ABO and MN grouping of bloodstains. *J. Forensic Sci.* **8:** 419–455 and 535–567

Fiori, A., G. Panari, G.V. Giusti and G. Brandi. 1973. Gel filtration of ABH blood group substances. IV. Fractions 4 and 5 of ABH substances in human red cells and their secretion patterns in saliva. *J. Chromatogr.* **84:** 335–346

Fiori, A., G. Panari, G. Rossi and D. DeMercurio. 1978. Polymorphism of A, B and H substances in human urine. *J. Chromatogr.* **145:** 51–62

Fiori, A., A. Serra, G.P. Panari and G.V. Giusti. 1972. Studio su popolazioni e famiglie di sostanze a basso peso moleculare con specificita antigene A, B e H separate mediante gel-filtrazione da saliva e globuli rossi umani. *Atti Assoc. Genet. Ital.* **17:** 38–42

Fischer, W. and F. Hahn. 1935. Über auffallende Schwäche der gruppenspezifischen Reaktionsfähigkeit bei einem Erwachsenen. *Z. Immunitaetsforsch. Exp. Ther.* **84:** 177–188 and see Camp *et al.*, 1974

Fisher, N. and A. Cahan. 1962. An addition to the family in which A_x is transmitted through a person of the blood group A_2B. *Vox Sang.* **7:** 484

Fisher, R.A. 1947. The Rhesus factor. A study in scientific method. *Am. Scientist* **35:** 95–102 and 113

Fisher, R.A. 1953. The variation in strength of the human blood group P. *Heredity* **7:** 81–89

Fisher, R.A. and R.R. Race. 1946. *Rh* gene frequencies in Britain. *Nature* **157:** 48–49

Fisk, R.T. and A.G. Foord. 1942. Observations on the Rh agglutinogen of human blood. *Am. J. Clin. Pathol.* **12:** 545–552

Fitzsimmons, J.M. and P.A. Morel. 1979. The effects of red blod cell suspending media on hemagglutination and the antiglobulin test. *Transfusion* (Phila) **19:** 81–85

Fletcher, K.S., E.G. Bremer and G.A. Schwarting. 1979. P blood group regulation of glycosphingolipid levels in human erythrocytes. *J. Biol. Chem.* **254:** 11196–11198

Florence, A. 1904. Peut-on distinguer le sang d'un homme du sang d'un autre homme? *Arch. Anthropol. Crim. Criminol. Psychol. Norm. Pathol.* **19** (N.S. **3**): 215–219

Folkerd, E.J., J.C. Ellory and N.C. Hughes-Jones. 1977. A molecular size determination of Rh(D) antigen by radiation inactivation. *Immunochemistry* **14:** 529–531

Fox, R.H. 1974. Absorption-elution testing for ABO blood group factors: preliminary collaborative study. *J. Assoc. Off. Anal. Chem.* **57:** 666–667

Francis, B.J. and D.E. Hatcher. 1966. MN blood types. The S-s-U+ and the M_1 phenotypes. *Vox Sang.* **11:** 213–216

Franks, D. and R. Liske. 1968. The specificity of the cross-reacting antibodies in blood group O sera which produce mixed agglutination. *Immunology* **14:** 433–444

Friedenreich, V. 1928a. Fortgesetzte Untersuchungen über bakterielle Umformung der Iso-Agglutinatorischen Verhältnisse der Blutkörperchen in Vitro (O. Thomsen). *Acta Med. Scand.*, Supp. No. 26: 308–315

Freidenreich, V. 1928b. Untersuchungen über das von O. Thomsen beschriebene vermehrungsfähige Agens als Veränderer des isoagglutinatorischen Verhaltens der roten Blutkörperchen. *Z. Immunitaetsforsch. Exp. Ther.* **55:** 84–101

Friedenreich., V. 1930. *The Thomsen Hemagglutination Phenomenon.* Production of a specific receptor quality in red corpuscles by bacterial activity. Levin and Munksgaard, Copenhagen

Freidenreich, V. 1931. Ueber die Serologie der Untergruppen A_1 and A_2. *Z. Immunitaetsforsch. Exp. Ther.* **71:** 283–313

Friedenreich, V. 1936a. Über einen eigentümlichen A-Receptor-Typus und seine Erblichkeitsverhältnisse. *Klin. Wochenschr.* **15**(9): 310–311

Friedenreich, V. 1936b. Eine bisher unbekannte Blutgruppeneigenschaft (A_3). *Z. Immunitaetsforsch. Exp. Ther.* **89:** 409–422 and see Camp *et al.*, 1974

Friedenreich, V. 1936c. Ein erblicher defekter N-Receptor, der wahrscheinlich eine bisher unbekannte Blutgruppeneigenschaft innerhalb des MN-Systems darstellt. *Dtsch. Z. Gesamte Gerichtl. Med.* **24:** 358–368 and see Camp *et al.*, 1974

Friedenreich, V. 1937. Ueber die Auffassung von der Ausscheidung und Nichtausscheidung serologischer Gruppensubstanzen. *Z. Immunitaetsforsch. Exp. Ther.* **91:** 39–49

Freidenreich, V. and G. Hartmann. 1938. Über die Verteilung der Gruppenantigene im Organismus der sogenannten 'Ausscheider' und 'Nichtausscheider'. *Z. Immunitaetsforsch. Exp. Ther.* **92:** 141–151 and see Camp *et al.*, 1974

Freidenreich, V. and A. Zacho. 1931. Die Differentialdiagnose zwischen den "Unter"-Gruppen A_1 und A_2. *Z. Rassenphysiol.* **4:** 164–191

Fujiwara, K. 1930. Einige Erfahrungen mit der Blutgruppenbestimmung an Flecken in Kriminalfällen. *Dtsch. Z. Gesamte Gerichtl. Med.* **15:** 470–477

Funatsu, Y. 1975. Identification of ABO blood group from dental calculus by elution test. *Jpn. J. Leg. Med.* **29:** 1–9 §'

Funk, H.J. and W. Towstiak. 1965. A practical method for detecting ABO agglutinins and agglutinogens in dried bloodstains. *J. Forensic Sci.* **10:** 455–465

Furthmayr, H. 1798. Structural comparison of glycophorins and immunochemical analysis of genetic variants. *Nature* **271:** 519–524

Furuhata, T. 1927. On the heredity of the blood groups. *Japan Med.* World English edition of *Nippon-no-ikai* **7:** 197–208

Furuhata, T. 1929. A summarized review on the gen-hypothesis of the blood groups. *Am. J. Phys. Anthropol.* **13:** 109–130

Furuhata, T. and H. Hasebe. 1955. Der Q-Blutfaktor. *Dtsch. Z. Gesamte Gerichtl. Med.* **44:** 356–361

Furuhata, T. and E. Matsunaga. 1950. Studies on the heredity of the group-specific isoagglutinin in normal human serum. I. The heredity of anti-A agglutinin. II. The heredity of anti-B agglutinin. III. On the heredity of anti-Q agglutinin. IV. On the heredity of anti-C agglutinin. *Proc. Japan Acad.* **26**, Suppl. to No. 1: 35–38 and **26**(2): 39–40 and **26**(3): 87–88 and 89–90

Galli-Valerio, B. 1905. Die Agglutination der roten Blutkörperchen des Menschen durch homologe und heterologe Sera and ihre Verwendung in der gerichtlichen Medicin. *Allgemeine Med. Central-Ztg.* **74**(3): 41–43

Gammelgaard, A. 1942. Om sjaeldne, svage A-receptorer (A_3, A_4, A_5 og A_x) hos menneskret. Nyt Nordisk Forlag. Arnold Busck, Copenhagen, and see Camp *et al.*, 1974

Gammelgaard, A. and P.V. Marcussen. 1940. Nachweis eines vierten allelomorphen A-Gens (A_4). *Z. Immunitaetsforsch. Exp. Ther.* **98:** 411–419

Ganaway, R.L. and P. Lux. 1978. ABO, PGM, EAP and Hp distribution in three ethnic groups in Bexar County, Texas. *Forensic Serol. News* **4**(1) §'

Gardas, A. 1978. Structure of an (A-blood-group)-active glycolipid isolated from human erythrocytes. *Eur. J. Biochem.* **89:** 471–473

Gardas, A. and J. Koscielak. 1974. I-active antigen of human erythrocyte membrane. *Vox Sang.* **26:** 227–237

Gavin, J., G.L. Daniels, H. Yamaguchi, Y. Okubo and T. Seno. 1979. The red cell antigen once called Levay is the antigen Kp^c of the Kell system. *Vox Sang.* **36:** 31–33

Gedde-Dahl, T., Jr., A.L. Grimstad, S. Gundersen and E. Vogt. 1967. A probably crossing over or mutation in the MNSs blood group system. *Acta Genet. Statist. Med.* **17:** 193–210

Gelpi, A.P. and M-C. King. 1976. Duffy blood group and malaria. *Science* **191:** 1284

Gershowitz, H., S.J. Behrman and J.V. Neel. 1958. Hemagglutinins in uterine secretions. *Science* **128:** 719–720

Gershowitz, H. and K. Fried. 1966. Anti-M^v, a new antibody of the MNS blood group system. I. M^v, a new inherited variant of the M gene. *Am. J. Hum. Genet.* **18:** 264–281

Gettler, A.O. and H.E. Kramer. 1936. Blood grouping in forensic medicine. *J. Immunol.* **31:** 321–329

Gibb, B. 1965. Vergleichende Untersuchungen zur Ausscheidung gruppengeprägter Substanzen des ABO-(H) Systems im Speichel und Urin (Eine Revision der Putkonenschen Daten). *Z. Aerztl. Fortbild.* **59:** 185–189

Gibb, B. and A. Vogt. 1965. Untersuchungen zum Nachweis der Blutgruppeneigenschaften A, B und O(H) in Urinflecken mittels "mixed agglutination." *Z. Aerztl. Fortbild.* **59:** 190–197

Gibbs, M.B. and J.H. Akeroyd. 1959. Quantitative immunohematologic studies of hemagglutination. I. Assay of the isoagglutinin anti-A II. Assay of the agglutinogen A. *J. Immunol.* **82:** 568–576 and 577–584

Giblett, E.R. 1958. Js, a 'new' blood group antigen found in Negroes. *Nature* **181:** 1221–1222

Giblett, E.R. 1969. *Genetic Markers in Human Blood,* Blackwell Scientific Publications, Oxford

Giblett, E.R. 1977. Blood group alloantibodies: an assessment of some laboratory practices. *Transfusion* (Phila.) **17:** 299–308

Giblett, E.R. and J. Chase. 1959. Js^a, a 'new' red-cell antigen found in Negroes; evidence for an eleventh blood group system. *Br. J. Haematol.* **5:** 319–326

Giblett, E.R., S.J. Klebanoff, S.H. Pincus, J. Swanson, B.H. Park and J. McCullough. 1971. Kell phenotypes in chronic granulomatous disease: a potential transfusion hazard. *Lancet* **1:** 1235–1236

Giles, C.M, J.D. Crossland, W.K. Haggas, and G. Longster. 1971. An Rh gene complex which results in a 'new' antigen detectable by a specific antibody, anti-Rh33. *Vox Sang.* **21:** 289–301

Giles, C.M., T. Darnborough, P. Aspinall and M.W. Fletton. 1970. Identification of the first example of anti-Co^b. *Br. J. Haematol.* **19:** 267–269

Giles, C.M. and M.N. Metaxas. 1964. Identification of the predicted blood group antibody anti-Yt^b. *Nature* **202:** 1122–1123

Giles, C.M., M. Metaxas-Bühler, Y. Romanski and M.N. Metaxas. 1967. Studies on the Yt blood group system. *Vox Sang.* **13:** 171–180

Giles, C.M. A.E. Mourant, D.M. Parkin, J.F. Horley and K.J. Tapson. 1959. A weak B antigen, probably acquired. *Br. Med. J.* **2:** 32–34

Giles, C.M. and F. Skov. 1971. The CDe Rhesus gene complex; some considerations revealed by the study of a Danish family with an antigen of the Rhesus gene complex (C)D(e) defined by a 'new' antibody. *Vox Sang.* **20:** 328–334

Ginsburg, V. 1972. Enzymatic basis for blood groups in man, *in: Advances in Enzymology* **36:** 131–149, Interscience Publishers, Division of John Wiley & Sons, New York, London etc.

Glass, B. and C.C. Li. 1953. The dynamics of racial intermixture—an analysis based on the American Negro. *Am. J. Hum. Genet.* **5:** 1–20

Gmyrek, D. 1962. Zur Serologie der Blutgruppen A_1 and A_2. *Z. Immunitaetsforsch. Exp. Ther.* **123:** 79–95

Gold, E.R., W.J. Luckyer and G.H. Tovey. 1958. Use of lyophilized

formol-treated red cells in blood group serology. *Nature* **182:** 950–951

Gonano, F., G. Modiano and M. DeAndreis. 1961. Relationships between the somatic antigens of E. coli $O_{86}B:7$ and blood group A and B substances. *Vox Sang.* **6:** 683–691

Goodman, H.O. and J.J. Thomas. 1968. Kell types in Down's syndrome. *Ann. Hum. Genet.* **31:** 369–372

Goodman, H.S. 1962. Chromatographic fractionation and characterization of antibody based on thermal dissociation of the specific antigen-antibody complex. *Nature* **193:** 350–353

Goodwin, S.A. and W.M. Watkins. 1974. The peptide moiety of bloodgroup-specific glycoproteins: some amino acid sequences in the regions carrying the carbohydrate chains. *Eur. J. Biochem.* **47:** 371–382

Göring, H.-D., J. Gross, A. Simon and I. Nause. 1969. Vergleichende experimentelle Untersuchungen zur Leistungsfähigkeit verschiedener Methoden der Blutgruppenbestimmung aus geringem Spuren-material. *Z. Aerztl. Fortbild.* **63:** 399–402

Goryanina, E.E. 1973. Optimal'nye ucloviya provedeniya reaktsii absorbtsii-elyutsii dlya vyyavleniya antigena Rh_o(D) v pyatnakh krovi. *Sud. Med. Ekspert.* (Moscow) **16**(2): 26–29

Gottschalk, A. 1972 (ed.) *Glycoproteins. Their Composition Structure and Function,* 2nd ed., BBA Library, vol. 5, Elsevier, Amsterdam, London, etc.

Grace, H.J. 1969. A potent snail haemagglutinin with anti-A specificity *J. Forensic Med.* **16:** 100–104

Grace, H.J. and G. Uhlenbruck. 1969. The agglutination of A_{bantu} and other human erythrocytes by reagents from snails. *J. Forensic Med.* **16:** 139–142

Gralnik, M.A., D. Goldfinger, P.A. Hatfield, M.E. Reid and W.L. Marsh. 1974. Anti-Lu 11: another antibody defining a high-frequency antigen related to the Lutheran blood group system. *Vox Sang.* **27:** 52–56

Gramer, L. 1975. Der Einfluss von Reinungsmitteln auf das Ergebnis der ABO-Blutgruppenbestimmung im Agglutinin-Bindungstest nach HOLZER bei Blutspuren an Kleidungsstücken. *Z. Rechtsmed.* **76:** 267–281

Gramer, L. and D. Tausch. 1973. Zur ABO-Blutgruppenbestimmung an Haaren. *Z. Rechtsmed.* **72:** 63–67

Granata, M. 1955. Controlli sperimentali sull'assorbimento della emoagglutinine da parte di alcune stoffe usate in Sardegna. *Rass. Med. Sarda* **57:** 541–546

Green, F.A. 1965. Studies on the Rh(D) antigen. *Vox Sang.* **10:** 32–53

Green, F.A. 1967. Disaggregated human erythrocyte membranes and blood group activity. *J. Immunol.* **99:** 56–60

Green, F.A. 1968a. Rh antigenicity: an essential component soluble in butanol. *Nature* **219:** 86–87

Green, F.A. 1968b. Phospholipid requirement for Rh antigenic activity. *J. Biol. Chem.* **243:** 5519–5521

Green, F.A. 1972. Erythrocyte membrane lipids and Rh antigen activity. *J. Biol. Chem.* **247:** 881–887

Greenwalt, T.J. 1961. Confirmation of linkage between the Lutheran and Secretor genes. *Am. J. Hum. Genet.* **13:** 69–88

Greenwalt, T.J. and R. Sanger. 1955. The Rh antigen E^w. *Br. J. Haematol.* **1:** 52–54

Greenwalt, T.J. and T. Sasaki. 1957. The Lutheran blood groups: a second example of anti-Lu^b and three further examples of anti-Lu^a. *Blood* **12:** 998–1003

Greenwalt, T.J., T. Sasaki, R. Sanger, J. Sneath and R.R. Race. 1954. An allele of the S(s) blood group genes. *Proc. Nat. Acad. Sci. USA* **40:** 1126–1129

Greenwalt, T.J., T. Sasaki and J. Sneath. 1956. Haemolytic disease of the newborn caused by anti-Jk^a. *Vox Sang* **1:** 157–160

Greenwalt, T.J., T.T. Sasaki and E.A. Steane. 1967. The Lutheran blood groups: a progress report with observations on the development of the antigens and characteristics of the antibodies. *Transfusion* (Phila.) **7:** 189–200

Greenwalt, T.J., R.H. Walker, C.I. Argall, E.A. Steane, R.T. Can and T.T. Sasaki. 1962. Js^b of the Sutter blood group system, *in*: Proc. 9th Congr. Int. Soc. Blood Transf., Mexico City, *Bibliotheca Haematol.,* Fasc. **19:** 235–237, S. Karger, Basel and N.Y. (1964)

Grollman, E.F., A. Kobata and V. Ginsburg. 1970. Enzymatic basis of blood types in man. *Ann. N.Y. Acad. Sci.* **169:** 153–158

Grosjean, R. 1952. Nouvelles recherches sur l'agglutinogène "P.". *Sang* **23:** 490–507

Grove-Rasmussen, M., L. Soutter and P. Levine. 1952. A new blood subgroup (A_o) identifiable with group O serums. *Am. J. Clin. Pathol.* **22:** 1157–1163

Grubb, R. 1948. Correlation between Lewis blood group and secretor character in man. *Nature* **162:** 933

Grubb, R. 1949. Some aspects of the complexity of the human ABO blood groups. *Acta Pathol. Microbiol. Scand.,* Suppl. **84:** 1–72

Grubb, R. 1950. Quelques aspects de la complexité des groupes ABO. *Rev. Hematol.* **5:** 268–275

Grubb, R. 1951. Observations on the human group system Lewis. *Acta Pathol. Microbiol. Scand.* **28:** 61–81

Grubb, R. and W.T.J. Morgan. 1949. The "Lewis" blood group characters of erythrocytes and body fluids. *Br. J. Exp. Pathol.* **30:** 198–208

Grunbaum, B.W., S. Selvin, N. Pace and D.M. Black. 1978. Frequency distribution and discrimination probability of twelve protein genetic variants in human blood as functions of race, sex and age. *J. Forensic Sci.* **23:** 577–587

Grundbacher, F.J. 1964. Immunohemolysins as a method for quantitation of the A antigen of human erythrocytes. *J. Immunol.* **93:** 205–212

Grundbacher, F.J. 1965. Sources of quantitative variation of the human A antigen of erythrocytes. *Am. J. Hum. Genet.* **17:** 399–409

Grundbacher, F.J. 1973. Lectins in precipitation reactions with soluble H substances from human saliva and serum. *Science* **181:** 461–463

Grundbacher, F.J. 1976. Genetics of anti-A and anti-B levels. *Transfusion* (Phila) **16:** 48–55

Grundbacher, F.J. and D.C. Summerlin. 1971. Inherited differences in blood group A subtypes in Caucasians and Negroes. *Human Hered.* **21:** 88–96

Grünwald, P. and E. Lackovic. 1963. The detection of genotypes of the ABO systems by phytagglutination in forensic practice, *in*: *Forensic Immunology, Medicine, Pathology and Toxicology,* Excerpta Medica Int. Congr. Ser. No. 80: 58–59, Excerpta Medica Foundation, Amsterdam, New York, etc.

Guareschi, G. 1937. Determinazione delle proprieta gruppo specifiche del sudore e delle secrezioni cutanee a scopo medico-legale. *Arch. Antropol. Crim. Psichiatr. Med. Leg.* **57:** Suppl., Atti VI Congr. Naz. Assoz. Ital. Med. Leg.: 420–432

Gunson, H.H. and V. Latham. 1972. An agglutinin in human serum reacting with cells from Le(a–b–) non-secretor individuals. *Vox Sang.* **22:** 344–353

Guthrie, C.G. and J.G. Huck. 1923. On the existence of more than four isoagglutinin groups in human blood. *Bull. Johns Hopkins Hosp.* **34:** 37–48 and 80–88 and 128–135

Guthrie, C.G. and J.F. Pessell. 1924a. Further studies on blood grouping. III. The varied types of "group IV" blood. *Bull. Johns Hopkins Hosp.* **35:** 81–85

Guthrie, C.G. and J.F. Pessell. 1924b. Further studies on blood grouping. IV. The demonstration of two addition isoagglutinins (D and Q) in human blood. *Bull. Johns Hopkins Hosp.* **35:** 126–131

Guthrie, C.G., J.F. Pessell and J.G. Huck. 1924. Further studies on blood grouping. V. The recognition of three types of "group II" blood. *Bull. Johns Hopkins Hosp.* **35:** 221–226

Habibi, B., M.T. Fouillade, N. Duebari, P.D. Issitt, P. Tippett and C. Salmon. 1978. The antigen Duclos. A new high frequency red cell antigen related to Rh and U. *Vox Sang.* **34:** 302–309

Hackel, E. 1964. Anti-Rh inhibition by RNA derivatives and amino acids. *Vox Sang* **9:** 56–59

Hackel, E. and R.E. Smolker. 1960. Effect of ribonuclease on erythrocyte antigens. *Nature* **187:** 1036–1037

Hackel, E., R.E. Smolker and S.A. Fenske. 1958. Inhibition of anti-Rh and anti-Lutheran sera by ribonucleic acid derivatives. *Vox Sang.* **3:** 402–408

Hackel, E. and K.S. Spolyar. 1960. Anti-c and anti-e inhibition by ribonucleic acid derivatives. *Vox Sang* **5:** 517–522

Hakim, S.A. and H.M. Bhatia. 1965. Serological specificity of anti-A on the basis of inhibition reactions. *Indian J. Med. Res.* **53**: 291-297

Hakomori, S-I. 1978. Isolation of blood-group ABH-active glycolipids from human erythrocyte membranes, *in*: V. Ginsberg (ed.) *Meth. Enzymol.* **50**: 207-211, Academic Press, New York, San Francisco & London

Hakomori, S-I. and A. Kobata. 1974. Blood group antigens, *in*: M. Sela (ed.) *The Antigens* **2**: 79-140, Academic Press, New York, San Francisco and London

Halban, J. and K. Landsteiner. 1902. Über Unterschiede des fötalen and mütterlichen Blutserums and über eine agglutinations- und fällungshemmende Wirkung des Normalserums. *Muench. Med. Wochenschr.* **49**(12): 473-476 and see Camp *et al.*, 1974

Halvorsen, K. and R. Nordhagen. 1967. A modified method of determining the ABO group of blood stains on textiles. *Vox Sang.* **12**: 312-316

Hamaguchi, H. and H. Cleve. 1972. Solubilization of human erythrocyte membrane glycoproteins and separation of the MN glycoprotein from a glycoprotein with I, S and A activity. *Biochim. Biophys. Acta* **278**: 271-280

Hamilton, C. and W. Kimberling. 1973. Genetics of sephadex gel fractions of ABH secretor substance. *Am. J. Hum. Genet.* **25**: 31A [Abstr.]

Hamilton, C., W. Kimberling and R. Taylor. 1974. Genetic and biochemical analysis of the expanded secretor system. *Am. J. Hum. Genet.* **26**: 38A [Abstr.]

Hanfland, P., R-G. Kladetzky and H. Egli. 1978. Isolation and purification of Le^a blood-group active and related glycolipids from human plasma of blood-group ALe^a individuals. *Chem. Phys. Lipids* **22**: 141-151

Happ, W.M. 1920. Appearance of isoagglutinins in infants and children. *J. Exp. Med.* **31**: 313-334

Hara, M. and R. Wakao. 1926. Isohämagglutination beim Kinde und in der Frauenmilch. *Jahrb. Kinderheilkd. Phys. Erziehung* **114** (3F **64**): 313-321

Harley, D. 1936. Determining the group of human blood-stains: notes on an anomalous group O serum. *Br. J. Exp. Pathol.* **17**: 35-38

Harley, D. 1943. *Medico-legal Blood Group Determination*, Wm. Heinemann Medical Books, Ltd., London

Harris, H., D.A. Hopkinson and E.B. Robson. 1974. The incidence of rare alleles determining electrophoretic variants: data on 43 enzyme loci in man. *Ann. Hum. Genet.* **37**: 237-253

Hartmann, G. 1941. *Group Antigens in Human Organs.* A discussion of the "secretor" "nonsecretor" phenomenon. *Ejnar Munksgaard Forlag*, Copenhagen and see Camp *et al.*, 1974

Hasebe, H. 1962a. Identification of blood group antigens A, B and $D(Rh_o)$ on human erythrocytes by the fluorescent antibody technique. *Jpn. J. Leg. Med.* **16**: 311-324 §[1]

Hasebe, H. 1962b. Determination of ABO blood groups in human blood stains by the fluorescent antibody technique. *Jpn. J. Leg. Med.* **16**: 325-329 §[1]

Haseeb, M.A. 1972. Studies on human bloodstains in the Sudan. *Med. Sci. Law* **12**: 129-130

Hausbrandt, F. 1938. Blutgruppenbestimmung an kleinsten Trockenblutmengen durch Agglutininbindung in Capillaren. *Dtsch. Z. Gesamte Gerichtl. Med.* **29**: 501-510

Hawes, M.D. and R.R.A. Coombs. 1960. Cellular distribution of Forssman (membrane) antigen in the guinea pig determined by the mixed agglutination reaction. *J. Immunol.* **84**: 586-589

Hayashi, N. 1958. Blood group substances in human ear wax. *Ochanomizu Med. J.* **6**(6): 776-786 (56-66 of issue) §[3]

Hayashi, S. 1974. Determination of blood group from the mixture of semen and vaginal fluid by electrophoresis. Part 3. Determination of blood group by disc electrophoresis. *Jpn. J. Leg. Med.* **28**: 286-293 §[1]

Hayward, J.W. 1969. The sub-typing of group A bloodstains. *J. Forensic Sci. Soc.* **9**: 147-149

Hegner, C.A. 1916. Ueber das Vorkommen von Agglutininen in der Tränenflüssigkeit. *Klin. Montasbl. Augenheilkd.* **57**: 48-50

Heiken, A. 1966. Observations on the blood group receptor P_1 and its development in children. *Hereditas* **56**: 83-98

Heiken, A. 1967. Demonstration of the Rh gene complex D(c)(E). *Vox Sang.* **13**: 158-164

Heim, K. 1926. Über menschiliche Isoantikörper in Blut and Milch. *Monatsschr. Geburtshülfe Gynakol.* **74**: 52-65

Heisto, H., M. van der Hart, G. Madsen, M. Moes, J. Noades, M.M. Pickles, R.R. Race, R. Sanger and J. Swanson. 1967. Three examples of a new red cell antibody, anti-Co^a. *Vox Sang.* **12**: 18-24

Hektoen, L. 1907. Isoagglutination of human corpuscles. *J. Infect. Dis.* **4**: 297-303

Helpern, M. and A.S. Wiener. 1961. Grouping semen in cases of rape. *Fertil. Steril.* **12**: 551-553

Henke, J. and D. Kasulke. 1976. The first example of the Rh antigen E^w in Western Europe. *Vox. Sang.* **30**: 305-307

Hennig, A. and A. Rackwitz. 1961. Untersuchungen über die praktische Anwendung der Sekretorbestimmung mittels einer Filterstreifenmethode. *Heilberufe* **13**: 11-13

Henningsen, K. 1949a. Investigations on the blood factor P. *Acta Pathol. Microbiol. Scand.* **26**: 639-654

Henningsen, K. 1949b. On the heredity of blood factor P. *Acta Pathol. Microbiol. Scand.* **26**: 769-785

Henningsen, K. 1958. A new 'deleted' Rh-chromosome. *Nature* **181**: 502

Henningsen, K. and T. Jacobsen. 1954. A probable mutation within the MN blood group system. *Acta Pathol. Microbiol. Scand.* **35**: 240-248

Henningsen, K. and T. Jacobsen. 1955. Relationship between blood factors P and Q. *Nature* **176**: 1179-1180

Herrman, W.P. and G. Uhlenbruck. 1972. Studies on a seminal plasma protein with blood group A activity. *Arch. Dermatol. Forsch.* **245**: 170-176

Higuchi, S. 1929. Ueber den Nachweis der vier menschlichen Blutgruppen in Blutflecken. *Z. Immunitaetsforsch. Exp. Ther.* **60**: 246-269

Hilgermann, R. 1971. Neue Untersuchungen zur A-Untergruppen-Differenzierung an Blutzpuren. *Z. Rechtsmed.* **68**: 79-85

Hirsch, H.F. and H.A. Graham. 1980. Adsorption of Le^c and Le^d from plasma onto red blood cells. *Transfusion* (Phila.) **20**: 474-475

Hirsch, W., P. Moores, R. Sanger and R.R. Race. 1957. Notes on some reactions of human anti-M and anti-N sera. *Br. J. Haematol.* **3**: 134-142

Hirschfeld, J. 1973. Some notes on the Rh system—a complex-complex model. *Vox Sang.* **24**: 21-32

Hirszfeld, L. 1928. *Konstitutionsserologie und Blutgruppenforschung*, Springer, Berlin and see Camp *et al.*, 1974

Hirszfeld, L. 1947. The transition forms of blood groups. *J. Immunol.* **55**: 141-151

Hirszfeld, L. and R. Amzel. 1932. Beitrag zur gerichtlich-medizinischen Verwertung der Blutgruppen. *Dtsch. Z. Gesamte Gerichtl. Med.* **19**: 133-148

Hirszfeld, L. and M. Grabowska. 1952. Über individuelle Bluteigenschaften. *Experientia* **8**: 355-357

Hirszfeld, L. and Z. Kostuch. 1938. Über das Wesen der Blutgruppe O. *Klin. Wochenschr.* **17**(30): 1047-1051

Hohorst, H.-J. 1954. Zur Kenntnis der Natur der M- and N-Substanz menschlichen Erythrocyten. *Z. Hyg. Infektionskr.* **139**: 561-564

Holborow, E.J., P.C. Brown, L.E. Glynn, M.D. Hawes, G.A. Gresham, T.F. O'Brien and R.R.A. Coombs. 1960. The distribution of the blood group A antigen in human tissues. *Br. J. Exp. Pathol.* **41**: 430-437

Holburn, A.M. and C.A. Masters. 1974. The radioimmunoassay of serum and salivary blood group A and Le^a glycoproteins. *Br. J. Haematol.* **28**: 157-167

Holzer, F.J. 1931. Ein einfaches Verfahren zur Gruppenbestimmung an vertrocknetem Blut durch Agglutininbindung. *Dtsch. Z. Gesamte Gerichtl. Med.* **16**: 445-458 ¶

Holzer, F.J. 1936. Zum Nachweis der Bluteigenschaften M und N. *Dtsch. Z. Gesamte Gerichtl. Med.* **26**: 515-518

Holzer, F.J. 1937. Untersuchungen über die gerichtlich-medizinische Verwertbarkeit der Ausscheidung Blutgruppensubstanzen. *Dtsch. Z. Gesamte Gerichtl. Med.* **28**: 234-248 ¶

Holzer, F.J. 1953. Der gegenwärtige Stand der Blutgruppenserologie und deren forensische Bedeutung. *Dtsch. Z. Gesamte Gerichtl. Med.* **42**: 416-437 ¶

Hooker, S.B. and L.M. Anderson. 1921. The specific antigenic properties

of the four groups of human erythrocytes. *J. Immunol.* **6**: 419–444

Hossaini, A. A. 1977. Selected topics in immunohematology. I. Advances in the Lewis system, *in*: M. Stefanini, A.A. Hossaini and H.D. Isenberg (eds.) *Progr. Clin. Pathol.* **7**: 165–181. Grune & Stratton, New York

Hotta, K. and G. F. Springer. 1965. Isolation and partial characterization of blood group N specific haptens from human blood group M and N substances *in*: Proc. 10th Congr. Int. Soc. Blood Transf., *Bibliotheca Haematol.*, Fasc. **23**: 505–509, S. Karger, Basel and New York

Howard, H.D. and P.D. Martin. 1969. An improved method for ABO and MN grouping of dried bloodstains using cellulose acetate sheets. *J. Forensic Sci. Soc.* **9**: 28–30

Hrubiško, M., Z. Čalkovska, O. Mergancová and K. Gallova. 1966. Beobachtungen über Varianten des Blutgruppensystems ABO. I. Studie der Variante A_m. II. Beitrag zur Erblichkeit der A_m-Variante. *Blut* **13**: 137–142 and 232–239

Hübener, G. 1926. Untersuchungen über Isoagglutination, mit besonderer Berücksichtigung scheinbarer Abweichungen vom Gruppenschema. *Z. Immunitaetsforsch. Exp. Ther.* **45**: 223–248

Huber, O. 1957. Nachweis der Blutgruppen in Flecken, *in:* A. Ponsold (ed.) *Lehrbuch der Gerichtlichen Medizin*, 680–684, G. Thieme, Stuttgart

Huck, J.G. and C.G. Guthrie. 1924. Further studies on blood grouping I. The antigenic properties of two types of "group II" erythrocytes. *Bull. Johns Hopkins Hosp.* **35**: 23–27

Huestis, D.W., M.L. Catino and S. Busch. 1964. A "new" Rh antibody (anti-Rh26) which detects a factor usually accompanying hr'. *Transfusion* (Phila.) **4**: 414–418

Huggins, C.E. 1963. Reversible agglomeration used to remove dimethylsulfoxide from large volumes of frozen blood. *Science* **139**: 504–505

Hughes, R.C. 1975. The complex carbohydrates of mammalian cell surfaces and their biological role. *Essays Biochem.* **11**: 1–36

Hughes-Jones, N.C., B. Gardner and P.J. Lincoln. 1971. Observations of the number of available c, D and E antigen sites on red cells. *Vox Sang.* **21**: 210–216

Hughes-Jones, N.C., B. Gardner and R. Telford. 1963. Comparison of various methods of dissociation of anti-D using ^{131}I-labelled antibody. *Vox Sang.* **8**: 531–536

Hughes-Jones, N.C., B. Gardner and R. Telford. 1964a. The effect of pH and ionic strength on the reaction between anti-D and erythrocytes. *Immunology* **7**: 72–81

Hughes-Jones, N.C., M.J. Polley, R. Telford, B. Gardner and G. Kleinschmidt. 1964b. Optimal conditions for detecting blood group antibodies by the antiglobulin test. *Vox Sang.* **9**: 385–395

Hunter, L., M. Lewis and B. Chown. 1951. A further example of Kidd (Jk^a) haemagglutinin. *Nature* **168**: 790–791

Huntoon, F.M. 1921. Antibody studies. I. Reversal of the antigen-antibody reaction. *J. Immunol.* **6**: 117–122

Huntoon, F.M. and S. Estris. 1921. Antibody studies. II. The recovery of antibody from sensitized antigens: technic. *J. Immunol.* **6**: 123–184

Huntoon, F.M., P. Masucci and E. Hannum. 1921. Antibody studies. III. Chemical nature of antibody. *J. Immunol.* **6**: 185–200

Ikin, E.W. 1966. The production of anti-M^g in rabbits. *Vox Sang.* **11**: 217–218

Ikin, E.W., H.E.M. Kay, J.H.L. Playfair and A.E. Mourant. 1961. P_1 antigen in the human foetus. *Nature* **192**: 883

Ikin, E.W., A.E. Mourant, H.J. Pettenkofer and G. Blumenthal. 1951. Discovery of the expected haemagglutinin anti-Fy^b. *Nature* **168**: 1077–1078

Illchmann-Christ, A. and V. Nagel. 1954. Zum Problem der Beziehungen von Bakterienantigenen und blutgruppenspezifischen Substanzen. *Dtsch. Z. Gesamte Gerichtl. Med.* **43**: 391–398

Iseki, S. 1977. ABH blood group substances in living organisms, *in*: J.F. Mohn, R.W. Plinkett, R.K. Cunningham and R.M. Lambert (eds.) *Human Blood Groups*, Proc. 5th Int. Convoc. Immunol., S. Karger, Basel, Munich, etc.

Iseki, S., S. Masaki and K. Shibasaki. 1957. Studies on Lewis blood group system. I. Le^c blood group factor. II. Distribution and heredity of Le^c blood group factor. *Proc. Japan Acad.* **33**: 492–497 and 681–686

Ishimori, T. and H. Hasekura. 1967. A Japanese with no detectable Rh blood group antigens due to silent Rh alleles or delected chromosomes. *Transfusion* (Phila.) **7**:84–87

Ishiyama, I. and T. Okada. 1975. Anwendung der modifizierten Mischzell-agglutination (mixed cell agglutination reaction, MCAR nach Davidsohn) in der forensischen serologie; MCAR auf dem Klebebandstreifen. *Z. Rechtsmed.* **77**: 25–40

Ishiyama, I., M. Orui, K. Ogawa and T. Kimura. 1977. The detection of isoantigenic activity from latent fingerprints: mixed cell agglutination reaction in forensic serology. *J. Forensic Sci.* **22**: 365–375

Ishigu, H. 1968a. Studies on blood group B_m from medico-legal aspects. Part 1. Serological study on group B_m. *Jpn. J. Leg. Med.* **22**: 618–626 §[1]

Ishigu, H. 1968b. Studies on blood group B_m from medico-legal aspects. Part 2. Study on the identification of blood group B_m. *Jpn. J. Leg. Med.* **22**: 627–634 §[1]

Issitt, P.D. 1970. *Applied Blood Group Serology,* Spectra Biologicals, Divn. of Becton, Dickinson & Co., Oxnard, CA

Issitt, P.D., J.M. Haber and F.H. Allen, Jr. 1965. Anti-Tm, an antibody defining a new antigenic determinant within the MN blood-group system. *Vox Sang.* **10**: 742–743

Issitt, P.D., J.M. Haber and F.H. Allen, Jr. 1968. Sj, a new antigen in the MN system, and further studies on Tm[1]. *Vox Sang.* **15**: 1–14

Issitt, P.D. and C.K. Issitt. 1975. *Applied Blood Group Serology,* 2nd ed., Spectra Biologicals, Divn. of Becton, Dickinson and Co., Oxnard, CA

Issitt, P.D., B.G. Pavone and M. Shapiro. 1978. Anti-Rh39: a "new" specificity Rh system antibody. *Transfusion* (Phila.) **18**: 644

Issitt, P.D., B.G. Pavone and M. Shapiro. 1979. Anti-Rh39—a "new" specificity Rh system antibody. *Transfusion* (Phila.) **19**: 389–397

Jack, J.A., P. Tippett, J. Noades, R. Sanger and R.R. Race. 1960. M_1, a subdivision of the human blood-group antigen M. *Nature* **186**: 642

Jackson, L.J., M.B. Rittenberg and G.V.F. Seaman. 1972. On the isolation of materials with Rh antigenic properties from the erythrocyte membrane. *Clin. Chim. Acta* **39**: 463–465

Jadin, J. 1934. L'identification des taches de sang. *Arch. Int. Med. Exp.* **9**: 325–339

Jakobowicz, R., J.J. Graydon and R.T. Simmons. 1966. Observations on saliva agglutinins. *Med. J. Aust.* **1**(10): 399–401

Jansky, J. 1907. Haematologicke studie u psychotikû[Études hématologiques dans les malades mentales]. *Sbornik Klinický* **8**: 85–139 §[4]

Jarkowski, T.L., C.P. Hinshaw, K.M. Beattie and B. Silberberg. 1962. Another example of anti-Js^a. *Transfusion* (Phila.) **2**: 423–424

Jenkins, G.C., J. Brown, P.J. Lincoln and B.E. Dodd. 1972. The problem of acquired B antigen in forensic serology. *J. Forensic Sci. Soc.* **12**: 597–603

Jenkins, W.J., W.L. Marsh, J. Noades, P. Tippett, R. Sanger and R.R. Race. 1960. The I antigen and antibody. *Vox Sang.* **5**: 97–106

Jensen, K.G. 1959. Elution of incomplete antibodies from red cells. A comparison of different methods. *Vox Sang.* **4**: 230–239

Jervell, F. 1921. The influence of temperature upon the agglutination of the red blood corpuscles. *J. Immunol* **6**: 445–451

Johnson, G.A. and R.H. McCluer. 1961. Relation of sialic acid to Rh_o (D) antigen. *Proc. Soc. Exp. Biol. Med.* **107**: 692–694

Joint AMA-ABA Guidelines. 1976. Joint AMA-ABA Guidelines: Present status of serologic testing in problems of disputed parentage 1976. *Family Law Q.* **10**: 247–285

Jones, A.R. and L.K. Diamond. 1955. Identification of the Kell factor in dried blood stains. A case report. *J. Forensic Med.* **2**: 243–246

Jones, A.R. and L. Kaneb. 1959. A new property of iso-agglutinins of the ABO blood group system. *Blood* **14**: 1094–1102

Jones, A.R. and L. Kaneb. 1960. Some properties of cross reacting antibody of the ABO blood group system. *Blood* **15**: 395–403

Jones, A.R., L. Kaneb and A. Abrahamov. 1959. A technique for the titration of "cross-reacting antibody" in group O serum. *J. Lab. Clin. Med.* **54**: 779–783

Jones, A.R., A.G. Steinberg, F.H. Allen, Jr., L.K. Diamond and B. Kriete. 1954. Observations on the new Rh agglutinin anti-f. *Blood* **9**: 117–122

Jonsson, B. 1944. Blutgruppenstudien mit japanischen und schwedischen Aalseren. *Acta Pathol. Microbiol. Scand.*, Suppl 54: 456–464

Jonsson, B. and K. Fast. 1948. Ein eigenartiger, familiärer Typus von extrem schwachem A-Faktor "A_4". *Acta Pathol. Microbiol. Scand.* **25:** 649–655

Jørgensen, J., M. Nielsen, J. Laugesen and S. Stoltenberg. 1979b. The influence of ionic strength and incubation time of the sensitivity of methods using papainized red cells. *Vox Sang.* **37:** 111–115

Jørgensen, J., M. Nielsen, C.-B. Nielsen and J. Nørmark. 1979a. The influence of ionic strength, albumin and incubation time on the sensitivity of the indirect Coombs test. *Vox Sang.* **36:** 186–191

Jorpes, E. 1934. Über die gruppenspezifischen isoagglutininbindenen Receptoren des Harns. *Acta Pathol. Microbiol. Scand.* **11:** 99–118

Jorpes, E. and G. Norlin. 1933. Ueber die chemische Natur der Blutgruppenmerkmale. *Z. Immunitaetsforsch. Exp. Ther.* **81:** 152–162

Jorpes, E. and G. Norlin. 1934. Über die Blutgruppenmerkmale im Harn des Menschen, Ihre quantitative Ausmessung und Anreicherung in Trockenpräparaten. *Acta Pathol. Microbiol. Scand.* **11:** 91–98

Joshi, S.R. and H.M. Bhatia. 1979. A new red cell phenotype I-i-: red cells lacking in both I and i antigens. *Vox Sang.* **36:** 34–38

Juberg, R.C. 1970. Blood-group gene frequencies in West Virginia. *Am. J. Hum. Genet.* **22:** 96–99

Judd, W.J., P.D. Issitt and B.G. Pavone. 1979b. The Can serum: demonstrating further polymorphism of M and N blood group antigens. *Transfusion* (Phila.) **19:** 7–11

Judd, W.J., P.D. Issitt, B.G. Pavone, J. Anderson and D. Aminoff. 1979a. Antibodies that define NANA-independent MN-system antigens. *Transfusion* (Phila.) **19:** 12–18

Judd, W.J., W.L. Marsh, R. Øyen, M.E. Nichols, F.H. Allen, Jr., M. Contreras and M. Stroup. 1977. Anti-Lu14: a Lutheran antibody defining the product of an allele at the Lu8 blood group locus. *Vox Sang.* **32:** 214–219

Judson, P.A. and D.J. Anstee. 1977. Comparative effect of trypsin and chymotrypsin on blood group antigens. *Med. Lab. Sci.* **34:** 1–6

Juel, E. 1959. Anti-A agglutinins in sera from A_2B individuals. *Acta Pathol. Microbiol. Scand.* **46:** 91–95

Jungwirth, J. 1955. Vergleichende Untersuchungen über die Ausscheidung von gruppensubstanzen bei Trägern der Blutgruppe O. *Dtsch. Z. Gesamte Gerichtl. Med.* **43:** 524–527

Kabat, E.A. 1949. Immunochemical Studies on Blood Group Substances, *in*: B. Cohen (ed.) *Bacteriol. Rev.* **13:** 189–202, Williams and Wilkins, Baltimore

Kabat, E.A. 1956. *Blood Group Substances. Their Chemistry and Immunochemistry.* Academic Press, New York

Kaita, H., M. Lewis, B. Chown and E. Gard. 1959. A further example of the Kell blood group phenotype. K-,k-, Kp(a-b-). *Nature* **183:** 1586

Kaklamanis, E.J., E.J. Holborow and L.E. Glynn. 1964. A method for differentiating homozygous from heterozygous secretors of ABH bloodgroup substances. Its application to the study of secretor status in rheumatic fever. *Lancet* **1:** 788–790.

Kao, Y.S., S. Frank and D.S. De Jongh. 1978. Anti-M in children with acute bacterial infections. *Transfusion* (Phila.) **18:** 320–322

Kaplan, S., C.C. Li, N. Wald and W. Borges. 1964. ABO frequencies in Mongols. *Ann. Hum. Genet.* **27:** 405–412

Karamihova-Tsacheva, L. 1966. Titre fluctuations of the A and B antigens in the spermal plasma of secretors. *Dokl. Bolgar. Akad. Nauk.* [C. R. Acad. Bulg. Sci.] **19(8):** 767–770

Karsner, H.T. and H.L. Koeckert. 1919. The influence of dessication on human normal isohemagglutinins. *J. Am. Med. Assoc.* **73:** 1207–1210

Karsznia, R., R. Spielmann and J. Trinkaus. 1969. Zum Nachweis der Blutgruppenantigene A und B an meschlichen Spermien. *Gynaecologia* **167:** 14–22

Kato, M., S. Kubo and M. Naiki. 1978. Complement fixation antibodies to glycosphingolipids in sera of rare blood group p and P^k phenotypes. *J. Immunogenet.* **5:** 31–40

Katsura, S., K. Sagisaka, H. Sugisawa, K. Tokiwa and K. Mochizuki. 1971. Studies on the preparation method of the specific anti-M and anti-N antibodies by various means of absorption-elution. *Jpn. J. Leg. Med.* **25:** 91–102 §¹

Kayssi, A.I. and W.G. Millar. 1937. Effect of heat and ultra-violet radiation on iso-agglutinins in dried bloodstains. *Br. Med. J.* **2:** 114–115

Kelso, J. 1968. Quantitative aspects of Lewis-Secretor interaction in saliva. *Vox Sang.* **14:** 282–288

Kennedy, J.A. 1928. Blood group classifications used in hospitals in the United States and Canada. *J. Am. Med. Assoc.* **90:** 1323–1324

Kennedy, J.A. 1931. Isohemagglutination: The work of Jan Jansky with a critical analysis. *J. Immunol.* **20:** 117–141

Kent, S.P., J.M. McKibbin, R. Boggio and J.B. McKibbin. 1977. Blood group A antigen in human erythrocytic membranes and membrane fractions. *Vox Sang.* **33:** 193–201

Kerde, Ch. 1961. Über einige Familiendaten zur Vererbung der Rezeptoren des Rh-Systems, ferner, Kell, P und Se. *Wiss. Z. Humboldt-Universitaet,* Naturwiss. Reihe, cited by Prokop and Uhlenbruck, 1969.

Kerde, C. 1965. Die Bilding von MN-Antikörpern bei 16 Schafen aus 6 verschiedener Tierrassen und das Verhalten der Antikörper bei langfristiger Immunisierung sowie deren Eignung zur Verwendung als Testseren. *Z. Aerztl. Fortbild.* **59:** 201–203

Kerék, G. and R. Eliasson. 1975. Absorption activity of the blood group substances in various fractions of split ejaculates from man. *Int. J. Fertil.* **20:** 125–127

Khalap, S. and G.B. Divall. 1979. A_1-A_2 subtyping of bloodstains using *Helix aspersa. Forensic Sci. Int.* **14:** 103 [Abstr.]

Kidd, P. 1949. Elution of an incomplete type of antibody from the erythrocytes in acquired haemolytic anemia. *J. Clin. Pathol.* **2:** 103–108

Kijewsky, S. and U. von Mülmann. 1975. Der Einfluss pedologischer Unterschiede auf die Mengenbestimmung und Erfassbarkeit serologischer Eigenschaften von Blutspuren im Erdboden. *Z. Rechtsmed.* **75:** 253–263

Kim, Z., G. Uhlenbruck and G.I. Pardoe. 1970. Studies on the nature of the T-antigen and the problem of panagglutination. *Jpn. J. Leg. Med.* **24:** 231–237 §¹

Kimberling, W. 1979. The genetics of an expanded secretor system *J. Immunogenet.* **6:** 75–82

Kind, S.S. 1955. A modified absorption technique of determining the ABO group of bloodstains. *Vox Sang.* **5:** (O.S.): 15–19 §¹

Kind, S.S. 1956. Prevention of haemolysis in bloodstain grouping. *J. Crim. Law Criminol. Police Sci.* **46:** 905

Kind, S.S. 1960a. Absorption-elution grouping of dried blood smears. *Nature* **185:** 397–398

Kind, S.S. 1960b. Absorption-elution grouping of dried bloodstains on fabrics. *Nature* **187:** 789–790

Kind, S.S. 1962. The ABO grouping of bloodstains. *J. Crim. Law Criminol. Police Sci.* **53:** 367–374

Kind, S.S. 1963. Some properties of ABH antisera considered in relation to bloodstain grouping. *J. Forensic Med.* **10:** 51–56

Kind, S.S. and R.M. Cleevely. 1969. The use of ammoniacal bloodstain extracts in ABO groupings. *J. Forensic Sci. Soc.* **9:** 131–134

Kind, S.S. and R.M. Cleevely. 1970. The fluorescent antibody technique. Its application to the detection of blood group antigens in stains. *J. Forensic Med.* **17:** 121–129

Kind, S.S. and B.G. Lang. 1975. An investigation into the possible sources of adventitious substances in bloodstains grouping. *Forensic Sci.* **5:** 143 [Abstr.]

Kind, S.S. and B.G. Land. 1976a. Some observations on the ammoniacal extraction method in bloodstain grouping and its comparison with other extractive and non-extractive methods. *J. Forensic Sci. Soc.* **16:** 47–54

Kind, S.S. and B.G. Lang. 1976b. An investigation into the possible sources of adventitious ABH substances in bloodstain grouping *J. Forensic Sci. Soc.* **16:** 155–161

Kind, S.S. B. Lang, E. Rutter and P.H. Whitehead. 1979a. A chromatographic study of ABH blood group substances in "non-secretor" salivas. *Forensic Sci. Int.* **14:** 112 [Abstr.]

Kind, S.S. B.G. Lang, E.R. Rutter, P.H. Whitehead and T.J. Rothwell. 1979b. The molecular polymorphism of ABH blood-group substances in human saliva—a scientific enigma? *J. Forensic Sci. Soc.* **19:** 287–291

King, H.K. and W.T.J. Morgan. 1944. The isolation of a blood group A substance from pseudo-mucinous ovarian cyst fluids. *Biochem. J.* **38,** Proc. Biochem. Soc.: x–xi

King, J.W., M.G. Berthier and C.J. Farinacci. 1955. Frequency of blood

antigens in a Texas population having Mexican surnames. *Am. J. Clin. Pathol.* **25:** 755–760

Kirihara, S. 1924. Über die Isohämagglutination beim menschlichen Blute. *Z. Klin. Med.* **99:** 522–545

Kirk, P.L., C. Brown and A.B. Conners. 1954. Some problems in blood testing and grouping. *J. Crim. Law Criminol. Police Sci.* **45:** 80–84

Kishi, K. and S. Iseki. 1977. Immunochemical studies on blood group A substance from human hair. *Z. Rechtsmed.* **80:** 191–195

Kishi, K. and S. Iseki. 1978. Immunochemical studies on blood group H and B substances from human hair. *Z. Rechtsmed.* **83:** 153–155

Kishino, H. 1955. Contributions to the blood grouping method of blood stains, body fluids and excretes. I. A few basic considerations for identifying the groups of blood stains. II. Preliminary treatment of blood stains in determining their group. *Shikoku Acta Med.* **7:** 148–157 and 189–195

Kissling, E. and W. Neumann. 1972. Beitrag zum Nachweis von H-Substanzen an Trockenblutspuren im Absorptionsverfahren und zur Erfassung schwacher Agglutinine im Agglutininwirkungsversuch nach Lattes. *Arch. Kriminol.* **150:** 141–145

Kitahama, M., H. Yamaguchi, Y. Okubo and E. Hazama. 1967. An apparently new B_h-like human blood-type. *Vox Sang.* **12:** 354–360

Klenk, E. and G. Uhlenbruck. 1960. Über neuraminsaurehaltige Mucoide aus Menschenerythrocytenstroma, ein Beirag zur Chemie der Agglutinogene. *Hoppe-Seyler's Z. Physiol. Chem.* **319:** 151–160

Kliman, A. and E.P. Smith. 1966. Large-scale routine automatic blood typing, *in: Automation in Analytical Chemistry,* Technicon Symp., v. I 1966: 106–111, Mediad, Inc., New York (1967)

Knox, E.G. 1966. A notional structure for Rhesus antigens. *Br. J. Haematol.* **12:** 105–114

Knox, E.G. 1977. Distribution of Rhesus antigens on red cell surfaces. *Br. J. Haematol.* **37:** 537–541

Kobiela, J.S., B. Turowska and B. Opolska. 1972. Oznaczanie cech MNS w plamach krwi ludzkiej. *Arch. Med. Sadowej. Kryminol.* **22:** 117–119

Kochetov, N.K., V.A. Derevitskaya and N.P. Arbatsky. 1976. The structure of pentasaccharides and hexasaccharides from blood group substance H. *Eur. J. Biochem.* **67:** 129–136

Kochwa, S. and R.E. Rosenfield. 1964. Immunochemical studies of the Rh system. I. Isolation and characterization of antibodies. *J. Immunol.* **92:** 682–692

Koeckert, H.L. 1920. A study of the mechanism of human isohemagglutination. *J. Immunol.* **5:** 529–537

Kolmer, J.A. 1919a. The influence of dessication upon natural hemolysins and hemagglutinins in human sera. *J. Immunol.* **4:** 393–402

Kolmer, J.A. 1919b. The influence of dessication on human normal isoagglutinins. *J. Am. Med. Assoc.* **73:** 1459–1460

Kolmer, J.A. 1920. The influence of dessication upon natural hemolysins and hemagglutinins in human sera. *Proc. Pathol. Soc. Philadelphia* **40:** (N.S. **22**): 64–65

Komai, T. 1950. Semi-allelic genes. *Am. Naturalist* **84:** 381–392

Konugres, A.A., L.S. Brown and P.A. Corcoran. 1966. Anti-M^A, and the phenotype M^aN, of the MN blood-group system (A new finding). *Vox Sang.* **11:** 189–193

Konugres, A.A. and N.M. Winter. 1967. Sul, a new blood group antigen in the MN system. *Vox Sang.* **12:** 221–224

Kornstad, L. and K. Halvorsen. 1958. Haemolytic disease of the newborn caused by anti-Jk^b. *Vox Sang.* **3:** 94–99

Korszun, A-K. B.E. Causton and P.J. Lincoln. 1978. Thermostability of ABO(H) blood-group antigens in human teeth. *Forensic Sci.* **11:** 231–239

Kortekangas, A.E., E. Kaarsald, L. Melartin, P. Tippett, J. Gavin, J. Noades, R. Sanger and R.R. Race. 1965. The red cell antigen P^k and its relationship to the P system: the evidence of three more P^k families. *Vox Sang.* **10:** 385–404

Kortekangas, A.E., J. Noades, P. Tippett, R. Sanger and R.R. Race. 1959. A second family with the red cell antigen P^k. *Vox Sang.* **4:** 337–349

Koscielak, J., T. Pacuszka and W. Dzierzkowa-Borodej. 1976. Activity of B-gene-specified galactosyltransferase in individuals with B_m phenotypes. *Vox Sang.* **30:** 58–67

Kovarik, S., I. Davidsohn and R. Stejskal. 1968. ABO antigens in cancer. Detection with the mixed cell agglutination reaction. *Arch. Pathol.* **86:** 12–21

Krah, E. 1949a. Beobachtungen über den schwachen N-Receptor. *Dtsch. Z. Gesamte Gerichtl. Med.* **39:** 213–221

Krah, E. 1949b. Zur Probleme des schwachen N. *Dtsch. Z. Gesamte Gerichtl. Med.* **39:** 222–230

Krainskaja-Ignatowa, V.N. 1929. Über die Gruppeneigenschaften des Spermas. (Zur Frage der individuellen Zugehörigkeit von Samenflecken). *Dtsch. Z. Gesamte Gerichtl. Med.* **13:** 441–447

Krefft, S. 1953. Über das Vorkommen von Gruppensubstanzen in menschlichen Haaren. *Dtsch. Z. Gesamte Gerichtl. Med.* **42:** 395–408

Kuhns, W.J. and A. Bailey. 1950. Use of red cells modified by papain for detection of Rh antibodies. *Am. J. Clin. Pathol.* **20:** 1067–1069

Kundu, S.K., S.M. Steane, J.E.C. Bloom and D.M. Marcus. 1978. Abnormal glycolipid composition of erythrocytes with a weak P antigen. *Vox Sang.* **35:** 160–167

LaCavera, A. and E.-M. Scafidi. 1972. Identification des groupes sanguins ABO au moyen d'une microméthode d'absorption-élution. *Med. Leg. Domm. Corpor.* **5:** 55–56

Lambert, J. 1941. L'antigène O. Sa répartition et ses rapports avec les antigènes de groupe. *Rev. Belge Sci. Med.* **13:** 1–52

Landé, K.E. 1938. Typing of blood and seminal stains by means of the absorption test. *Arch. Pathol.* **25:** 463–473

Landsteiner, K. 1900. Zur Kenntnis der antifermentativen, lytischen und agglutinierenden Wirkungen des Blutserums und der Lymphe. *Centralbl. Bakteriol. Parasitenkd. Infektionskr.,* Abt. I **27:** 357–362 and see Camp *et al.,* 1974

Landsteiner, K. 1901. Über Agglutinationserscheinungen normalen menschlichen Blutes. *Wein. Klin. Wochenschr.* **14**(46): 1132–1134 and see Camp *et al.,* 1974

Landsteiner, K. 1902. Ueber Serumagglutinine. *Muench. Med. Wochenschr.* **49**(46): 1905–1908

Landsteiner, K. 1903. Ueber Beziehungen zwischen dem Blutserum und den Körperzellen. *Muench. Med. Wochenschr.* **50**(42): 1812–1814

Landsteiner, K. 1936. On the group specific A substance in horse saliva. *J. Exp. Med.* **63:** 185–190

Landsteiner, K. and M.W. Chase. 1936. On group specific A substances. III. The substance in commercial pepsin. *J. Exp. Med.* **63:** 813–817

Landsteiner, K. and R.A. Harte. 1941. Group-specific substances in human saliva. *J. Biol. Chem.* **140:** 673–674

Landsteiner, K. and N. Jagic. 1903. Ueber die Verbindungen und die Entstehung von Immunkörpern. *Muench. Med. Wochenschr.* **50**(18): 764–768

Landsteiner, K. and P. Levine. 1926a. On group specific substances in human spermatozoa. *J. Immunol.* **12:** 415–418

Landsteiner, K. and P. Levine. 1926b. On the cold agglutinins in human serum. *J. Immunol.* **12:** 441–460

Landsteiner, K. and P. Levine. 1927a. A new agglutinable factor differentiating individual human bloods. *Proc. Soc. Exp. Biol. Med.* **24:** 600–602

Landsteiner, K. and P. Levine. 1927b. Further observations on individual differences of human blood. *Proc. Soc. Exp. Biol. Med.* **24:** 941–942

Landsteiner, K. and P. Levine. 1928a. On individual differences in human blood. *J. Exp. Med.* **47:** 757–775

Landsteiner, K. and P. Levine, 1928b. On the inheritance of agglutinogens of human blood demonstrable by immune agglutinins. *J. Exp. Med.* **48:** 731–749

Landsteiner, K. and P. Levine. 1929. On the racial distribution of some agglutinable structures of human blood. *J. Immunol.* **16:** 123–131

Landsteiner, K. and P. Levine. 1930. On the inheritance and racial distribution of agglutinable properties of human blood. *J. Immunol.* **18:** 87–94

Landsteiner, K. and P. Levine. 1931. The differentiation of a type of human blood by means of normal animal serum. *J. Immunol.* **20:** 179–185

Landsteiner, K. and C.P. Miller. 1925. Serological studies on the blood of primates. II. The blood groups in anthropoid apes. *J. Exp. Med.* **42:** 853–862

Landsteiner, K. and M. Richter. 1903. Ueber die Verwertbarkeit Blutdif-

ferenzen für die forensische Praxis. *Z. Medizinalbeamte* **16**(3): 85–89

Landsteiner, K. and J. Van der Scheer. 1925a. On the antigens of red blood corpuscles. The question of lipoid antigens. *J. Exp. Med.* **41**: 427–437

Landsteiner, K. and J. van der Scheer. 1925b. On the antigens of red blood corpuscles. II. Flocculation reactions with alcoholic extracts of erythrocytes. *J. Exp. Med.* **42**: 123–142

Landsteiner, K. and J. van der Scheer. 1936. On cross reactions of immune sera to azoproteins. *J. Exp. Med.* **63**: 325–339

Landsteiner, K., J. van der Scheer and D.H. Witt. 1925. Group specific flocculation reactions with alcoholic extracts of human blood. *Proc. Soc. Exp. Biol. Med.* **22**: 289–291

Landsteiner, K. and A.S. Wiener. 1940. An agglutinable factor in human blood recognized by immune sera for Rhesus blood. *Proc. Soc. Exp. Biol. Med.* **43**: 223

Landsteiner, K. and A.S. Wiener. 1941. Studies on an agglutinogen (Rh) in human blood reacting with anti-Rhesus sera and with human isoantibodies. *J. Exp. Med.* **74**: 309–320

Landsteiner, K. and D.H. Witt. 1924. Observations on human isoagglutinins. *Proc. Soc. Exp. Biol. Med.* **21**: 389–392

Landsteiner, K. and D.H. Witt. 1926. Observations on the human blood groups. Irregular reactions. Isoagglutinins in the sera of group IV. The factor A^1. *J. Immunol.* **11**: 221–247

Lang, B.G. 1975a. The assay of ABO antisera intended for use in the grouping of bloodstains. *Forensic Sci.* **5**: 146 [Abstr.]

Lang, B.G. 1975b. The use of bovine serum albumin for H antigen typing in bloodstains. *Forensic Sci.* **5**: 147 [Abstr.]

Lang, B.G. 1976a. The assay of ABO antisera intended for use in the grouping of bloodstains. *J. Forensic Sci. Soc.* **16**: 55–60

Lang, B.G. 1976b. The use of bovine serum albumin for H antigen typing in bloodstains. *J. Forensic Sci. Soc.* **16**: 61–66

Lang, B.G., E. Rutter and P.H. Whitehead. 1979a. The chromatography of blood group substances in non-secretor salivas. *HOCRE Rept.* No. 314, Home Office Central Res. Estab., Aldermaston, Reading, Berks., England

Lang, B.G., E. Rutter and P.H. Whitehead. 1979b. The chromatography of blood group substances in non-secretor salivas. *J. Forensic Sci. Soc.* **19**: 293–299

Langer, J. 1903. Ueber Isoagglutinine beim Menschen, mit besonderer Berücksichtigung des Kindesalters. *Z. Heilkd., Abt. Int. Med.* **24**(N.F. 4): 111–141

Lanset, S., C. Ropartz, P.-Y. Rousseau, Y. Guerbet and C. Salmon. 1966. Une famille comportant les phenotypes Bombay: O_h^{AB} et O_h^B. *Transfusion* (Paris) **9**: 255–263

Lattes, L. 1913. Sull'applicazione pratica della prova di agglutinazione per la diagnosi specifica ed individuale di sangue umano. *Arch. Antropol. Crim. Psichiatr. Med. Leg.* **34** (4 ser. **5**): 310–325 ¶

Lattes, L. 1915a. L'individualita del sangue umano e la sua dimostrazione medico-legale. *Arch. Antropol. Crim. Psichiatr. Med. Leg.* **36** (4 ser. **7**): 422–447 and 538–554

Lattes, L. 1915b. L'individualité su sang humain et sa demonstration médico-légale. *Arch. Ital. Biol.* **64** (N.S. **4**): 388–404

Lattes, L. 1916a. Due casi pratici di diagnosi individuale di sangue umano. *Arch. Antropol. Crim. Psichiatr. Med. Leg.* **37** (4 ser. **7**): 298–303) ¶

Lattes, L. 1916b. Sulla tecnica della prova di isoagglutinazione per la diagnosi individuale del sangue. *Arch. Antropol. Crim. Psichiatr. Med. Leg.* **37** (4 ser. 7): 400–408 ¶

Lattes, L. 1923. Le diagnostic individuel des taches de sang. *Ann. Med. Leg. Criminol. Police Scientif.* **3**: 213–226

Lattes, L. 1927. Praktische Erfahrungen über Blutgruppenbestimmung in Flecken. *Dtsch. Z. Gesamte Gerichtl. Med.* **9**: 402–419 ¶

Lattes, L. 1928. Blutgruppendiagnose von Blutflecken. *Ukrain. Zentralbl. Blutgruppenforsch.* **2**(2): 36–46

Lattes, L. 1932. *Individuality of the Blood in Biology and in Clinical and Forensic Medicine.* Oxford Univ. Press, London

Lattes, L. and G. Canuto. 1926. Ancora un caso di diagnosi individuale di macchie sanguigne (con nuovo procedimento tecnico). *Rass. Int. Clin. Terap.* **7**: 248–252

Lattes, L. and A. Cavazzuti. 1924. Sur l'existence d'une troisième élément d'isoagglutination. *J. Immunol.* **9**: 407–425

Laudanna, A.A. and M. Segre. 1959. A proposito della diagnosi individuale (gruppo sanguigno) di macchie di sangue mediante l'elettroforesi su carta. *Minerva Medicoleg.* **79**: 182–183

Lauer, A. 1928. Zur Kenntnis der erblichen Blutstrukturen. *Dtsch. Z. Gesamte Gerichtl. Med.* **11**: 264–277

Lauer, A. 1933. Zur Technik der Blutfleckdiagnose nach M und N. *Dtsch. Z. Gesamte Gerichl. Med.* **22**: 86–94

Lauer, A. 1964. Die Vererbungsweise im Rh-System. *Blut* **10**: 99–112

Lawler, S.D. 1950. The inheritance of the Lutheran blood groups in forty-seven English families. *Ann. Eugenics* **15**: 255–257

Layrisse, M. and T. Arends. 1956. The "Diego" blood factor distribution. Genetic, clinical and anthropological significance, *in*: Proc. 6th Congr. Int. Soc. Blood Transf. Boston, *Bibliotheca Haematol.*, Fasc. **7**: 114–116, S. Karger, Basel and N.Y. (1958)

Layrisse, M., T. Arends and R. Dominguez Sisco. 1955. Nuevo grupo sanguineo encontrada en descendientas de Indios. Su capacidad productora de incompatibilidad materno-fetal e importancia antropologica. *Acta Med. Venezolana* **3**: 132–138

Layrisse, M. and J. Wilbert. 1966. *Indian Societies of Venezuela. Their Blood Group Types.* Inst. Caribe Antropol. Sociol. Fundac. la Salle Cienc. Natural. Monogr. No. 13, Editorial Sucre, Caracas

League of Nations. Health Organization. 1930. Permanent Standardization Commission. Conference on Blood Groups. August 6, 1930. Document C.H. 885, from conference held July 21–25, 1930 at the Pasteur Institute, Paris.

Lecha-Marzo, A. and A. Piga. 1914. Algunos experimentos sobre la diferencia medico-legal de la sangre de diversos individuos. *Rev. Criminol. Psiquiatr. Med. Leg.* **1**: 668–671

Lee, C.L. 1975. Current status of paternity testing. *Family Law Q.* **9**: 615–633

Lehrs, H. 1930. Über gruppenspezifische Eigenschaften des menschilichen Speichels. *Z. Immunitaetsforsch. Exp. Ther.* **66**: 175–192

Leister, C.I., Jr. and P.L. Kirk. 1961. Purification of plant lectins by continuous electrophoresis and their application to the grouping of dry blood stains. *J. Forensic Med.* **8**: 42–46

Leister, C.I., Jr., F.R. Sylvia and P.L. Kirk. 1961. Possible application of plant lectins to genotype determination. *J. Forensic Med.* **8**: 85–91

Lenkiewicz, B. and B. Sarul. 1971. Anti-A_1 in blood donors in the Warsaw region. *Arch. Immunol. Ther. Exp.* **19**: 643–647

LeRoux, M.E. and M. Shapiro. 1969. The antigen M_1. *J. Forensic Med.* **16**: 135–138

Levine, P. 1932. The application of blood groups in forensic medicine. *Am. J. Police Sci.* **3**(2): 157–168

Levine, P. 1943. The pathogenesis of erythroblastosis fetalis. *J. Pediatrics* **23**: 656–675

Levine, P. 1945. On the Hr factor and the Rh genetic theory. *Science* **102**: 1–4

Levine, P., M. Becker, M. Wigod and R. Ponder. 1949. A new human hereditary blood property (Cellano) present in 99.8% of all bloods. *Science.* **109**: 464–466

Levine, P., O.B. Bobbitt, R.K. Waller and A. Kuhmichel. 1951a. Isoimmunization by a new blood factor in tumor cells. *Proc. Soc. Exp. Biol. Med.* **77**: 403–405

Levine, P., L. Burnham, E.M. Katzin and P. Vogel. 1941b. The role of isoimmunization in the pathogenesis of erythroblastosis fetalis. *Am. J. Obstet. Gynecol.* **42**: 925–937

Levine, P., M.J. Cellano, F. Falkowski, J.W. Chambers, O.B. Hunter, Jr., and C.T. English. 1964. A second example of —/— blod or Rh_{null}. *Nature* **204**: 892–893

Levine, P., M. Celano, R. Fenichel, W. Pollack and H. Singher. 1961b. A "D-like" antigen in Rhesus monkey, human Rh positive and human Rh negative red blood cells. *J. Immunol.* **87**: 747–752

Levine, P., M. Celano, R. Fenichel and H. Singher. 1961a. A "D-like" antigen in Rhesus red blood cells and in Rh-positive and Rh-negative red cells. *Science* **133**: 332–333

Levine, P., M.J. Celano, G.H. Vos and J. Morrison. 1962. The first human blood, —/—, which lacks the 'D-like' antigen. *Nature* **194**: 304–305

Levine, P., M.J. Celano, J. Wallace and R. Sanger. 1963. A human 'D-like' antigen. *Nature* **198:** 596–597

Levine, P., E.M. Katzin and L. Burnham. 1941a. Isoimmunization in pregnancy. Its possible bearing on the etiology of erythroblastosis fetalis. *J. Am. Med. Assoc.* **116:** 825–827

Levine, P., E.A. Koch, R.T. McGee and G.H. Hill. 1954b. Rare human isoagglutinins and their identification. *Am. J. Clin. Pathol.* **24:** 292–304

Levine, P., A.B. Kuhmichel, M. Wigod and E. Koch. 1951b. A new blood factor, s, allelic to S. *Proc. Soc. Exp. Biol. Med.* **78:** 218–220

Levine, P., F. Ottensooer, M.J. Celano and W. Pollitzer. 1955a. On reactions of plant anti-N with red cells of chimpanzees and other animals. *Am. J. Phys. Anthropol.* **13**(N.S.): 29–36

Levine, P., E.A. Robinson, B. Pryer and O. Michel. 1954a. Anti-Tj^a in second pair of U.S. sibs with observations on the original sibs. *Vox Sang.* **4:** (O.S.): 143–148 §²

Levine, P., E. Robinson, M. Celano, O. Briggs and L. Falkinburg. 1955b. Gene interaction resulting in suppression of blood group substance B. *Blood* **10:** 1100–1108

Levine, P., E. Robinson, M. Layrisse, T. Arends and R. Domingues Sisco. 1956. The Diego blood factor. *Nature* **177:** 40–41

Levine, P., R.E. Rosenfield and J. White. 1961c. The first example of the Rh phenotype r^Gr^G. *Am. J. Hum. Genet.* **13:** 299–305

Levine, P. J.S. Sneath, E.A. Robinson and P.W. Huntington, Jr. 1955c. A second example of anti-Fy^b. *Blood* **10:** 941–944

Levine, P. and R.E. Stetson. 1939. An unusual case of intra-group agglutination. *J. Am. Med. Assoc.* **113:** 126–127 and see *Vox Sang.* **38:** 297–300 (1980)

Levine, P., M. Uhlíř and J. White. 1961d. A_h, an imcomplete suppression of A resembling O_h. *Vox Sang.* **6:** 561–567

Lewis, M., H. Kaita, P.W. Allderdice, S. Bartlett, W.G. Squires and R.G. Huntsman. 1979. Assignment of the red cell antigen, Targett (Rh40), to the Rh blood group system. *Am. J. Hum. Genet.* **31:** 630–633

Lewis, M., H. Kaita and B. Chown. 1957. The blood groups of a Japanese population. *Am. J. Hum. Genet.* **9:** 274–283

Lewis, M., H. Kaita and B. Chown. 1972. The Duffy blood group system in Caucasians. A further population sample. *Vox Sang.* **23:** 523–527

Lewis, M., H. Kaita, B. Chown, E.R. Giblett, J. Anderson and G.B. Côté. 1977. The Lutheran and Secretor loci: genetic linkage analysis. *Am. J. Hum. Genet.* **29:** 101–106

Lewis, M., H. Kaita, D. Duncan and B. Chown. 1960. Failure to find the hypothetic K^a (KKp^a) of the Kell blood group system *Vox Sang.* **5:** 565–567

Libich, M., M. Kout and C.M. Giles. 1978. Fy(a–b–) phenotype in Czechoslovakia. *Vox Sang* **35:** 423–425

Lillie, R.D. 1977. (ed.). *H. J. Conn's Biological Stains,* 9th ed., Williams and Wilkins, Baltimore

Limber, G.K., R.F. Davis and S. Bakerman. 1970. Acrylamide gel electrophoresis studies of human erythrocyte membranes. *Blood* **36:** 111–118

Lincoln, P.J. 1973. A study of elution of blood group antibodies from red cells and its useful application to forensic serology. *The London Hosp. Gaz.,* Clin. Scientif. Suppl., June 1973

Lincoln, P.J. and B.E. Dodd. 1968a. Mixed agglutination as a method for the determination of A, B and H blood groups of hair. *Med. Sci. Law* **8:** 38–40

Lincoln, P.J. and B.E. Dodd. 1968b. The detection of the Rh antigens C, C^w, c, D, E and e and the antigen S of the MNSs system in bloodstains. *Med. Sci. Law* **8:** 288–295

Lincoln, P.J. and B.E. Dodd. 1969. Antigen-antibody studies in the ABO blood group system with particular reference to cross-reacting antibodies in group O sera. *Immunology* **16:** 301–310

Lincoln, P.J. and B.E. Dodd. 1973. An evaluation of the factors affecting the elution of antibodies from bloodstains. *J. Forensic Sci. Soc.* **13:** 37–45

Lincoln, P.J. and B.E. Dodd. 1975a. A micro-elution technique using anti-human globulin for the detection of the S, s, K, Fy^a, Fy^b and Jk^a antigens in stains and their useful application to casework. *Forensic Sci.* **5:** 147 Abstr.

Lincoln, P.J. and B.E. Dodd. 1975b. The application of a microelution technique using anti-human globulin for the detection of the S, s, K, Fy^a, Fy^b and Jk^a antigens in stains. *Med. Sci. Law* **15:** 94–101

Lincoln, P.J. and B.E. Dodd. 1978. The use of low ionic strength solution (LISS) in elution experiments and in combination with papain-treated cells for the titration of various antibodies, including eluted antibody. *Vox Sang* **34:** 221–226

Lisowska, E. 1960. Badania nad antygenami grupowymi M i N. Doniesienie VI. Rozkted wydzielonyck substancji grupowych M i N enzymami proteolitycznymi. *Arch. Immunol. Terap. Doswiad.* **8:** 235–242

Lisowska, E. and M. Duk. 1975. Effect of modification of amino groups of human erythrocytes on M, N and N_{Vg} blood group specificities. *Vox Sang.* **28:** 392–397

Lisowska, E. and M. Kordowicz. 1977. Specific antibodies for desialized M and N blood group antigens. *Vox Sang.* **33:** 164–169

Lisowska, E. and A. Morawiecki. 1967. The role of free amino groups in the blood group activity of M and N mucoids. *Eur. J. Biochem.* **3:** 237–241

Litten, J., R. Culpepper and S. Bakerman. 1978. Studies on the characterization of the Rh_o (D) antigen. *Biochim. Biophys. Acta* **543:** 226–234

Lodge, T.W., J. Andersen and E.R. Gold. 1965. Observations on antibodies reacting with adult and cord Le(a–b–) cells, with O_h Le (a–b–) cells and a soluble antigen present in certain salivas. *Vox Sang.* **10:** 73–81

Lodge, T.W. and A. Usher. 1962. Lewis blood group substances in seminal fluid. *Vox Sang.* **7:** 329–333

Lopez, M., J. Benali, V. Bony and Ch. Salmon. 1979. Activity of IgG and IgM antibodies against some weak A (A_3, A_x, A_{end}) and weak B (B_3, B_x) red cells. *Vox Sang.* **37:** 281–285

Lorusso, D.J., J.P. Binette and F.A. Green. 1977. The Rh antigen system and disaggregated human erythrocyte membranes. *Immunochemistry* **14:** 503–508

Lorusso, D.J. and F.A. Green. 1975. Reconstitution of Rh(D) antigen activity from human erythrocyte membranes solubilized by deoxycholate. *Science* **188:** 66–67

Lovett, D.A. and M.N. Crawford. 1967. Js^b and Go^a screening of Negro donors. *Transfusion* (Phila.) **7:** 442

Löw, B. 1955. A practical method using papain and incomplete Rh-antibodies in routine Rh blood-grouping. *Vox Sang.* **5** (O.S.): 94–98 §²

Löw, B. and L. Messeter. 1974. Antiglobulin test in low-ionic strength salt solution for rapid antibody screening and cross matching. *Vox Sang.* **26:** 53–61

Lund, H. 1941. Relation of the concentration of red blood cells to the sensitivity of the isoagglutination reaction. *Arch. Pathol.* **31:** 458–466

Lundquist, F. 1949. Aspects of the biochemistry of human semen. *Acta Physiol. Scand.* **19,** Suppl. 66: 1–105

MacDonald, K.A., M.E. Nichols, W.L. Marsh and W.J. Jenkins. 1967. The first example of anti-Henshaw in human serum. *Vox Sang.* **13:** 346–348

MacIlroy, M., J. McCreary and M. Stroup. 1972. Anti-Lu8, an antibody recognizing another Lutheran-related antigen. *Vox Sang.* **23:** 455–457

Macmahon, B. and J.C. Folusiak. 1958. Leukemia and ABO blood group. *Am. J. Hum. Genet.* **10:** 287–293

Macvie, S.I., J.A. Morton and M.M. Pickles. 1967. The reactions and inheritance of a new blood group antigen, Sd^a. *Vox Sang.* **13:** 485–492

Madivale, M.S., V.M. Chitale and H.S. Mahal. 1971. Eine verbesserte Absorptions-Elutions-Methode zur ABO-Gruppenbestimmung an Blutflecken. *Arch. Kriminol.* **147:** 168–175

Mainwaring, U.R. and M.M. Pickles. 1948. A further case of anti-Lutheran immunization, with some studies on its capacity for human sensitization. *J. Clin. Pathol.* **1:** 292–294

Mäkelä, O. 1957. Studies in hemagglutinins of Leguminosae seeds. *Ann. Med. Exp. Biol. Fenn.* **35,** Suppl. No. 11: 1–133

Mäkelä, O. and K. Cantell. 1958. Destruction of M and N blood group receptors of human red cells by some influenza viruses. *Ann. Med. Exp. Biol. Fenn.* **36:** 366–374

Mäkelä, O. and P. Mäkelä. 1956a. Le^b antigen. Studies on its occurrence in red cells, plasma and saliva. *Ann. Med. Exp. Biol. Fenn.* **34:** 157–162

Mäkelä, O. and P. Mäkelä. 1956b. Some new blood group specific phytagglutinins. A preliminary report. *Ann. Med. Exp. Biol. Fenn.* **34:** 402–404

Mäkelä, O., P.H. Mäkelä and A. Kortekangas. 1967. In vitro transformation of the Lewis blood groups of erythrocytes. *Ann. Med. Exp. Biol. Fenn.* **45:** 159–164

Mäkelä, O., E. Ruoslahti and C. Ehnholm. 1969. Subtypes of human ABO blood groups and subtype-specific antibodies. *J. Immunol.* **102:** 763–771

Maniatis, A., B. Frieman and J.F. Bertles. 1977. Increased expression in erythrocytic Ii antigens in sickle cell disease and sickle cell trait. *Vox Sang.* **33:** 29–36

Marchesi, V.T. 1979. Functional proteins of the human red blood cell membrane. *Sem. Hematol.* **16:** 3–20

Marcinkowski, T. 1959. Praktische Modifizierung der Lattesmethode. *Dtsch. Z. Gesamte Gerichtl. Med.* **48:** 246–267

Marcinkowski, T. 1970. Detection of antigens of the system ABO in blood stains by means of red blood corpuscles E-Li. *J. Forensic Med.* **17:** 69–71

Marcus, D.M. 1969. The ABO and Lewis blood-group system. Immunochemistry, genetics and relation to human disease. *New Engl. J. Med.* **280:** 994–1006

Marcus, D.M. 1970. The nature of the Le^a and Le^b antigens in human plasma. *Ann. N.Y. Acad. Sci.* **169:** 161–163

Marcus, D.M. 1971. Isolation of a substance with blood-group P_1 activity from human erythrocyte stroma. *Transfusion* (Phila.) **11:** 16–18

Marcus, D.M., E.A. Kabat and R.E. Rosenfield. 1963. The action of enzymes from Clostridium tertium on the I antigenic determinant of human erythrocytes. *J. Exp. Med.* **118:** 175–194

Marcus, D.M., M. Naiki and S.K. Kundu. 1976. Abnormalities in the glycosphingolipid content of human P^k and p erythrocytes. *Proc. Nat. Acad. Sci. USA* **73:** 3263–3267

Maresch, W. and E. Wehrschütz. 1963. Moderne Methode der Blutflecken-diagnostik (Agargeldiffusion and Mischagglutination). *Arch. Kriminol.* **132:** 1–9

Margni, R.A., J. Leoni and M. Bazzurro. 1977. The incomplete anti-Rh antibody agglutination mechanism of trypsinized ORh+ red cells. *Immunology* **33:** 153–160

Marsh, W.L. 1960. The pseudo B antigen. A study of its development. *Vox Sang.* **5:** 385–397

Marsh, W.L. 1961. Anti-i: a cold antibody defining the Ii relationship in human red cells. *Br. J. Haematol.* **7:** 200–209

Marsh, W.L. 1970. Letter to the Editor. *Transfusion* (Phila.) **10:** 41

Marsh, W.L. 1972. Anti-Lu5, anti-Lu6 and anti-Lu7. Three antibodies defining high frequency antigens related to the Lutheran blood group system. *Transfusion* (Phila.) **12:** 27–34

Marsh, W.L. 1975a. Studies on the Kell blood group system. *Med. Lab. Technol.* **32:** 1–18

Marsh, W.L. 1975b. Present status of the Duffy blood group system. *CRC Crit. Rev. Clin. Lab. Sci.* **5:** 387–412

Marsh, W.L. and W.J. Jenkins. 1960. Anti-i: a new cold antibody. *Nature* **188:** 753

Marsh, W.L., W.J. Jenkins and W.W. Walther. 1959. Pseudo-B: an acquired group antigen. *Br. Med. J.* **2:** 63–66

Marsh, W.L., M.E. Nichols and M.E. Reid. 1971. The definition of two I antigen components. *Vox Sang.* **20:** 209–217

Marsh, W.L., R. Øyen and M.E. Nichols. 1974. Kidd blood-group antigens of leukocytes and platelets. *Transfusion* (Phila.) **14:** 378–381

Marsh, W.L., R. Øyen and M.E. Nichols. 1976. Kx antigen, the McLeod phenotype, and chronic granulomatous disease: further studies. *Vox Sang.* **31:** 356–362

Marsh, W.L., R. Øyen, M.E. Nichols and F.H. Allen, Jr. 1975a. Chronic granulomatous disease and the Kell blood groups. *Br. J. Haematol.* **29:** 247–262

Marsh, W.L., H.F. Taswell, R. Øyen, M.E. Nichols, M.S. Vergara and A.A. Pineda. 1975b. Kx antigen of the Kell system and its relationship to chronic granulomatous disease. Evidence that the Kx gene is X-linked. *Transfusion* (Phila.) **15:** 527 [Abstr.]

Marsters, R.W. and F.C. Schlein. 1958. Factors affecting the deterioration of dried bloodstains. *J. Forensic Sci.* **3:** 288–302

Martin, P.D. 1977. A manual method for the detection of Rh antigens in dried bloodstains. *J. Forensic Sci. Soc.* **17:** 139–142

Martin, P.D., S.P. Rand and M. Pereira. 1975. Comparison and evaluation of methods of detection of Rh antigens in dried bloodstains. *Forensic Sci.* **5:** 150–151 [Abstr.]

Masis, T.M. 1964. Secretion of agglutinogens of the ABO isoserological system, *in*: M. V. Kryukov (ed.) *Mezhdunar. Kongr. Antropol. Etnogr. Nauk.*, 7th, 1964, **1:** 472–478 (publ. 1968) through *Chem. Abstr.* **72:** 20115 (1970)

Masis, T.M. 1971. Postmortem determination of ABO secretor status by studying the properties of cadaver bile and urine. *Sud. Med. Ekspert.* (Moscow) **14**(2): 25–29

Masis, T.M. and V.P. Ol'khovik. 1970. The medico-legal examination of a blood sample with an uncommon group characteristic. *Sud. Med. Ekspert.* (Moscow) **13**(2): 55–56

Masis, T.M., G.S. Yudina and V.P. Ol'khovic. 1973. Detection of the A and B antigens in saliva and sperm by the absorption-elution method. *Sud. Med. Ekspert.* (Moscow) **16**(2): 31–33

Masouredis, S.P. 1960. Relationship between $Rh_0(D)$ genotype and quantity of I^{131} anti-$Rh_0(D)$ bound to red cells. *J. Clin. Invest.* **39:** 1450–1462

Masouredis, S.P. and P. Sturgeon. 1965. Quantitative serologic and isotopic studies on the Rh_0 variant—D^u. *Blood* **25:** 954–975

Masouredis, S.P., E.J. Sudora and E.J. Victoria. 1977. Immunological and electron microscopic analysis of IgG anti-D saline hemagglutination of neuraminidase- and protease-modified red cells. *J. Lab. Clin. Med.* **90:** 929–948

Matson, G.A., E.A. Koch and P. Levine. 1954. A study of the hereditary blood factors among the Chippewa Indians of Minnesota. *Am. J. Phys. Anthropol.* **12**(N.S.): 413–426

Matson, G.A., J. Swanson, J. Noades, R. Sanger and R.R. Race. 1959. A "new" antigen and antibody belonging to the P blood group system. *Am. J. Hum. Genet.* **11:** 26–34

Matsuzawa, S., N. Yoshikawa and Y. Hata. 1972. Secretion of anti-A and anti-B agglutinins into human saliva. *Jpn. J. Leg. Med.* **26:** 19–23 §¹

Matta, D. 1937. A critical examination of the blood groups and their medico-legal application. The Egyptian University, Cairo, Faculty of Medicine Publ. No. 11

Matuhasi, T. 1966. Evidence of monospecificity of anti-A and anti-B in group O serum, *in*: Proc. 11th Congr. Int. Soc. Blood Transf., Sydney, *Bibliotheca Haematol.*, Fasc. **29**, Part I: 378–383, S. Karger, Basel and N.Y. (1968)

Mayeda, K. 1966. Study of tolerance to the ABO blood group antigens. *Vox Sang.* **11:** 33–37

McConnell, R.B. 1961. Lewis blood group substances in body fluids, *in*: 2nd Int. Conf. Human Genetics, Rome, Excerpta Medica Int. Congr. Ser. No. 32, Excerpta Medica Foundation, Amsterdam, London, etc.

McDowall, M.J., P.J. Lincoln and B.E. Dodd. 1978a. Increased sensitivity of tests for the detection of blood group antigens in stains using a low ionic strength medium. *Med. Sci. Law* **18:** 16–23

McDowall, M.J., P.J. Lincoln and B.E. Dodd. 1978b. Observations on the use of an autoanalyser and a manual technique for the detection of the red cell antigens C, D, E, c, K and S in bloodstains. *Forensic Sci.* **11:** 155–164

McGinnis, M.H., A.J. Grindon and P.J. Schmidt. 1974. Evidence for two anomalous I blood group determinants. *Transfusion* (Phila.) **14:** 257–260

McIlvaine, T.C. 1921. A buffer solution for colorimetric comparison. *J. Biol. Chem.* **49:** 183–186

McKusick, V. 1975. *Mendelian Inheritance in Man.* Catalogues of Autosomal Dominant, Autosomal Recessive and X-Linked Phenotypes, 4th ed., The Johns Hopkins Univ. Press, Baltimore

McNeil, C., E.F. Trentelman, C.D. Fullmer, V.O. Kreutzer and R.B. Orlob. 1957b. The significance of blood group conflicts and aberrant salivary secretions in spontaneous abortions. *Am. J. Clin. Pathol.* **28:** 469–480

McNeil, C., E.F. Trentelman, W.M. Helmick, R. Orlob and J.G. Haskell. 1960. Family blood group and secretion studies. *Vox Sang.* **5:** 164–170

McNeil, C., E.F. Trentelman, V.O. Kreutzer and C.D. Fullmer. 1957a. Aberrant secretion of salivary A, B and H group substances in human beings. *Am. J. Clin. Pahol.* **28:** 145–151

McWright, C.G. 1961. The study of group specific substances in keratinized tissues. *J. Forensic Sci.* **6:** 351–353

Merkeley, D.K. 1953. Isoagglutinins in dried blood stains. *Am. J. Clin. Pathol.* **23**: 190–192

Metaxas, M.N. and M. Metaxas-Bühler. 1964. M^k: an apparently silent allele at the MN locus. *Nature* **202**: 1123

Metaxas-Bühler, M., E.W. Ikin and J. Romanski. 1961. Anti-N in the serum of a healthy blood donor of group MN. *Vox Sang.* **6**: 574–582

Michailow, R. 1977. Methode zur Blutgruppenbestimmung von unvollständig hämolysiertem Blut nach Verlust seiner Agglutinationsfähigkeit. *Z. Rechtsmed.* **80**: 35–38

Mikami, Y., K. Haba, H. Morioka, M. Tanaka, T. Habara, K. Satoh, S. Tobe, T. Kamada, S. Ueno, T. Kiyotani and Y. Minagi. 1966. Consecutive application of preliminary blood test, fibrin plate method, and group specific double combination method for the identification of human blood type with a minute quantity of blood stained material. *Jpn. J. Leg. Med.* **20**: 207–212 §'

Miller, E.B., R.E. Rosenfield and P. Vogel. 1951. On the incidence of some of the new blood agglutinogens in Chinese and Negroes. *Am. J. Phys. Anthropol.* **9**(N.S.): 115–126

Miller, E.B., R.E. Rosenfield, P. Vogel, G. Haber and N. Gibbel. 1954. The Lewis blood factors in American Negroes. *Am. J. Phys. Anthropol.* **12**(N.S.): 427–443

Miller, L.H., S.J. Mason, J.A. Dvorak, M.H. McGinnis and I.K. Rothman. 1975. Erythrocyte receptors for (Plasmodium knowlesi) malaria: Duffy blood group determinants. *Science* **189**: 561–563

Milne, G.R., J. Wallace, E.W. Ikin and A.E. Mourant. 1953. The Kidd (anti-Jk^a) haemagglutinin: a third example. *Lancet* **1**: 627

Milne, R.W. and C. Dawes. 1973. The relative contributions of different salivary glands to the blood group activity of whole saliva in humans. *Vox Sang* **25**: 298–307

Mino, P. 1924. Ueber die angebliche Existenz von mehr als zwei isoagglutininen im menschlichen Blute. *Muench. Med. Wochenschr.* **71**(33): 1129–1130

Mishakova, M.V. 1961. Poluchenie immunnykh syvorotok anti-P, metodika primeneniya i izuchenie ikh svoystv. (Soobshchenie II). *Sud. Med. Ekspert.* (Moscow) **4**(2): 35–40

Mitra, A.K. and H.N. Ganguly. 1973. Simplified mixed agglutination technique for determination of blood group of dried blood stains. *Indian J. Exp. Biol.* **11**: 433–435

Mohr, J. 1951a. A search for linkage between the Lutheran blood group and other hereditary characteristics. *Acta Pathol. Microbiol. Scand.* **28**: 207–210

Mohr, J. 1951b. Estimation of linkage between the Lutheran and the Lewis blood groups. *Acta Pathol. Microbiol. Scand.* **29**: 339–343

Mollison, P.L. 1972. *Blood Transfusion in Clinical Medicine,* 5th ed., Blackwell Scientific Publications, Oxford, London, etc.

Molthan, L. 1980. The second example of anti-M_1 antibodies produced by a group MN person. *Vox Sang.* **38**: 210–212

Molthan, L. and M.N. Crawford. 1966. Three examples of anti-Lu^b and related data. *Transfusion* (Phila.) **6**: 584–589

Molthan, L., M.N. Crawford, W.L. Marsh and F.H. Allen, Jr. 1973a. Lu9, another new antigen of the Lutheran blood-group system. *Vox Sang.* **24**: 468–471

Molthan, L., M.N. Crawford and P. Tippett. 1973b. Enlargement of the Dombrock blood group system: the finding of anti-Do^b. *Vox Sang.* **24**: 382–384

Moore, H.C. and P.L. Mollison. 1976. Use of low-ionic-strength medium in manual tests for antibody detection. *Transfusion* (Phila.) **16**: 291–296

Moore, R.E. 1955. Distribution of blood factors ABO, MN and Rh in a group of American Negroes. *Am. J. Phys. Anthropol.* **13**(NS): 121–128

Moores, P., M. Marks and M.C. Botha. 1980. S-s-U- phenotype in the coloured population of Cape Town: problems encountered in paternity tests. *Forensic Sci. Int.* **15**: 207–214

Morgan, B.R.J. and S.L. Richards. 1967. Subtyping of group A bloodstains. *Med. Sci. Law* **7**: 82–83

Morgan, R.J. 1964. The examination of an unusual bloodstain. *Med. Sci. Law* **4**: 122

Morgan, W.T.J. 1943. An artificial antigen with blood-group A specificity. *Br. J. Exp. Pathol.* **24**: 41–49

Morgan, W.T.J. 1970. Molecular aspects of human blood group specificity. *Ann. N.Y. Acad. Sci.* **169**: 188–130

Morgan, W.T.J. and H.K. King. 1943. Studies in immunochemistry. 7. The isolation from hog gastric mucin of a polysaccharide-amino acid complex possessing blood group A specificity. *Biochem. J.* **37**: 640–651

Morgan, W.T.J. and R. van Heyningen. 1944. The occurrence of A, B and O blood group substances in pseudo-mucinous ovarian cyst fluids. *Br. J. Exp. Pathol.* **25**: 5–15

Morgan, W.T.J. and M.B.R. Waddell. 1945. A specific blood group O substance. *Br. J. Exp. Pathol.* **26**: 387–396

Morgan, W.T.J. and W.M. Watkins. 1948. The detection of a product of the blood group *O* gene and the relationship of the so-called O-substance to the agglutinogens A and B. *Br. J. Exp. Pahtol.* **29**: 159–173

Morgan, W.T.J. and W. Watkins. 1951. The inactivation of the blood group receptors on the erythrocyte surface by the periodate ion. *Br. J. Exp. Pathol.* **32**: 34–48

Morgan, W.T.J. and W.M. Watkins. 1962. Blood group P_1 substance: (I) Chemical properties, *in*: Proc. 9th Congr. Int. Soc. Blood Transf., Mexico City, *Bibliotheca Haematol.*, Fasc. **19**: 225–229 (1964)

Morgan, W.T.J. and W.M. Watkins. 1969. Genetic and biochemical aspects of human blood-group A-, B-, H-, Le^a- and Le^b- specificity. *Br. Med. Bull.* **25**(1): 30–34

Morley, G. 1978. A study of different Rhesus phenotypes and their binding characteristics. *Vox Sang.* **35**: 382–386

Morton, J.A. 1962. Some observations on the action of blood-group antibodies on red cells treated with proteolytic enzymes. *Br. J. Haematol.* **8**: 134–148

Morton, J.A. and M.M. Pickles. 1947. Use of trypsin in the detection of incomplete anti-Rh antibodies. *Nature* **159**: 779–780

Morton, J.A. and M.M. Pickles. 1965. Screening for blood group antibodies with the autoanalyzer, *in*: L.T. Skeggs (ed.) *Automation in Analytical Chemistry*, Technicon Symp., 1965, pp. 535–538, Mediad Inc., New York (1966)

Morton, J.A., M.M. Pickles and A.M. Terry. 1970. The Sd^a blood group antigen in tissues and body fluids. *Vox Sang.* **19**: 472–482

Morton, J.A. and A.M. Terry. 1970. The Sd^a blood group antigen. Biochemical properties of urinary Sd^a. *Vox Sang.* **19**: 151–161

Morville, P. 1929. Investigations on isohemagglutination in mothers and newborn children. The development of the blood characteristics during the 1st year of life. *Acta Pathol. Microbiol. Scand.* **6**: 39–65

Moskow, Iw. 1935. Untersuchungen über Bestandigkeit und Vererbung der Isoagglutinationsempfindlichkeit der roten Blutkörperchen. (Untergruppen in der Merkmalen B, M und N). *Dtsch. Z. Gesamte Gerichtl. Med.* **24**: 333–342

Moskowitz, M. and M. Calvin. 1952. On the components and structure of the human red cell membrane. *Exp. Cell Res.* **3**: 33–46

Moskowitz, M. and S. Carb. 1957. Surface alterations and the agglutinability of red cells. *Nature* **180**: 1049–1050

Moskowitz, M., W.B. Dandliker, M. Calvin and R.S. Evans. 1950. Studies on the antigens of human red cells. I. The separation from human erythrocytes of a water soluble fraction containing the Rh, A and B factors. *J. Immunol.* **65**: 383–392

Moss, W.L. 1910a. Studies on isoagglutinins and isohemolysins. *Bull. Johns Hopkins Hosp.* **21**: 63–70

Moss, W.L. 1910b. Studien über Isoagglutinine and Isohämolysine. *Folia Serol.* **5**: 267–281

Moulds, J.J., D. Dykes and H.F. Polesky. 1974. A silent allele in the Colton blood group system. *Transfusion* (Phila.) **14**: 508 [Abstr.]

Moullec, J. and B. LeChevrel. 1959. Un facteur B faible dans un sang du groupe AB. *Transfusion* (Paris) **2**: 47–56

Mourant, A.E. 1945. A new Rhesus antibody. *Nature* **155**: 542–543

Mourant, A.E. 1946. A "new" human blood group antigen of frequent occurrence. *Nature* **158**: 237–238

Mourant, A.E., A.C. Kopeć and K. Domaniewska-Sobczak. 1976. *The Distribution of the Human Blood Groups and Other Polymorphisms,* Oxford Univ. Press, London

Moureau, P. 1935a. Contribution à l'étude des facteurs d'individualisation du sang humain et leurs applications en médecine légale. 1. Les groupes d'isoagglutination de Landsteiner. 2. Les groupes M et N d'hémoagglutination de Landsteiner et Levine. 3. Applications pratiques des

propriétés individuelle du sang. *Rev. Belge Sci. Med.* **7**: 177–233 and 540–588 and 589–629

Moureau, P. 1935b. Peut-on distinguer sérologiquement le génotype des hématies A et B? *Sang, Biol. Pathol.* **9**: 484–499

Moureau, P. 1946. L'antigene O dans ses rapports avec la genetique et la serologie. *Rev. Hematol.* **1**: 266–277

Moureau, P. 1963. Determination of blood groups in blood stains, *in*: F. Lundquist (ed.) *Methods of Forensic Science* **2**: 187–202, Interscience, New York and London

Moureau, P., A. Andre, J. Warin and J. Joiris. 1963. B-like antigen in blood-A of decomposed body, *in: Forensic Immunology, Medicine, Pathology and Toxicology,* Excerpta Medica Int. Congr. Ser. No. 80: 55, Excerpta Medica Foundation, Amsterdam

Mueller, B. 1953. *Gerichtliche Medizin,* Springer Verlag, Berlin, Göttingen and Heidelberg

Mukai, S., T. Takei, R. Mukoyama, T. Odagiri, T. Maruyama and T. Miyazawa. 1975. Studies on blood groups of human teeth. *Jpn. J. Leg. Med.* **29**: 27–38 §[1]

Mukherjee, J.B. and P.K. Chattopadhyay. 1976. Blood grouping from teeth by the absorption-elution technique and its role in establishing identity. *Med. Sci. Law* **16**: 232–234

Mulet, C., J.-P. Cartron, H. Schenkel-Brunner, D. Duchet, P. Sinay and C. Salmon. 1979. Probable biosynthetic pathway for the synthesis of the B antigen from B_h variants. *Vox Sang.* **37**: 272–280

Müller, M.A. 1927. Das Agglutinin-Anreichungsverfahren, ein neues Verfahren zur Blutgruppenbestimmung an altem, eingetrocknetem Blute. *Dtsch. Z. Gesamte Gerichtl. Med.* **11**: 120–128

Murray, J. 1944a. A nomenclature of subgroups of the Rh factor. *Nature* **154**: 701–702

Murray, J. 1944b. Rh antenatal testing. A suggested nomenclature. *Lancet* **2**: 594–596

Murray, J. and E.C. Clarke. 1952. Production of anti-*Rh* in guinea pigs from human erythrocyte extracts. *Nature* **169**: 886–887

Murray, J., R.R. Race and G.L. Taylor. 1945. Serological reactions caused by the rare human gene Rh_z. *Nature* **155**: 112–113

Myllyla, G., U. Furuhjelm, S. Nordling, A. Pirkola, P. Tippett, J. Gavin and R. Sanger. 1971. Persistent mixed field polyagglutinability. Electrophoretic and serological aspects. *Vox Sang.* **20**: 7–23

Nagano, T., T. Tsuji, S. Nishitani and N. Tanaka. 1975a. Thermostability of blood group A- and B-active glycolipids obtained from human red cells. I. Qualitative activity assay of heated glycolipids. *Jpn. J. Leg. Med.* **29**: 10–17 §[1]

Nagano, T., T. Tsuji and K. Ieda. 1975b. Blood group determination of badly charred bodies. I. Activity changes of A-, B-, AB- and O(H)-active glycolipids and A- and B-active glycoproteins by heat. *Forensic Sci.* **5**: 153 [Abstr.]

Nagano, T., T. Tsuji and K. Ieda. 1976. Blood group determinations of severely charred bodies—the effects of heating on the blood group activity of A, B, AB and O(H) active glycolipids and A and B active glycoproteins. *J. Forensic Sci. Soc.* **16**: 163–168

Naiki, M., J. Fong, R. Ledeen and D.M. Marcus. 1975. Structure of the human erythrocyte blood group P_1 glycosphingolipid. *Biochemistry* **14**: 4831–4837

Naiki, M. and M. Kato. 1979. Immunological identification of blood group P^k antigen on normal human erythrocytes and isolation of anti-P^k with different affinity. *Vox Sang* **37**: 30–38

Naiki, M. and D.M. Marcus. 1974. Human erythrocyte P and P^k blood group antigen: identification as glycosphingolipids. *Biochem. Biophys. Res. Comm.* **60**: 1105–1111

Naiki, M. and D.M. Marcus. 1975. An immunological study of the human blood group P_1, P and P^k glycosphingolipid antigens *Biochemistry* **14**: 4837–4841

Nakajima, H., T. Ogushi, K. Seki and T. Tsukamoto. 1968. Some notes on the absorption of Lewis blood group substances from plasma onto red cells. *Jpn. J. Leg. Med.* **22**: 144–152 §[1]

Napier, P.W., D.L. Everhart and F.J. Grundbacher. 1974. Characterization of a human saliva antigen precipitated by a lectin from *Lotus tetragonolobus. Vox Sang.* **27**: 447–458

Neel, J.V. and M.M. Hanig. 1951. The inheritance and frequency of the MNS factors in the American Negro. *Genetics* **36**: 84–92

Newman, E., G.S. Naifeh, J.E. Auer and J.A. Buckwalter. 1961. Secretion of ABH antigens in peptic ulceration and gastric carcinoma. *Br. Med. J.* **1**: 92–94

Newman, R.A. and G.G. Uhlenbruck. 1977. Investigations into the occurrence and structure of lectin receptors on human and bovine erythrocyte, milk-fat globule and lymphocyte plasma-membrane glycoproteins. *Eur. J. Biochem.* **76**: 149–155

Newman, W. and E.A. Kabat. 1976. Immunochemical studies on blood groups. Purification, chemical and immunochemical properties of blood group-active glycoproteins from horse gastric mucosae. Immunochemical properties of B-active and non-B-active blood group substances from horse gastric mucosae and the relative size distributions of oligosaccharides liberated by base-borohydride. Structures and immunochemical properties of nine oligosaccharides from B-active and non-B-active blood group substances of horse gastric mucosae. *Arch. Biochem. Biophys.* **172**: 510–523 and 524–534 and 535–550

Nickolls, L.C. 1956. *The Scientific Investigation of Crime,* Butterworth and Co., London

Nickolls, L.C. and M. Pereira. 1962. A study of modern methods of grouping dried blood stains. *Med. Sci. Law* **2**: 172–179

Nicolson, G.L., S.P. Masouredis and S.J. Singer. 1971. Quantitative two-dimensional ultrastructural distribution of Rh_o(D) antigenic sites on human erythrocyte membranes. *Proc. Nat. Acad. Sci. USA* **68**: 1416–1420

Niederman, J.C., E.C. Gilbert and H.M. Spiro. 1962. The relationship between blood pepsin level, ABO blood group and secretor status. *Ann. Int. Med.* **56**: 564–569

Nijenhuis, L.E. 1961. Comments on the genetical structure of the Rhesus system. *Vox Sang.* **6**: 229–232

Nordhagen, R. 1967. Possible sources of error in the blood grouping of dried blood stains on textiles. *Vox Sang.* **12**: 317–318

Nunn, H.D., C.M. Giles and K.M. Dormandy. 1966. A second example of anti-Ku in a patient who has the rare Kell phenotype K^o. *Vox Sang.* **11**: 611–619

Oepen, I. and H. Noever. 1980. Zur ABO Blutgruppenprägung des menschlichen Haares. *Z. Rechtsmed.* **85**: 205–209

Ogata, T. 1960. Hemagglutination on a smear. A new method. *Vox Sang.* **5**: 252–257

Ogata, T. and T. Matuhasi. 1960. Problems of specific and cross reactivity of blood group antibodies, *in*: Proc. 8th Congr. Int. Soc. Blood Transf., Tokyo, *Bibliotheca Haematol.*, Fasc. **13**: 208–211, S. Karger, Basel and N.Y. (1962)

Ogata, T. and T. Matuhasi. 1962. Further observations on the problems of specific and cross reactivity of blood group antibodies, *in:* Proc. 9th Congr. Int. Soc. Blood Transf., Mexico City, *Bibliotheca Haematol.,* Fasc. **19**: 528–531, S. Karger, Basel and N.Y. (1964)

Oguchi, Y., T. Kawaguchi, T. Suzuta and T. Osawa. 1978. The nature of human blood group A_1 erythrocytes. *Vox Sang.* **34**: 32–39

Okada, K., T. Inoue and I. Yuasa. 1974. Reagglutination-inhibition test using red blood cells sensitized with anti-A and anti-B antisera. *Jpn. J. Leg. Med.* **28**: 425–434 §[1]

Olbrich, S. and E. Walther. 1941. Vergleichende Untersunchungen über den A_1/A_2-Titer von Anti-A-Seren und seiene blutgruppendiagnostische Beudeutung. *Z. Immunitaetsforsch. Exp. Ther.* **99**: 194–209

Oppenheim, J.D., P. Owen, M.S. Nachbar, K. Colledge and H.S. Kaplan. 1977. Identification and quantitation of solubilized I blood group substance by wheat germ agglutinin using quantitative immunoelectrophoresis. *Immunol. Comm.* **6**(2): 167–181

Osborne, R.H. and F.V. DeGeorge. 1962. The ABO blood groups in parotid and submaxillary gland tumors. *Am. J. Hum. Genet.* **14**: 199–209

Ottenberg, R. 1921a. Medicolegal application of human blood grouping. *J. Am. Med. Assoc.* **77**: 682–683

Ottenberg, R. 1921b. Hereditary blood qualities. Medicolegal application of human blood grouping. *J. Immunol.* **6**: 363–385

Ottensooer, F. and K. Silberschmidt. 1953. Haemagglutinin anti-N in plant seeds. *Nature* **172**: 914

Ottensooer, F. and St. Zurukzoglu. 1932. Unterscheidung der Untergruppen A_1 and A_2 durch die Pepsin-Hemmungsreaktion. *Klin. Wochenschr.* **11**(41): 1715–1716

Outteridge, R.A. 1962a. Absorption-elution grouping of bloodstains. Modification and development. *Nature* **194**: 385–386, Title correction on page 534

Outterridge, R.A. 1962b. Absorption-elution method of grouping bloodstains. *Nature* **195**: 818–819

Outteridge, R.A. 1963a. Direct ABO group determination of bloodstains on a micro scale by batch procedure. *Nature* **198**: 698–699

Outteridge, R.A. 1963b. Bloodstain grouping—elution v. inhibition. *J. Forensic Sci. Soc.* **4**: 87–90

Outterridge, R.A. 1963c. Determination of the ABO group from fingernails. *Med. Sci. Law* **3**: 275–276

Outteridge, R.A. 1965a. The biological individuality of dried human bloodstains. *J. Forensic Sci. Soc.* **5**: 22–51

Outteridge, R.A. 1965b. Recent advances in the grouping of dried blood and secretion stains, *in:* A.S. Curry (ed.) *Methods of Forensic Science* **4**: 299–332, Interscience, London and N.Y.

Owen, R.D. 1954. Heterogeneity of antibodies to the human blood groups in normal and immune sera. *J. Immunol.* **73**: 29–39

Palatnik, M. and F.R. Carnese. 1970. Sustancias grupospecificas ABH en manchas experimentales. I. Su deteccion mediante una variante de "mixed agglutination". *Sangre* **15**: 362–368

Panari, G., G. Rossi and A. Fiori. 1976. Polymorphism of A, B and H substances in seminal fluid. *Forensic Sci.* **7**: 55–60

Parish, W.E., J.A. Carron-Brown and C.B. Richards. 1967. The detection of antibodies to spermatozoa and to blood group antigens in cervical mucus. *J. Reprod. Fertil.* **13**: 469–483

Pauls, F.P., B.B. Victors and M.W. Dodson. 1953. Distribution of blood groups among the Eskimos, Indians and Whites of western Alaska. *Am. J. Hum. Genet.* **5**: 252–256

Pemberton, J. de J. 1919. Practical considerations of the dangers associated with blood transfusion. *Collected Papers of the Mayo Clinic* **11**: 635–640

Pereira, M. 1963a. The identification of MN groups in dried bloodstains. *Med. Sci. Law* **3**: 268–271

Pereira, M. 1963b. Observations on the grouping of dried stains of body fluids, *in: Forensic Immunology, Medicine, Pathology and Toxicology,* Excerpta Medica Int. Congr. Ser. No. 80, Excerpta Medica Foundation, Amsterdam

Pereira, M. 1973. ABO grouping of decomposed human tissue. *J. Forensic Sci. Soc.* **13**: 33–36

Pereira, M., B.E. Dodd and J.V. Marchant. 1969. The detection of A, B and H group specific substances in stains from body fluids by absorption-elution and mixed agglutination techniques. *Med. Sci. Law* **9**: 116–121

Pereira, M. and P.D. Martin. 1975. Problems involved in the grouping of saliva, semen and other body fluids. *Forensic Sci.* **5**: 158 [Abstr.]

Pereira, M. and P.D. Martin. 1976. Problems involved in the grouping of saliva, semen and other body fluids. *J. Forensic Sci. Soc.* **16**: 151–154

Pereira, M. and P.D. Martin. 1977. Determination of Lewis secretions in body fluids and dried stains of body fluids. *J. Forensic Sci. Soc.* **17**: 135–138

Pereira, M.E.A. and E.A. Kabat. 1974. Specificity of purified hemagglutinin (lectin) from *Lotus tetragonolobus. Biochemistry* **13**: 3184–3192

Pereira, M.E.A. and E.A. Kabat. 1976. Immunological studies on blood groups. LXII. Fractionation of hog and human A, H and AH blood group active substance on insoluble immunoadsorbents of Dolichos and Lotus lectins. *J. Exp. Med.* **143**: 422–436

Perkins, H.A. and P.A. Morel. 1980. Problems in paternity testing. Subgroups of AB. *Am. J. Clin. Pathol.* **73**: 263–266

Pettenkofer, H.J. 1953a. Serologie und Vererbung des Lewis-Blutgruppensystems. *Z. Immunitaetsforsch. Exp. Ther.* **110**: 217–227

Pettenkofer, H.J. 1953b. Zur Genetik des Lewis-Blutgruppensystems. *Naturwissenschaften* **40**: 321–322

Pettenkofer, H.J. and R. Bickerich. 1955. Untersuchungen über den Gehalt des blutgruppenfaktors P im menschlichen Gewebe. *Z. Immunitaetsforsch. Exp. Ther.* **112**: 140–143

Pettenkofer, H.J., W. Maassen and R. Bickerich. 1960. Antigengemeinschaften zwischen menschlichen Blutgruppen und Enterobacteriaceen. *Z. Immunitaetsforsch Exp. Ther.* **119**: 415–429

Pickles, M.M. 1946. Effect of cholera filtrate on Rh cells as demonstrated by incomplete *Rh* antibodies. *Nature* **158**: 880

Pietrusky, F. 1937. Über eingeengte Seren und über andere Untersuchungsmethoden zum Nachweis des schwachen N-Recptors (N_1) im Blute. *Dtsch. Z. Gesamte Gerichtl. Med.* **28**: 468–473

Pigman, W. 1957. (ed.) *The Carbohydrates. Chemistry, Biochemistry and Physiology,* Academic Press, New York

Piner, S.C. and M.S. Sänger. 1980. Lewis grouping of human secretion stains. *Forensic Sci. Int.* **15**: 87–92

Pinkerton, F.J., L.E. Mermod, B.A. Liles, J.A. Jack, Jr. and J. Noades. 1959. The phenotype Jk(a–b–) in the Kidd blood group system. *Vox Sang.* **4**: 155–160

Planques, J. and J. Ducos. 1957. Précautions à prendre pour le prélèvement d'échantillons de sang et de taches de sang desséché en vue d'expertises médico-légales. *Ann. Med. Leg. Criminol.* **37**: 202–206

Plapp, F.V., M.M. Kowalski, L. Tilzer, P.J. Brown, J. Evans and M. Chiga. 1979. Partial purification of Rh_o(D) antigen from Rh positive and negative erythrocytes. *Proc. Nat. Acad. Sci. USA* **76**: 2964–2968

Plato, C.C. and H. Gershowitz. 1961. Specific differences in the inhibition titers of the anti-H lectins from Cystisus sessilifolius and Ulex europaeus. *Vox Sang.* **6**: 336–347

Plaut, G., E.W. Ikin, A.E. Mourant, R. Sanger and R.R. Race. 1953. A new blood-group antibody, anti-Jk^b. *Nature* **171**: 431

Polesky, H.F. and H.D. Krause. 1977. Blood typing in disputed paternity cases. Capabilities of American laboratories. *Transfusion* (Phila.) **17**: 521–524

Polesky, H.F. and J.L. Swanson. 1966. Studies on the distribution of the blood group antigen Do^a (Dombrock) and the characteristics of anti-Do^a. *Transfusion* (Phila.) **6**: 268–270

Pollet, Ph. 1969. Adaptation des méthodes immuno-fluorescentes à la détermination directe des facteurs de groupes érythrocytaires dans le sang hémolysé et les taches de sang. Application médico-légale. *Arch. Belge Med. Soc. Hyg. Med. Trav. Med. Leg.* **27**: 447–465

Polley, M.J. and P.L. Mollison. 1961. The role of complement in the detection of blood group antibodies: special reference to the antiglobulin test. *Transfusion* (Phila.) **1**: 9–22

Ponsold, A. 1934. Eine Mikromethode zur quantitativen Auswertung kleinster serummengen. (Die Bestimmung des Agglutiningehaltes und der Receptorempfindlichkeit mittels Capillarröhrchen). *Dtsch. Z. Gesamte Gerichtl. Med.* **23**: 46–60

Ponsold, A. 1937. Ein Mikroabsorptionsverfahren zum Nachweis der Blutgruppen A_1 and A_2. *Dtsch. Z. Gesamte Gerichtl. Med.* **28**: 248–256

Poon, W.L. and B.E. Dodd. 1964. The subdivision of blood stains into A_1 and A_2 and the detection of H on human epidermal cells. *Med. Sci. Law* **4**: 258–264

Popoff, N.W. 1927. Isoagglutination and ihre forensische Anwendung in Russland. *Dtsch. Z. Gesamte Gerichtl. Med.* **9**: 411–425

Popoff, N.W. 1929. Zur Frage der individuellen Blutfleckendiagnosi mit Hilfe der Isoagglutination. *Ukrain. Zentralbl. Blutgruppenforsch.* **3**(3): 177–222

Potapow [Potapov], M.I. 1970a. Untersuchungen von Blutflecken und menschlichen Ausscheidungsprodukten mit den Gruppenlektin Anti-B (Evonymus alata). *Folia Haematol.* **93**: 458–464

Potapov, M.I. 1970b. Obnaruzhenie antigena sistemy Lewis, svoystvennogo eritrotsitam vydeliteley gruppy Le(a–b–). *Prob. Gematol. Pereliv. Krovi.* **15**(11): 45–49

Potapov, M.I. 1976. Production of immune anti-Lewis sera in goats. *Vox Sang.* **30**: 211–213

Poulik, M.D. and P.K. Lauf. 1965. Heterogeneity of water-soluble structural components of human red cell membrane. *Nature* **208**: 874–876

Poulik, M.D. and P.K. Lauf. 1969. Some physico-chemical and serological properties of isolated protein components of red cell membranes. *Clin. Exp. Immunol.* **4**: 165–175

Prager, M.D., G. Hopkins and M. Foster. 1963. The effect of phosphatases and organic phosphates on Rh reactions. *Vox Sang.* **8**: 410–419

Prager, M.D. and M.E. Lowry. 1966. On the possible relation of sialic acid

to the Rh factor. *Transfusion* (Phila). **6**: 577–583

Prodanov, P. 1979. Etude quantitative des antigènes ABH dans la salive des nouveau-nés et des adultes. *Rev. Fr. Transf. Immunohematol.* **22**: 387–397

Profili, M. and R. Hurley. 1979. ABO Blood group distributions in the Baltimore, Maryland metropolitan area. *Forensic Serol. News* 5(1) §[4]

Prohaska, R., H. Schenkel-Brunner and H. Tuppy. 1978. Enzymatic synthesis of blood-group Lewis-specific glycolipids. *Eur. J. Biochem.* **84**: 161–166

Prokop, O. 1961. Untersuchungen über die Antikörper-Ausscheidung im Speichel. *Lecture Int. Akad. Gerichtl. Med.*, Vienna, cited by Prokop and Uhlenbruck, 1969.

Prokop, O. 1963. On the agglutination content in human saliva and lacrimal fluid, *in: Forensic Immunology, Medicine, Pathology and Toxicology*, Excerpta Medica Int. Congr. Ser. No. 80, p. 54, Excerpta Medica Foundation, Amsterdam

Prokop, O., G. Bundschuh and G. Geserick. 1963. Über Blutgruppenreaktionen in der Tränenflüssigkeit. *Dtsch. Gesundheitswes.* **27**: 1162 cited by Prokop and Uhlenbruck, 1969

Prokop, O. and G. Geserick. 1972. An "anti-secretor"-reagent for the direct estimation of Se-status by precipitation. *Haematologia* **6**: 135–137

Prokop, O. and B. Gibb. 1965. Ergänzende Bemerkungen zu der Arbeit von Maresch und Wehrschütz über die Ausscheidereigenschaft. *Dtsch. Z. Gesamte Gerichtl. Med.* **56**: 221–225

Prokop, O., K. Jarosch and G. Sorgo. 1976. Aspekte der Blutgruppe "CAD". *Beitr. Gerichtl. Med.* **34**: 141–144

Prokop, O., A. Rackwitz and D. Schlisinger. 1965. A "new" human blood group receptor A_{hel} tested with saline extracts from *Helix hortensis* (garden snail). *J. Forensic Med.* **12**: 108–110

Prokop, O. and D. Schlesinger 1965a. Über das Vorkommen von P_1-Blutgruppensubstanz oder einer "P_1-like substance" bei Lumbricus terretris. *Acta Biol. Med. German.* **15**: 180–181

Prokop, O. and D. Schlesinger. 1965b. Über das Vorkommen von P_1-Blutgruppensubstanz bei einigen Metazoen, insbesondere Ascaris suum and Lumbricus terrestris. *Z. Immunitaetsforsch. Allergieforsch.* **129**: 344–353

Prokop, O. and G. Uhlenbruck. 1969. *Human Blood and Serum Groups.* Wiley Interscience, New York

Putkonen, T. 1930. Über die gruppenspezifischen Eigenschaften verschiedener Köperflüssigkeiten. *Acta Soc. Med. Fenn. Duodecim*, Ser. A, **14**(12) and see Camp *et al.*, 1974

Race, R.R. 1944. An 'incomplete' antibody in human serum. *Nature* **153**: 771–772

Race, R.R. 1946. A summary of present knowledge of human blood groups, with special reference to serological incompatibility as a cause of congential disease. *Br. Med. Bull.* **4**: 188–193

Race, R.R. 1948. The Rh genotypes and Fisher's theory. *Blood*, special issue No. 2 in 1947–48: 27–42

Race, R.R. 1957. Editorial. *Vox Sang.* **2**: 2–7

Race, R.R., H.A. Holt and J.S. Thompson. 1951a. The inheritance and distribution of the *Duffy* blood groups. *Heredity* **5**: 103–110

Race, R.R. and R. Sanger. 1950. *Blood Groups in Man.* Blackwell Scientific Publications, Oxford

Race, R.R. and R. Sanger. 1951. The *Rh* antigen C^u. *Heredity* **5**: 285–287

Race, R.R. and R. Sanger. 1952. The inheritance of the *Duffy* blood groups: an analysis of 110 English families. *Heredity* **6**: 111–119

Race, R.R. and R. Sanger. 1958. *Blood Groups in Man*, 3rd ed., Blackwell Scientific Publications, Oxford

Race, R.R. and R. Sanger. 1962. *Blood Groups in Man*, 4th Ed., Blackwell Scientific Publications, Oxford

Race, R.R. and R. Sanger. 1968. *Blood Groups in Man*, 5th ed., Blackwell Scientific Publications, Oxford and Edinburgh

Race, R.R. and R. Sanger. 1975. *Blood Groups in Man*, 6th ed., Blackwell Scientific Publications, Oxford, London, etc.

Race, R.R., R. Sanger. F.H. Allen, Jr., L.K. Diamond and D. Niedziela. 1951b. Inheritance of the human blood group antigen Jk^a. *Nature* **168**: 207–208

Race, R.R., R. Sanger and S.D. Lawler. 1948a. *Rh* genes allelomorphic to D. *Nature* **162**: 292–293

Race, R.R., R. Sanger and S.D. Lawler. 1948b. The *Rh* antigen D^u. *Ann. Eugenics* **14**: 171–184

Race, R.R., R. Sanger and S.D. Lawler, 1948c. *Rh* genes allelomorphic to C. *Nature* **161**: 316–317

Race, R.R., R. Sanger and S.D. Lawler. 1948d. Allelomorphs of the Rh gene C. *Heredity* **2**: 237–250

Race, R.R., R. Sanger and S.D. Lawler. 1960. The Rh antigen called c^v: a revocation. *Vox Sang.* **5**: 334–336

Race, R.R., R. Sanger, P. Levine, R.T. McGee, J.J. van Loghem, M. van der Hart and C. Cameron. 1954. A position effect of the *Rh* blood-group genes. *Nature* **174**: 460–461

Race, R.R., R. Sanger and J.G. Selwyn. 1950. A probable deletion in a human *Rh* chromosome. *Nature* **166**: 520

Race, R.R. and G.L. Taylor. 1943. A serum that discloses the genotype of some *Rh*-positive people. *Nature* **152**: 300

Race, R.R., G.L. Taylor, K.E. Boorman and B.E. Dodd. 1943. Recognition of *Rh* genotypes in man. *Nature* **152**: 563–564

Race, R.R., G.L. Taylor, D.F. Cappell and M.N. McFarlane. 1944a. Recognition of a further common Rh phenotype in man. *Nature* **153**: 52–53

Race, R.R., G.L. Taylor, E.W. Ikin and A.M. Prior. 1944b. The inheritance of allelomorphs of the *Rh* gene in fifty-six families. *Ann. Eugenics* **12**: 206–210

Randeria, K.J. and H.M. Bhatia. 1971. Quantitative inhibition studies on the ABH and Lewis antigens in saliva. *Indian J. Med. Res.* **59**: 1737–1753

Rangnekar, K.N. and S.S. Rao. 1970. The source of blood group antigens on spermatozoa and their significance. *Int. Arch. Allergy Appl. Immunol.* **37**: 49–53

Raszeja, S. and J. Dziedzic. 1971. Études comparées sur l'applicabilité de quelques extraits de plantes et de quelques sérums pour la differenciation "universelle" des sécréteurs et non-sécréteurs de substances de groupe. *Med. Leg. Domm. Corpor.* **4**: 115–118

Reed, T.E. 1967. Research on blood groups and selection from the child health and development studies, Oakland California. I. Infant birth measurements. *Am. J. Hum. Genet.* **19**: 732–746

Reed, T.E. 1968. Distribution and tests of independence of seven blood group systems in a large multiracial sample from California. *Am. J. Hum. Genet.* **20**: 142–150

Reepmaker, J. and J.J. van Loghem. 1953. Anti-A and anti-B immune antibodies in pregnancy. *Vox Sang.* 3 (O.S.): 143–161 §[2]

Rees, B., H.D. Howard and S.K. Strong. 1975. The persistence of blood-group factors in stored blood samples. *J. Forensic Sci. Soc.* **15**: 43–49

Rega, A.F., A.I. Weed, C.F. Reed, G.G. Berg and A. Rothstein. 1967. Changes in the properties of human erythrocyte membrane protein after solubilization by butanol extraction. *Biochim. Biophys. Acta* **147**: 297–312

Rekvig, O.P. and K. Hannestad. 1977. Acid elution of blood group antibodies from intact erythrocytes. *Vox Sang.* **33**: 280–285

Renkonen, K.O. 1948. Studies on hemagglutinins present in seeds of some representatives of the Family of Leguminoseae. *Ann. Med. Exp. Biol. Fenn.* **26**: 66–72

Renton, P.H. and J.A. Hancock. 1955. An individual of unusual Rh type. *Vox Sang.* **5** (O.S.): 135–142 §[2]

Renton, P.H., P. Howell, E.W. Ikin, C.M. Giles and K.L.G. Goldsmith. 1967. Anti-Sd^a, a new blood group antibody. *Vox Sang.* **13**: 493–501

Reviron, J., A. Jacquet, F. Delarue. G. Liberge, D. Salmon and Ch. Salmon. 1967. Interactions alléliques des gènes de groupes sanguins ABO. Résultats préliminaires avec l'anti-B d'un sujet A_1O. *Nouv. Rev. Fr. Hematol.* **7**: 425–433

Reviron, J., A. Jacquet and Ch. Salmon. 1968. Un example de chromosome "cis A_1B". Étude immunologique et génétique du phénotype induit. *Nouv. Rev. Fr. Hematol.* **8**: 323–338

Robinson, M.C., D. Tolchin and C. Halpern. 1971. Enteric bacterial agents and the ABO blood groups. *Am. J. Hum. Genet.* **23**: 135–145

Rochna, E. and N.C. Hughes-Jones. 1965. The use of purified ^{125}I-labelled anti-γ-globulin in the determination of the number of D antigen sites on red cells of different phenotypes. *Vox Sang.* **10**: 675–686

Rogers, M.J., P.A. Stiles and J. Wright. 1974. A new minus-minus phenotype: three Co(a-b-) individuals in one family. *Transfusion* (Phila.) **14:** 508 [Abstr.]

Romano, E.L., P.L. Mollison and J. Linares. 1978. Number of B sites generated on group O red cells from adults and newborn infants. *Vox Sang.* **34:** 14–17

Romanowska, E. 1964. Reactions of M and N blood-group substances natural and degraded with specific reagents of human and plant origin. *Vox Sang.* **9:** 578–588

Rosenfield, R. 1955. A-B hemolytic disease of the newborn. Analysis of 1480 cord blood specimens with special reference to the direct antiglobulin test and to the group O mother. *Blood* **10:** 17–28

Rosenfield, R.E., F.H. Allen, Jr. and P. Rubinstein. 1973. Genetic model for the Rh blood-group system. *Proc. Nat. Acad. Sci. USA* **70:** 1303–1307

Rosenfield, R.E., F.H. Allen, Jr., S.N. Swisher and S. Kochwa. 1962. A review of Rh serology and presentation of a new terminology. *Transfusion* (Phila.) **2:** 287–312

Rosenfield, R.E., F.H. Allen, S.N. Swisher and S. Kochwa. 1979a. Rh nomenclature. *Transfusion* (Phila.) **19:** 487

Rosenfield, R.E. and G.V. Haber. 1958. An Rh blood factor, **rh$_i$** (Ce), and its relationship to **hr** (ce). *Am. J. Hum. Genet.* **10:** 474–480

Rosenfield, R.E. and G.V. Haber. 1965. Detection and measurement of homologous human hemagglutinins, *in*: L.T. Skeggs, Jr. (ed.) *Automation in Analytical Chemistry,* Technicon Symp., 503–506, Mediad, Inc., New York (1966)

Rosenfield, R.E., G.V. Haber, R. Schroeder and R. Ballard. 1960. Problems in Rh typing as revealed by a single Negro family. *Am. J. Hum. Genet.* **12:** 147–159

Rosenfield, R.E. and S. Kochwa. 1964. Immunochemical studies of the Rh system. II Capacity of antigenic sites of different phenotypes to bind and retain Rh antibodies. *J. Immunol.* **92:** 693–701

Rosenfield, R.E. and S. Kochwa. 1965. Time and action: the kinetics of Rh notation. *Am. J. Hum. Genet.* **17:** 458

Rosenfield, R.E. and G. Ohno. 1955. A-B hemolytic disease of the newborn. *Rev. Hematol.* **10:** 231–235

Rosenfield, R.E., S.H. Shaikh, F. Innella, Z. Kaczera and S. Kochwa. 1979b. Augmentation of hemagglutination by low ionic conditions. *Transfusion* (Phila.) **19:** 499–510

Rosenfield, R.E., P. Vogel, N. Gibbel, G. Ohno and G. Haber. 1953a. Anti-Jk^a: three new examples of the isoantibody. Frequency of the factor in Caucasians, Negroes and Chinese in New York City. *Am. J. Clin. Pathol.* **23:** 1222–1225

Rosenfield, R.E., P. Vogel, N. Gibbel, R. Sanger and R.R. Race,. 1953b. A "new" Rh antibody, anti-f. *Br. Med. J.* **1:** 975

Rothwell, T.J. 1979. Studies on the blood group substances in saliva. *J. Forensic Sci. Soc.* **19:** 301–308

Roychowdhury, A.B. 1963. A new method of determination of blood groups from stains of blood, *in: Forensic Immunology, Medicine, Pathology and Toxicology,* Excerpta Medica Int. Congr. Ser. No. 80, 50–51, Excerpta Medica Foundation, Amsterdam

Roychowdhury, A.B. 1973. Application of the method of triple-bonded agglutination in blood stains of medico-legal exhibits. *Indian J. Exp. Biol.* **11:** 450–452

Roychowdhury, A.B. 1974. Triple-bonded agglutination in bloodstains on medicolegal exhibits. *J. Indian Med. Assoc.* **63:** 8–10

Roychowdhury, A.B. 1975. TBA method in forensic application *Forensic Sci.* **5:** 182 [Abstr.]

Rubin, H. 1963. Antibody elution from red blood cells. *J. Clin. Pathol.* **16:** 70–73

Ruffié, J. and J. Ducos. 1952. Sur l'identification de taches de sang sec. I) Recherche des facteurs C D E du système Rhesus. II) Mise en évidence des agglutinines irrégulières anti-M, anti-D, anti-C. *Ann. Med. Leg. Criminol.* **32:** 351–361

Ruffié, J. and J. Ducos. 1953. Les groupes sanguins en médecine légale. Etat actuel de la question. *Toulouse Med.* **54:** 551–568

Ruffié, J. and J. Ducos. 1957. Absorption des anticorps anti-Fy^a par le sang desséché. *C. R. Soc. Biol.* **151:** 393–395

Rule, A.H. and W.C. Boyd. 1964. Relationship between blood group agglutinogens: role of sialic acid. *Transfusion* (Phila.) **4:** 449–456

Rutter, E.R. and P.H. Whitehead. 1975. The fractionation of ABH blood group substances in saliva. *Forensic Sci.* **5:** 163 [Abstr.]

Rutter, E.R. and P.H. Whitehead. 1976. The fractionation of ABH blood group substances in saliva. *J. Forensic Sci. Soc.* **16:** 241–246

Sabo, B., J. McCreary, M. Stroup, D.E. Smith and J.G. Weidner. 1979. Another Kell-related antibody, anti-K19. *Vox Sang.* **36:** 97–102

Sachs, M.S., L.J. Unger and A.S. Wiener. 1960. Present status of the Rh-Hr blood types. A report of the Committee on Medico-legal Problems, A.M.A. *J. Am. Med. Assoc.* **172:** 1158–1162

Sadler, J.E., J.C. Paulson and R.L. Hill. 1979. The role of sialic acid in the expression of human MN blood group antigens. *J. Biol. Chem.* **254:** 2112–2119

Sagisaka, K., K. Tokiwa, H. Sugisawa, K. Suzuki and W. Matsuo. 1971. Detection of the MN blood group of bloodstains by means of the elution test in combination with the anti-globulin test. *Jpn. J. Leg. Med.* **25:** 103–113 §[1]

Salmon, Ch. 1965. Etude quantitative et thermodynamique de l'isohémagglutination. Méthodes er résultats récents. *Nouv. Rev. Fr. Hematol.* **5:** 191–196

Salmon, Ch. 1969. A tentative approach to variations in ABH and associated erythrocyte antigens. *Ser. Haematol.* **2**(1): 3–33

Salmon, Ch., P. Borin and R. André. 1958. Le groupe sanguin A_m dans deux générations d'une même famille. *Rev. Hematol.* **13:** 529–537

Salmon, Ch., J. de Grouchy and G. Liberge. 1965. Un nouvel antigène du système ABO: $A_{1(80)}$. *Nouv. Rev. Fr. Hematol.* **5:** 631–638

Salmon, Ch., M. Lopez, A. Gerbal, A. Bouguerra and J.P. Cartron. 1973. Current genetic problems in the ABO blood group system. *Biomedicine* **18:** 375–386

Salmon, Ch. and J. Reviron. 1964. Trois phénotypes "B faible": B_{80}, B_{60}, B_0 définis par leur agglutinabilité, comparée a celle du phénotype normal "B_{100}". *Nouv. Rev. Fr. Hematol.* **4:** 655–668

Salmon, Ch., D. Salmon, G. Liberge, R. André, P. Tippett and R. Sanger. 1961. Un nouvel antigène de groupe sanguin erythrocytaire présent chez 80% des sujets de race blanche. *Nouv. Rev. Fr. Hematol.* **1:** 649–661

Salmon, Ch., D. Salmon and J. Reviron. 1964. Données quantitatives et thermodynamiques comparées concernant les antigènes B_{100}, B_{80}, B_{60} et B_0. Applications a l'étude de l'agglutination. *Nouv. Rev. Fr. Hematol.* **4:** 739–754

Salmon, Ch., D. Salmon and J. Reviron. 1965. Étude immunologique et génétique de la variabilité du phénotype A_x. *Nouv. Rev. Fr. Hematol.* **5:** 275–290

Salmon, D., S. Bouchmel, A. Hafsia, S. Boussen, M. Boussen and A. Bougverra. 1979. p Phenotypes observed in two generations of a Tunisian family with a high rate of inbreeding. *Rev. Fr. Transf. Immunohematol.* **22:** 563–571

Salmon, D.B. and J. Brocteur. 1978. Probability of paternity exclusion when relatives are involved. *Am. J. Hum. Genet.* **30:** 65–75

Saneshige, Y., I. Yuasa and K. Okada. 1980. Blood typing of blood stains on sweat stains by the isoagglutinins detection method. *Forensic Sci. Int.* **15:** 161–168

Sanford, A.H. 1918. A modification of the Moss method of determining isohemagglutination groups. *J. Am. Med. Assoc.* **70:** 1221

Sanger, R. 1955. An association between the *P* and *Jay* systems of blood groups. *Nature* **176:** 1163–1164

Sanger, R., J. Gavin, P. Tippett, P. Teesdale and K. Eldon. 1971. Plant agglutinin for another human blood-group *Lancet* **1:** 1130

Sanger, R., J. Noades, P. Tippett, R.R. Race, J.A. Jack and C.A. Cunningham. 1960. An Rh antibody specific for V and R$'^s$. *Nature* **186:** 171

Sanger, R. and R.R. Race. 1947. Subdivisions of the MN blood groups in man. *Nature* **160:** 505

Sanger, R. and R.R. Race. 1951. The MNSs blood group system. *Am. J. Hum. Genet.* **3:** 332–343

Sanger, R., R.R. Race and J.J. Jack. 1955. The Duffy blood groups of New York Negroes: the phenotype Fy(a-b-). *Br. J. Haematol.* **1:** 370–374

Sanger, R., R.R. Race, R.E. Rosenfield and P. Vogel. 1953. A serum containing anti-s and anti-Jk^b. *Vox Sang.* **3** (O.S.): 71 §[2]

Sanger, R., R.R. Race, R.J. Walsh and C. Montgomery. 1948. An antibody which subdivides the human MN blood groups. *Heredity* **2:** 131–139

Sasaki, H. 1932. Ueber das Vorkommen gruppenspezifischen Eigenschaften im Speichel und anderen Körperflüssigkeiten und den Nachweis zweier "Ausscheidungstypen". *Z. Immunitaetsforsch. Exp. Ther.* **77:** 101–129

Sato, M. and F. Ottensooer. 1967. Blood group substances in body fluids. Comparison of the concentrations in semen and in saliva. *J. Forensic Med.* **14:** 30–38

Schaidt, G. 1958. Auswertung von Blutspuren in Flüssigkeiten und Schmutz. Verfahren zur Blutgruppenbestimmung in Urin und Kot. *Arch. Kriminol.* **122:** 66–68

Schenkel-Brunner, H., J.-P. Cartron and C. Doinel. 1979. Localization of blood-group A and I antigenic sites on inside-out and rightside out human erythrocyte membrane vesicles. *Immunology* **36:** 33–36

Schiebe, E., B. Gibb and E. Ulrich. 1961. Eine einfache Methode zum Nachweis von blutgruppenaktiven Substanzen im menschlichen Zahngewebe. *Arch. Kriminol.* **128:** 155–163

Schiff, F. 1924a. Zur Kenntnis blutgruppenspezifischer Antigene und Antikörper. *Klin. Wochenschr.* **3:** 679 and see Camp *et al.*, 1974

Schiff, F. 1924b. Über gruppenspezifische serumpräcipitine. *Klin. Wochenschr.* **3**(16): 697–680

Schiff, F. 1926. *Die Technik der Blutgruppenuntersuchungen für Kliniker und Gerichtsärzte. Nebst Berücksichtigung ihrer Anwendung in der Anthropologie und der Vererbungs- und Konstitutionsforschung.* Julius Springer Verlag, Berlin

Schiff, F. 1927a. Die Blutgruppen und ihre Anwendung von Gericht. *Dtsch. Z. Gesamte Gerichtl. Med.* **9:** 369–401

Schiff, F. 1927b. Über den serologischen Nachweis der Blutgruppeneigenschaft *O*. *Klin. Wochenschr.* **6**(7): 303–304

Schiff, F. 1930. Die Vererbungsweise der Faktoren M und N von Landsteiner und Levine. *Klin. Wochenschr.* **9**(42): 1956–1959

Schiff, F. 1931. *Über die gruppenspezifischen Substanzen des menschlichen Körpers,* Gustav Fischer Verlag, Jena and see Camp *et al.*, 1974

Schiff, F. 1932a. *Die Technik der Blutgruppenuntersuchungen für Kliniker und Gerichtsärzte,* 3rd Aufl., Julius Springer, Berlin

Schiff, F. 1932b. Die gerichtlich-medizinische Bedeutung der serologischen Eigenschaften M und N von Landsteiner und Levine. *Dtsch. Z. Gesamte Gerichtl. Med.* **18:** 41–65

Schiff, F. 1933a. Die forensische Bedeutung der Faktoren M und N und anderer neuerer serologischer Typenmerkmale. *Dtsch. Z. Gesamte Gerichtl. Med.* **21:** 404–434

Schiff, F. 1933b. *Die Blutgruppen und Ihre Anwendungsgebiete,* Julius Springer, Berlin and see Camp *et al.*, 1974

Schiff, F. and L. Adelsberger. 1924. Ueber blutgruppenspezifische Antikörper und Antigene. I. Mittheil. *Z. Immunitaetsforsch. Exp. Ther.* **40:** 335–367

Schiff, F. and M. Akune. 1931. Blutgruppen und Physiologie. *Muench. Med. Wochenschr.* **78**(16): 657–660

Schiff, F. and W.C. Boyd. 1942. *Blood Grouping Technic.* A manual for clinicians, serologists, anthropologists and students of legal and military medicine, Interscience Publishers, New York

Schiff, F. and F.A. Burón. 1935. Zur Kenntnis der sogenannten Blutgruppenfermente. *Klin. Wochenschr.* **14**(20): 710–712

Schiff, F. and W. Halberstaedter. 1926. Ueber Aglutinationserscheinung bei gealterten Blutkorperchen. *Z. Immunitaetsforsch. Exp. Ther.* **48:** 414–428

Schiff, F. and H. Sasaki. 1932. Der Ausscheidungstypus, ein auf serologischem Wege nachweisbares Mendelndes Merkmal. *Klin. Wochenschr.* **11:** 1426–1429 and see Camp *et al.*, 1974

Schiff, F. and G. Weiler. 1931. Fermente und Blutgruppen. I und II. *Biochem. Z.* **235:** 454–465 and **239:** 489–492

Schiffman, G. and C. Howe. 1965. The specificity of blood group A-B cross-reacting antibody. *J. Immunol.* **94:** 197–204

Schimmack, L., I. Müller and L. Kornstad. 1971. A contribution to the Ny^a problem. *Human Hered.* **21:** 346–350

Schlesinger, D. and M. Osińka. 1964. The influence of pregnancy on salivary secretion of group isoantibodies. *Arch. Immunol. Ther. Exp.* **12:** 297–307

Schleyer, F. 1957. Vergleichende Untersuchungen über den optimalen Nachweis von ABO-Agglutinogenen in Blutflecken. *Z. Immunitaetsforsch. Exp. Ther.* **114:** 224–252

Schleyer, F. 1961. Weitere Untersuchungen über den optimalen ABO-Gruppennachweis in Blutflecken auf Textilien. *Arch. Kriminol.* **127:** 161–166

Schmidt, P.J. and G.H. Vos. 1967. Multiple phenotypic abnormalities associated with Rh_{null} (–/–). *Vox Sang.* **13:** 18–20

Schnug, G. 1952. Über den P-Nachweis an Blutflecken. *Dtsch. Z. Gesamte Gerichtl. Med.* **41:** 451–453

Schreffler, D.C., C.F. Sing, J.V. Neel, H. Gershowitz and J.A. Napier. 1971. Studies on genetic selection in a completely ascertained Caucasian population. I. Frequencies, age and sex effects, and phenotype associations for 12 blood group systems. *Am. J. Hum. Genet.* **23:** 150–163

Schulz, E. 1970. Ein Beitrag zur Durchführung der Mischagglutination in der Spurendiagnostik. *Arch. Kriminol.* **146:** 95–98

Schulz, E. 1974. Absättigungsversuch und Mischagglutination. Vergleichende Untersuchungen zum Nachweis der Ausscheidereigenschaft. *Z. Rechtsmed.* **74:** 87–98

Schütze, H. 1921. Haemagglutination and its medico-legal bearing, with observations upon the theory of isoagglutinins. *Br. J. Exp. Pathol.* **2:** 26–33

Schwarting, G.A., D.M. Marcus and M. Metaxas. 1977. Identification of sialosylparagloboside as the erythrocyte receptor for an 'anti-p' antibody. *Vox Sang.* **32:** 257–261

Schwarzmann, E.M. 1928. Über den Isoagglutiningehalt im Blute und in anderen physiologischen und pathologischen Flüssigkeiten und Ausscheidungen des weiblichen Organismus. *Z. Geburtshilfe Gynaekol.* **92:** 505–516

Schwerd, W. 1978. Zum Beweiswert der MN-Differenzierung aus Blutspuren. *Z. Rechtsmed.* **80:** 293–298

Seaman, M.J. D.G. Chalmers and D. Franks. 1968. Siedler: an antibody which reacts with A_1Le(a–b+) red cells. *Vox Sang.* **15:** 25–30

Seidl, S. 1976. Das Rh_{null}-Syndrom. *Blut* **33:** 271–274

Selvin, S., D.M. Black, B.W. Grunbaum and N. Pace. 1979. Racial classifications based on blood group protein systems. *J. Forensic Sci.* **24:** 376–383

Serebryanikov, P. 1927. Tekhnika i sudebno-meditsinskoye znacheniye opredeleniya krovyanykh grupp po krovyanym pyatnam. *Odesskiy Meditsinskiy Zhur.* **2:** 87–93

Seyfried, H., I. Walewska and B. Werblinska. 1964. Unusual inheritance of ABO group in a family with weak B antigens. *Vox Sang.* **9:** 268–277

Shaler, R.C. 1978. Forensic Implications of Genetic Population Data collected in Different Geographical Regions. First Report on Contract J-LEAA-025-73 for Law Enforcement Assistance Administration, Eastern Technical Divn., The Aerospace Corp., Report No. ATR-79(7910)-1

Shaler, R.C. A.M. Hagins and C.E. Mortimer. 1978. MN determinations in bloodstains—selective destruction of cross-reacting activity. *J. Forensic Sci.* **23:** 570–576

Shapiro, H.A. 1956. The medico-legal significance of the recently discovered Henshaw (He) blood factor. *J. Forensic Med.* **3:** 137–138

Shapiro, M. 1951. The ABO, MN, P and Rh blood group systems in the South African Bantu. A genetic study. *S. Afr. Med. J.* **25:** 187–192

Shapiro, M. 1956. Inheritance of the Henshaw (He) blood factor. *J. Forensic Med.* **3:** 152–160

Shapiro, M. 1960. Serology and genetics of a new blood factor: hr^S. *J. Forensic Med.* **7:** 96–105

Shapiro, M. 1964. Serology and genetics of a 'new' blood factor: hr^H. *J. Forensic Med.* **11:** 52–66

Shapiro, M., M. LeRoux and S. Brink. 1972. Serology and genetics of a new blood factor: hr^B *Haematologia* **6:** 121–128

Shattock, S.G. 1900. Chromocyte clumping in acute pneumonia and certain other diseases, and the significance of the buffy coat in the shed blood. *J. Pathol. Bacteriol.* **6:** 303–314

Siddiqui, B. and S.-I. Hakomori. 1973. A ceramide trisaccharide of human erythrocyte membrane reacting with anti-type XIV pneumococcal polysaccharide serum. *Biochim. Biophys. Acta* **330:** 147–155

Sievers, M.L. 1959. Hereditary aspects of gastric secretory function. Race and ABO blood groups in relation to acid and pepsin production. *Am. J. Med.* **27:** 246–255

Siglar, G. 1979. Personal Communication

Silveira, E. 1967. Exclusion of paternity by Rh-Hr and M-N-S genotyping. *J. Forensic Sci.* **12:** 77–81

Simon, A. 1964. Der Einfluss der gebräuchlichen Waschmittel "Fit" und "Persil" auf die Gruppenspezifität eingetockneter Blutspuren. *Arch. Kriminol.* **133:** 12–19

Simson, F.W. 1926. A study of the third agglutinating system in human blood. *J. Pathol. Bacteriol.* **29:** 279–291

Sinclair, M., D.I. Buchanan, P. Tippett and R. Sanger. 1973. Another antibody related to the Lutheran blood group system (Much.). *Vox Sang.* **25:** 156–161

Singer, S.A. and G.L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. *Science* **175:** 720–731

Siracusa, V. 1923. La sostanza isoagglutinabile del sangue. *Arch. Antropol. Crim. Psichiatr. Med. Leg.* **43** (4 ser. **14**): 362–364 ¶

Siracusa, V. 1927. Sull'applicazione pratica dei metodi per la diagnosi individuale del sangue. *Arch. Antropol. Crim. Psichiatr. Med. Leg.* **47** (4 ser. **18**): 307–339

Siracusa, V. 1937. Sulla mescolanza dei sieri gruppo-specifici per la diagnosi individuale delle macchie. *Riv. Patol. Sperim.* **19:** (N.S. **8**): 109–119

Sirai, S. 1925. Reference quoted in section 19.8.1

Skov, F. and N.C. Hughes-Jones. 1977. Observations on the number of available C antigen sites on red cells. *Vox Sang.* **33:** 170–174

Skutelsky, E., R. Lotan, N. Sharon and D. Danon. 1977. Distribution of the T-antigen on erythroid cell surfaces. Studies with peanut agglutinin, an anti-T specific lectin. *Biochim. Biophys. Acta* **467:** 165–174

Slavík. Vl. and Fr. Meluzín. 1972. Bestimmung der Gruppenzugehörigkeit im System ABO aus histologischem. Material. I. Histologische Präparate der sezierten Falle. *Z. Rechtsmed.* **70:** 79–88

Smith, J.A. and A.J. Sinclair. 1977. Rh_0 and the erythrocyte membrane. *Blood* **49:** 491

Smith, M.L. and M.L. Beck. 1979. The immunoglobulin structure of human anti-M agglutinins. *Transfusion* (Phila.) **19:** 472–474

Sneath, J.S. and P.H.A. Sneath. 1955a. Transformation of the Lewis groups of human red cells. *Nature* **176:** 172

Sneath, J.S. and P.H.A. Sneath. 1955b. Absorption of blood-group substances from serum onto red cells. *Br. Med. Bull.* **15**(2): 154–157

Solish, G.I., H. Gershowitz and S.J. Behrman. 1961. Occurrence and titer of isohemagglutinins in secretions of the human uterine cervix. *Proc. Soc. Exp. Biol. Med.* **108:** 645–649

Solomon, J.M., M.B. Gibbs and A.J. Bowdler. 1965. Methods in quantitative hemagglutination. *Vox Sang.* **10:** 54–72 and 133–148

Speiser, P. 1961. Sechzig Jahre Blutgruppen (Landsteiner Feier in Wien). *Med. Welt,* No. 50: 2666–2670

Springer, G.F. 1956. Inhibition of blood-group agglutinins by substances occurring in plants. *J. Immunol.* **76:** 399–407

Springer, G.F. 1965. Microbes and higher plants. Their relation to blood group substances, *in*: Proc. 10th Congr. Int. Soc. Blood Transf. *Bibliotheca Haematol.,* Fasc. **23:** 465–475, S. Karger, Basel and N.Y.

Springer, G.F. 1970. Importance of blood group substances in interactions between man and microbes. *Ann. N.Y. Acad. Sci.* **169:** 134–152

Springer, G.F. and N.J. Ansell. 1958. Inactivation of human erythrocyte agglutinogens M and N by influenza viruses and receptor destroying enzyme. *Proc. Nat. Acad. Sci. USA* **44:** 182–189

Springer, G.F. and N.J. Ansell. 1960. Acquisition of blood-group B-like bacterial antigens by human A and O erythrocytes. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **19:** 70

Springer, G.F. and N.J. Ansell Hahn. 1960. Acquisition of bloodgroup B-like bacterial antigens by human A and O erythrocytes, *in:* Proc. 8th Congr. Int. Soc. Blood Transf., Tokyo, *Bibliotheca Haematol.* Fasc. **13:** 219–221, S. Karger, Basel and N.Y. (1962)

Springer, G.F. and P.R. Desai. 1974. Common precursors of human blood group MN specificities. *Biochem. Biophys. Res. Comm.* **61:** 470–475

Springer, G.F. and P.R. Desai. 1975. Human blood-group MN and precursor specificities: structural and biological aspects. *Carbohyd. Res.* **40:** 183–192

Springer, G.F., P.R. Desai, M.S. Murthy, H.J. Yang and E.F. Scanlon. 1979. Precursors of the blood group MN antigens as human carcinoma-associated antigens. *Transfusion* (Phila.) **19:** 233–249

Springer, G.F., R.E. Horton and M. Forbes. 1959. Origin of antihuman blood group B agglutinins in White Leghorn chickens. *J. Exp. Med.* **110:** 221–244

Springer, G.F. and S.V. Huprikar. 1972. On the biochemical and genetic basis of the human blood-group MN specificities. *Haematologia* **6:** 81–92

Springer, G.F., Y. Nagai and H. Tegtmeyer. 1966. Isolation and properties of human blood-group NN and meconium-Vg antigens. *Biochemistry* **5:** 3254–3272

Springer, G.F. and K. Stalder. 1961. Action of influenza viruses, receptor-destroying enzyme and proteases on blood group agglutinogen Mg. *Nature* **191:** 187–188

Springer, G.F., H. Tegtmeyer and S.V. Huprikar. 1972. Anti-N reagents in elucidation of the genetical basis of human blood-group MN specificities. *Vox Sang.* **22:** 325–343

Springer, G.F. and H.J. Yang. Effect of periodate oxidation on specific activities and carbohydrate components of human blood group N- and M-specific glycoproteins and glycopeptides. *Vox Sang.* **35:** 255–264

Springer, G.F., H.J. Yang and I.-Y. Huang. 1977. Small glycopeptides and oligosaccharides with human blood group M- and N- specificities. *Naturwissenschaften* **64:** 393

Staal, G.E.J., M.C.M. van der Heijden, J. Troost, M. Moes and E. Borst-Eilers. 1977. Fucosidosis and Lewis substances. *Clin. Chim. Acta* **76:** 155–157

Stacey, M. 1946. The chemistry of mucopolysaccharides and mucoproteins, *in:* W.W. Pigman and M.L. Wolfrom (eds.) *Adv. Carbohyd. Chem.* **2:** 161–201, Academic Press, N.Y.

Stalder, K. and G.F. Springer. 1960. M and N agglutinin inhibition by human kidney and erythrocyte extracts. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **19:** 70

Stanbury, A. and B. Francis. 1967. The Lu(a–b–) phenotype: an additional example. *Vox Sang.* **13:** 441–443

Staveley, J.M. and G.L. Cameron. 1958. The inhibiting action of hydatid cyst fluid on anti-Tj^a sera. *Vox Sang.* **3:** 114–118

Steck, T.L., G. Fairbanks and D.F.H. Wallach. 1971. Disposition of the major proteins in the isolated erythrocyte membrane. Proteolytic dissection. *Biochemistry* **10:** 2617–2624

Steinberg, A.G. 1965. Evidence for a mutation or crossing-over at the Rh locus. *Vox Sang.* **10:** 721–724

Stern, K., I. Davidsohn, F.G. Jensen and R. Muratore. 1958. Immunologic studies on the Be^a factor. *Vox Sang.* **3:** 425–434

Steusing, Z. 1930. Classification sérologique des spermatozoides humains. *C.R. Soc. Biol.* **103:** 430

Stratton, F. 1946. A new *Rh* allelomorph. *Nature* **158:** 25–26

Stratton, F. 1953. A slide test for the detection of Rh-antibodies using papain treated red cells. *Vox Sang.* **3**(O.S.): 43–50 §²

Stratton, F. 1956. The value of fresh serum in the detection and use of anti-Jk^a antibody. *Vox Sang.* **1:** 160–167

Stratton, F. and P.H. Renton. 1948. *Rh* genes allelomorphic to D [Actually, no title, but follows immediately this title over a paper by Race, Sanger and Lawler] *Nature* **162:** 293–294

Stratton, F. and P.H. Renton. 1954. Haemolytic disease of the newborn caused by a new Rh antibody, anti-C^x. *Br. Med. J.* **1:** 962–965

Stratton, F. and P.H. Renton. 1959. Acquisition of B-like antigen *Br. Med. J.* **2:** 244

Stroup, M. 1977. Rh system. Genetics and function. *Mayo Clinic Proc.* **52:** 141–144

Stroup, M., M. MacIlroy, R. Walker and J.V. Aydelotte. 1965. Evidence that Sutter belongs to the Kell blood group system. *Transfusion* (Phila.) **5:** 309–314

Sturgeon, P. 1960. Studies on the relation of anti-$rh'^N(C^N)$ to Rh blood factor rh_i (Ce). *J. Forensic Sci.* **5:** 287–293

Sturgeon, Ph. and M.B. Arcilla. 1970. Studies on the secretion of blood

group substances. I. Observations on the red cell phenotype Le(a+b+x+). *Vox Sang.* **18:** 301–322

Sturgeon, P., M. DuCros, D. McQuiston and W. Smythe. 1965. Multichannel high-speed fully automated filter paper unit for routine blood typing, *in:* L.T. Skeggs (ed.) *Automation in Analytical Chemistry,* Technicon Symp., 1965, 515–524, Mediad, Inc., New York (1966)

Sturgeon, P., D. McQuiston and S. van Camp. 1973. Quantitative studies on salivary blood group substances. I. Methodology and physiological variables. II. Normal values. *Vox Sang.* **24:** 97–113 and 114–125

Stuver, W.C. 1979. Personal Communication

Sussman, L.N. 1956. Blood groups in Chinese of New York area. Studies with A-B-O, M-N, Rh-Hr, Kell and P groups. *Am. J. Clin. Pathol.* **26:** 471–476

Sussman, L.N. 1976. *Paternity Testing by Blood Grouping,* 2nd ed., C.C. Thomas, Springfield.

Süssman, Ph. O. 1925. Individuelle Eiweissdifferenzierung durch Präzipitation. *Wuerzburger Abhandl. Gesamtgebiet Med.* **22** (N.F. 2): 297–306

Suyama, H. and T. Imai. 1975. An identification of the blood groups and the isoenzyme phenotypes from teeth. *Forensic Sci.* **5:** 169 [Abstr.]

Suzuki, T. 1970. Blood grouping of bloodstains by immuno-electron microscopy. *Tohoku J. Exp. Med.* **101:** 1–7

Swanson, J., H.F. Polesky, P. Tippett and R. Sanger. 1965. A 'new' blood group antigen, Do^a. *Nature* **206:** 313

Swinburne, L.M. 1962. The identification of skin. *Med. Sci. Law* **3:** 3–12

Sylvia, F.R. and P.L. Kirk. 1961. Specificity in blood grouping with plant lectins and the application of N-lectin to dry blood stains. *J. Forensic Med.* **8:** 172–181

Takagi, M. 1968. Medicolegal studies on the group determinations of human blood and saliva stains by the elution method. *J. Tokyo Med. College* **26:** 453–471 §²

Takata, H. 1973. Studies on blood groups of human teeth. Part 1. Identification of ABO blood groups from permanent and deciduous teeth by means of elution. *Jpn. J. Leg. Med.* **27:** 46–54 §¹

Takata, H. 1974. Studies on blood groups of human teeth. Part 2. Identification of ABO blood groups of teeth left standing under various conditions. *Jpn. J. Leg. Med.* **28:** 417–421 §¹

Taliano, V., R.M. Guevin and P. Tippett. 1973. The genetics of a dominant inhibitor of the Lutheran antigens. *Vox Sang.* **24:** 42–47

Tan, T.T. and M.K. Wong. 1963. A modified method of ABO grouping of blood stains. *J. Forensic Sci. Soc.* **3:** 110–112

Tandon, K.N. and S.N. Naik. 1976. A, B and H substances in the saliva of dogs. *Indian J. Exp. Biol.* **14:** 97–99

Tanner, M.J. A., D.J. Anstee and P.A. Judson. 1977. A carbohydrate-deficient membrane glycoprotein in human erythrocytes of phenotype S-s-. *Biochem. J.* **165:** 157–161

Taylor, G.L., R.R. Race, A.M. Prior and E.W. Ikin. 1942. Frequency of the iso-agglutinin α_1 in the serum of the subgroups A_2 and A_2B. *J. Pathol Bacteriol.* **54:** 514–516

Tesař, J. 1954. Určování skupinových vlastností ve vlasech. *Časkopis Lékařu Českých* **93(39):** 1078–1080

Tha Aye, U. 1977. Detection of residual ABO substances in serologically used glass test tubes by absorption-elution method. *Forensic Sci.* **9:** 155–156

Tha Aye, U. 1978. The reliability of ABO grouping of bloodstains contaminated with sweat. *J. Forensic Sci. Soc.* **18:** 193–195

Therkelsen, F. 1934. Die gerchtsmedizinische Fleckuntersuchung durch Typenbestimmung. I. Über schwer nachweisbare Typeneigenschaften in Flecken, besonders im Hinblick auf den Nachweis von Typus A_2B in Blutflecken. II. Über den Nachweis des Receptors M in Blutflecken. *Dtsch. Z. Gesamte Gerichtl. Med.* **23:** 29–34 and 35–39

Therkelsen, F. 1936. Typenbestimmung bei gerichtsmedizinischen Fleckenuntersuchungen. *Z. Rassenphysiol.* **8:** 98–132 and **9:** 1–21

Thoma, K. 1954. Determination of the blood groups A, B, AB and O in the finger and toe nails. *Int. Crim. Police Rev.,* No. **77:** 107–115

Thompson, P.R., D.M. Childers and D.E. Hatcher. 1967. Anti-Di^b—first and second examples. *Vox. Sang.* **13:** 314–318

Thomsen, O. 1927. Ein vermehrungsfähiges Agens als Veränderer des isoagglutinatorischen Verhaltens der roten Blutkörperchen, eine bisher unbekannte Quelle der Fehlbestimmung. *Z. Immunitaetsforsch. Exp. Ther.* **52:** 85–107

Thomsen, O. 1930a. Untersuchungen über die serologische gruppendifferenzierung des Organismus. *Acta Pathol. Microbiol. Scand.* **7:** 250–278

Thomsen, O. 1930b. Recherches sur la différenciation des groups sérologiques dans l'organisme. Leucocytes; Les cellules organiques et specialment les cellules des tumeurs; Le sérum; Les urines. *C.R. Soc. Biol.* **104:** 499–501 and 501–503 and 504–506 and 506–508

Thomsen, O. 1932. Über die A_1 and A_2-Receptoren in der sogenannten A-Gruppe. *Acta Soc. Med. Fenn. Duodecim* **15** (Ser. A) (9): 1–15

Thomsen, O. 1936. Ueber das Erscheinen von Isoagglutininen bei Menschen. *Z. Immunitaetsforsch. Exp. Ther.* **89:** 435–453

Thomsen, O., V. Freidenreich and E. Worsaae. 1930. Über die Möglichkeit der Existenz zweier neuer Blutgruppen; auch ein Beitrag zur Beleuchtung sogennanter Untergruppen. *Acta Pathol. Microbiol. Scand.* **7:** 157–190

Thomsen, O. and K. Kettel. 1929. Die Starke der menschlichen Isoagglutinine und entsprechenden Blutkörperchenrezeptoren in verschieden Lebensaltern. *Z. Immunitaetsforsch. Exp. Ther.* **63:** 67–93

Tilley, C.A., M.C. Crookston, J.H. Crookston, J. Shindman and H. Schachter. 1978. Human blood-group *A*- and *H*-specified glycosyltransferase levels in the sera of newborn infants and their mothers. *Vox Sang.* **34:** 8–13

Tippett, P. 1963. Serological study of the inheritance of unusual Rh and other blood group phenotypes. Ph.D. Dissertation, Univ. of London

Tippett, P. 1967. Genetics of the Dombrock blood group sytsem. *J. Med. Genet.* **4:** 7–11

Tippett, P. 1971. A case of suppressed Lu^a and Lu^b antigens. *Vox Sang.* **20:** 378–380

Tippett, P., J. Gavin and R. Sanger. 1972. The Dombrock system; linkage relations with other blood group loci. *J. Med. Genet.* **9:** 392–393

Tippett, P., J. Noades, R. Sanger, R.R. Race, L. Sausais, C.A. Holman and R.J. Buttimer. 1960. Further studies of the I antigen and antibody. *Vox Sang.* **5:** 107–121

Tippett, P. and R. Sanger. 1962. Observations on subdivisions of the Rh antigen D. *Vox Sang.* **7:** 9–13

Tippett, P., R. Sanger, R.R. Race, J. Swanson and S. Busch. 1965. An aglutinin associated with the P and the ABO blood group systems. *Vox Sang.* **10:** 269–280

Toivanen, P. and T. Hirvonen. 1969a. Iso- and heteroagglutinins in human fetal and neonatal sera. *Scand. J. Haematol.* **6:** 42–48

Toivanen, P. and T. Hirvonen. 1969b. Fetal development of red cell antigens K, k, Lu^a, Lu^b, Fy^a, Fy^b, Vel and Xg^a. *Scand. J. Haematol.* **6:** 49–55

Toivanen, P. and T. Hirvonen. 1973. Antigens Duffy, Kell, Kidd, Lutheran and Xg^a on fetal red cells. *Vox Sang.* **24:** 372–376

Toma, V.A., M. Iturralde, J.D. Anderson and F.P. Retief. 1979. Is the d an allelic gene of D in the Rh blood group system? A quantitative study using ^{99m}Technitium-pyrophosphate-labelled antibody (TcLA) method. *J. Immunol. Meth.* **27:** 273–282

Tomita, K. 1967. On the detection of blood groups from bloodstains containing detergent. *Hiroshima J. Med. Sci.* **16:** 67–80

Tomita, M., H. Furthmayr and V.T. Marchesi. 1978. Primary structure of human erythrocyte glycophorin A. Isolation and characterization of peptides and complete amino acid sequence. *Biochemistry* **17:** 4756–4770

Trela, F. and B. Turowska. 1975. ABO(H) group substances in human inner ear fluid. *Forensic Sci.* **6:** 5–7

Tröger, H.D. 1973. Ein modifiziertes Absorptions-Elutions-Verfahren zur Schnellbestimmung der ABO-Gruppe an Blutspuren. *Beitr. Gerichtl. Med.* **30:** 445–448

Tröger, H.D., J. Jungwirth and W. Eisenmenger. 1976. Zeitliche Nachweisgrenze der ABO-Gruppenbestimmung an Hautspuren unter Fingernägeln zur Zuordnung von Abwehrverletzungen. *Beitr. Gerichtl. Med.* **34:** 151–154

Tumosa, C.S. 1977a. Apparent blood groups from animal bloodstains. *Forensic Serol. News* **3(2)** §¹

Tumosa, C.S. 1977b. The absorption and elution of some cold agglutinatinins from a dried bloodstain. *Forensic Serol. News* **3(5)** §¹

Ueda, S. 1974. Determination of ABH substances in the human saliva of secretors and non-secretors by thin layer chromatography. *Jpn. J. Leg. Med.* **28:** 401 §[1]

Ueno, S. 1963. An improved technique for the determination of the blood group of blood, saliva and semen stains. *Z. Immunitaetsforsch. Allergieforsch.* **125:** 230–235

Uhlenbruck, G. 1960. Neuraminsaurehaltige Mucoide aus menschlichen Erythrocyten und ihr Verhalten gegenuber verschiedenen Enzymen. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg.*, I Abt., Ref. **177:** 197–219

Uhlenbruck, G. 1965. Immunochemical studies on erythrocyte mucoids; the nature of the M and N specific substances, *in:* Proc. 10th Congr. Int. Soc. Blood Transf., *Bibliotheca Haematol.*, Fasc. **23:** 476–482, S. Karger, Basel and N.Y.

Uhlenbruck, G. 1969. Possible genetical pathways in the MNSsUu system. *Vox Sang.* **16:** 200–210

Uhlenbruck, G. and M. Krüpe. 1965. Cryptantigenic N_{Vg} receptor in mucoids from Mu/Mu cells. *Vox Sang.* **10:** 326–332

Uhlenbruck, G., I. Sprenger, M. Heggen and A.M. Leseney. 1971. Diagnosis of the "Cad" blood group with agglutinins from snails and plants. *Z. Immunitaetsforsch. Exp. Klin. Immunol.* **141:** 290–291

Uhlenbruck, G., G. Steinhausen and W.P. Hermann. 1973. Blood group substance A prepared from human seminal plasma. *Arch. Dermatol. Forsch.* **246:** 47–54

Unger, L.J. 1951. A method for detecting Rh_0 antibodies in extremely low titer. *J. Lab. Clin. Med.* **37:** 825–827

Unger, L.J. and A.S. Wiener. 1959. A "new" antibody, anti-**Rh^c** resulting from isosensitization by pregnancy with special reference to the heredity of a new Rh-Hr agglutinogen $\mathfrak{Rh}_i^c$. *J. Lab. Clin. Med.* **54:** 835–842 and see Wiener, 1961

Unger, L.J., A.S. Wiener and L. Weiner. 1959. New antibody (anti-Rh^B) resulting from blood transfusion in an Rh-positive patient. *J. Am. Med. Assoc.* **170:** 1380–1383 and see Wiener, 1961

Valdes, M.D., C. Zoes and A. Froker. 1978. Unusual inheritance in the ABO blood group system: a group O child from a group A_2B mother. *Vox Sang.* **35:** 176–180

Van Arsdel, P.O. and A.G. Motulsky. 1959. Blood groups and secretion of blood group substances. Comparison of allergic with nonallergic persons in a Pacific Northwest college population. *J. Allergy* **30:** 460–463

Vance, D.E. and C.C. Sweely. 1967. Quantitative determination of the neutral glycosyl ceramides in human blood. *J. Lipid Res.* **8:** 621–630

Van den Bosch, C. 1948. The very rare *Rh* genotype R_yr (CdE/cde) in a case of erythroblastosis fetalis. *Nature* **162:** 781

Van der Hart, M., A. Szaloky and J. J. van Loghem. 1968. A 'new' antibody associated with the Kell blood group system. *Vox Sang.* **15:** 456–458

Van der Hart, M. and J.J. van Loghem. 1953. A further example of anti-Jk^a. *Vox Sang.* **3** (O.S.): 72–73 §[2]

Van Loghem, J.J., Jr., A-M. Heier, M. van der Hart and V.R. Sanches. 1953. A serum containing anti-Jk^b, anti-C and anit-M. *Vox Sang.* **3** (O.S.): 115–117 §[2]

Van Loghem, J.J. and M. van der Hart. 1954. The weak antigen A_x occurring in the offspring of group O parents. *Vox Sang.* **4** (O.S.): 69–75 §[2]

Verdier, L. 1906. Contribution a l'étude de la différenciation individuelle du sang humain. Thesis, M.D., Faculty of Medicine and Pharmacy, Univ. Toulouse

Verzár, F. 1927. Die Unsicherheiten in der Nomenklatur der Blutgruppen and ihre praktischen Folgen. *Klin. Wochenschr.* **6:** 347–348

Vitullo, L.R. 1966. Use of enzyme-treated cells in grouping dried bloodstains. *J. Crim. Law Criminol. Police Sci.* **57:** 356–359

Vogt, A., B. Gibb and M. Eder. 1965. Nochmals zum Thema: Gruppengeprägte A-, B- und O(H) Substanzen in menschilichen Haar. *Z. Aerztl. Fortbild.* **59:** 183–185

Von Decastello, A. and A. Sturli. 1902. Über die Isoagglutinine im Serum gesunder und kranker Menschen. *Muench. Med. Wochenschr.* **49**(26): 1090–1095 and see Camp *et al.*, 1974

Von Dungern, E. 1910. Ueber Nachweis und Vererbung biochemischer Strukturen und ihre forensische Bedeutung. *Muench. Med. Wochenschr.* **57**(6): 293–295

Von Dungern, (E.) and (L.) Hirschfeld [Hirszfeld]. 1910a. Ueber eine Methode, das Blut verschiedener Menschen serologisch zu unterscheiden. *Muench. Med. Wochenschr.* **57**(14): 741–742

Von Dungern, E. and L. Hirschfeld [Hirszfeld]. 1910b. Ueber Vererbung gruppenspezifischer Strukturen des Blutes. *Z. Immunitaetsforsch. Exp. Ther.*, I Teil, Orig. **6:** 284–292

Von Dungern, (E.) and (L.) Hirszfeld. 1911. Über gruppenspezifische Strukturen des Blutes. *Z. Immunitaetsforsch. Exp. Ther.*, Orig. **8:** 526–562 and see Camp *et al.*, 1974

Vos, G.H. and G.A. Kelsall. 1956. A new elution technique for the preparation of specific immune anti-Rh serum. *Br. J. Haematol.* **2:** 342–344

Vos, G.H. and R.L. Kirk. 1958. The "protection" of sensitized A and B red cell antigens by water-soluble A and B substances. *J. Immunol.* **80:** 149–158

Vos, G.H., D. Vos, R.L. Kirk and R. Sanger. 1961. A sample of blood with no detectable Rh antigens. *Lancet* **1:** 14–15

Waissbluth, J.G. and M.J.S. Langman. 1971. ABO blood groups, secretor status, salivary protein, and serum and salivary immunoglobulin concentrations. *Gut* **12:** 646–649

Walker, M.E., P. Rubinstein and F.H. Allen, Jr. 1977. Biochemical genetics of MN. *Vox Sang.* **32:** 111–120

Walker, M.E., P.A. Tippett, J.M. Roper, M.D. Osthold, M.J. Munn, A. Matheson, S.J. Lewis, S.M.R. Krabbe, M. Gillis, C.F. Farrenkopf, J.M. Downs, P. Crawcour, P.A. Corcoran, K. Caspersen, T. von Bercken, R. Ball and F.H. Allen, Jr. 1961. Tests with some rare blood-group antibodies. *Vox Sang.* **6:** 357

Walker, R.H., C.I. Argall, E.A. Steane, T.T. Sasaki and T.J. Greenwalt. 1963. Anti-Js^b, the expected antithetical antibody of the Sutter blood group system. *Nature* **197:** 295–296

Walsh, R.J. and O. Kooptzoff. 1954. The human blood group Tj^a. *Aust. J. Exp. Biol. Med. Sci.* **32:** 387–392

Walsh, R.J. and C.M. Montgomery. 1947. A new human isoagglutinin subdividing the MN blood groups. *Nature* **160:** 504–505

Waśniowska, K., Z. Drzeniek and E. Lisowska. 1977. The amino acids of M and N blood group glycopeptides are different. *Biochem. Biophys. Res. Comm.* **76:** 385–390

Watanabe, K., S-I. Hakomori, R.A. Childs and T. Feizi. 1979. Characterization of a blood group I-active ganglioside. Structural requirements for I and i specificities. *J. Biol. Chem.* **254:** 3221–3228

Watkins, W.M. 1958. The multiple specificities of blood group mucopolysaccharides, *in:* Proc. 7th Congr. Int. Soc. Blood Transf., Rome, *Bibliotheca Haematol.*, Fasc. **10:** 692–696, S. Karger, Basel and N.Y. (1959)

Watkins, W.M. 1959. Some genetical aspects of the biosynthesis of human blood group substances, *in:* Ciba Foundation Symp. on *Biochemistry of Human Genetics,* 217–241, Little Brown & Co., Boston

Watkins, W.M. 1962. Changes in the specificity of blood-group mucopolysaccharides induced by enzymes from *Trichomonas foetus. Immunology* **5:** 245–266

Watkins, W.M. 1972. Blood-group specific substances, *in:* A Gottschalk (ed.) *Glycoproteins. Their Composition, Structure and Function,* 2nd ed., Pt. B: 830–891, BBA library, Vol. 5, Elsevier, Amsterdam, New York, etc.

Watkins, W.M. 1978. Genetics and biochemistry of some human blood groups. *Proc. R. Soc. Lond.* **B202:** 31–53

Watkins, W.M. and W.T.J. Morgan. 1955. Some observations on the O and H characters of human blood and secretions. *Vox Sang.* **5** (O.S.): 1–14 §[2]

Watkins, W.M. and W.T.J. Morgan. 1959. Possible genetical pathways for the biosynthesis of blood group mucopolysaccharides. *Vox Sang.* **4:** 97–119

Watkins, W.M. and W.T.J. Morgan. 1962. Blood group P_1 substance: (II) Immunological properties, *in:* Proc. 9th Congr. Int. Soc. Blood Transf., Mexico City, *Bibliotheca Haematol.*, Fasc. **19:** 230–234, S. Karger, Basel (1964)

Weicker, H. 1968. Isolation of a small molecular peptide from human erythrocyte membrane with Rh properties. *Clin. Chim. Acta* **21:** 513–514

Weicker, H. and J. Metz. 1971. Isolierung eines kleinmolekularen

Erythrocytenmembran-Proteins durch Anti-D-Immunadsorption. *Z. Klin. Chem. Klin. Biochem.* **9**: 367–374

Weicker, H. and D. Roelke. 1971. Untersuchungen über die Rh-(D)-Eigenschaft eines kleinmolekularen Erythrocytenmembranproteins mit der Schultz-Dale-Reaktion. *Z. Klin. Chem. Klin. Biochem.* **9**: 375–382

Weiner, W. 1957. Eluting red cell antibodies: a method and its application. *Br. J. Haematol.* **3**: 276–283

Weiner, W., H.B.M. Lewis, P. Moores, R. Sanger and R.R. Race. 1957. A gene, y, modifying the blood group antigen A. *Vox Sang.* **2**: 25–37

Werckmeister-Freund, R. 1932. Gruppenbestimmungen an verunreinigtem und physikalischen Einflüssen ausgesetztem Blut. *Dtsch. Z. Gesamte Gerichtl. Med.* **19**: 238–243

West, C.D., R. Hong and N.H. Holland. 1962. Immunoglobulin levels from the newborn period to adulthood and in immunoglobulin deficiency states. *J. Clin. Invest.* **41**: 2054–2064

White, B.J. 1954. Applications of blood grouping in the examination of blood stains. *J. Forensic Med.* **1**: 333–337

Wiener, A.S. 1935. *Blood Groups and Transfusion,* C.C. Thomas, Springfield and Baltimore

Wiener, A.S. 1939. *Blood Groups and Transfusion,* 2nd ed., C.C. Thomas, Springfield and Baltimore

Wiener, A.S. 1942. Hemolytic transfusion reactions. III. Prevention, with special reference to the Rh and cross-match tests. *Am. J. Clin. Pathol.* **12**: 302–311

Wiener, A.S. 1943a. Distribution and heredity of the Rh types. *Science* **98**: 182–184 and see Weiner, 1954

Wiener, A.S. 1943b. Genetic theory of the Rh blood types. *Proc. Soc. Exp. Biol. Med.* **54**: 316–319 and see Wiener, 1954

Wiener, A.S. 1943c. *Blood Groups and Transfusion,* 3rd ed., Hafner Pub. Co., New York & London

Wiener, A.S. 1944a. Forensic importance of blood grouping. *Exp. Med. Surg.* **2**: 44–51 and see Wiener, 1954

Wiener, A.S. 1944b. A new test (blocking test) for Rh sensitization. *Proc. Soc. Exp. Biol. Med.* **56**: 173–176

Wiener, A.S. 1945. Theory and nomenclature of the Hr blood groups. *Science* **102**: 479–482 and see Wiener, 1954

Wiener, A.S. 1946. Pathogenesis of congenital hemolytic disease (erythroblastosis fetalis) I. Theoretic considerations. *Am. J. Dis. Children* **71**: 14–24 and see Wiener, 1954

Wiener, A.S. 1950. Heredity of the Rh blood types. IX. Observations in a series of 526 cases of disputed parentage. *Am. J. Hum. Genet.* **2**: 177–197

Wiener, A.S. 1952. History of the Rhesus blood types. *J. Hist. Med. Allied Sci.* **7**: 369–383 and see Wiener, 1954

Wiener, A.S. 1953. The blood factor C of the A-B-O system, with special reference to the rare blood group *C. Ann. Eugenics* **18**: 1–8

Wiener, A.S. 1954. *Rh-Hr Blood Types.* Applications in Clinical and Legal Medicine and Anthropology. Grune & Stratton, New York

Wiener, A.S. 1961. *Advances in Blood Grouping,* Grune and Stratton, New York & London

Wiener, A.S. 1962. Blood groups and disease. A critical review. *Lancet* **1**: 813–816

Wiener, A.S. 1963. Isoagglutinins in dried blood stains. A sensitive technique for their demonstration. *J. Forensic Med.* **10**: 130–132

Wiener, A.S. 1965a. *Advances in Blood Grouping II.* Grune and Stratton, New York & London

Wiener, A.S. 1965b. Nomenclature of Rh groups. *Am. J. Hum. Genet.* **17**: 457–458

Wiener, A.S. 1968. Serology, genetics and nomenclature of the P blood group system. Preliminary report. *Lab. Digest* **31**: 6–9

Wiener, A.S. 1969. Problems and pitfalls in blood grouping tests for nonparentage. I. Distribution of the blood groups. *Am. J. Clin. Pathol.* **51**: 9–14

Wiener, A.S. 1970. *Advances in Blood Grouping III.* Grune and Stratton, New York & London

Wiener, A.S. 1974. Blood groups of Chinese in New York city: application to problems of disputed parentage. *J. Forensic Sci.* **19**: 735–743

Wiener, A.S., G.J. Brancato and E.B. Gordon. 1957a. Observations on the rare genes r'^{W} and R^{Z} of the Rh-Hr allelic series. *Acta Genet. Med. Gemellol.* **6**: 195–200 and see Wiener, 1961

Wiener, A.S., G.J. Brancato and R. Waltman. 1953a. Observations on isosensitization to the Kell factor. *J. Lab. Clin. Med.* **42**: 570–580 and see Wiener, 1961

Wiener, A.S., I, Davidsohn and E.L. Potter. 1945. Heredity of the Rh blood types. II. Observations on the relation of factor Hr to the Rh blood types. *J. Exp. Med.* **81**: 63–72

Wiener, A.S., N. Di Diego and S. Sokol. 1953b. Studies on the heredity of the human blood groups. I. The M-N types. *Acta Genet. Med. Gemellol.* **2**: 391–397

Wiener, A.S., J. Geiger and E.B. Gordon. 1957b. Mosaic nature of the Rh_0 factor of human blood. *Exp. Med. Surg.* **15**: 75–82 and see Wiener, 1961

Wiener, A.S. and E.B. Gordon. 1951. Répartition des facteurs de groupes sanguins dans une population de New York city, avec une étude spéciale des agglutinogènes rares. *Rev. Hematol.* **6**: 45–50

Wiener, A.S. and E.B. Gordon. 1956. A hitherto undescribed human blood group, A_m. *Br. J. Haematol.* **2**: 305–307

Wiener, A.S. and E.B. Gordon. 1967. Further observations on the blood factors associated with the blood factor $\mathbf{Rh_0}$. *J. Forensic Med.* **14**: 131–137 and see Wiener, 1970

Wiener, A.S. and E.B. Gordon. 1967. Further observations on the blood factors associated with the blood factor $\mathbf{Rh_0}$. *J. Forensic Med.* **14**: 131–137 and see Wiener, 1970

Wiener, A.S., E.B. Gordon and A. Evans. 1958. The value of anti-H reagents (Ulex europaeus) for grouping dried blood stains. *J. Forensic Sci.* **3**: 493–502

Wiener, A.S., E.B. Gordon and J. Moor-Jankowski. 1964a. The Lewis blood groups in man. A review with supporting data on non-human primates. *J. Forensic Med.* **11**: 67–83

Wiener, A.S., E.B. Gordon and J.P. Wexler. 1963. The M-N types, with special reference to the mating MN x MN. *Exp. Med. Surg.* **21**: 89–100 and see Wiener, 1965a

Wiener, A.S. and J.G. Hurst. 1947. A new sensitive test (direct test) for Rh blocking antibodies. *Exp. Med. Surg.* **5**: 285–298 and see Wiener, 1954

Wiener, A.S., J.G. Hurst and E.B. Sonn-Gordon. 1947. Studies on the conglutination reaction, with special reference to the nature of conglutinin. *J. Exp. Med.* **86**: 267–284 and see Wiener, 1954

Wiener, A.S. and M.A. Hyman. 1948. Observations on the rare genes R^{Z} and r^{y}. *Am. J. Clin. Pathol.* **18**: 921–926 and see Wiener, 1954

Wiener, A.S. and H.E. Karowe. 1944. Diagrammatic representation of the human blood-group reactions. *J. Immunol.* **49**: 51–61

Wiener, A.S. and I. Kosofsky. 1941a. Quantitative studies on the group-specific substances in human blood and saliva. I. Group-specific substance B. *J. Immunol.* **41**: 413–428

Wiener, A.S. and I. Kosofsky. 1941b. Quantitative studies on the group-specific substances in blood and saliva. II. Group-specific substance A, with special reference to the subgroups. *J. Immunol.* **42**: 381–393

Wiener, A.S. and K. Landsteiner. 1943. Heredity of variants of the Rh type. *Proc. Soc. Exp. Biol. Med.* **53**: 167–170

Wiener, A.S., J. Moor-Jankowski and E.B. Gordon. 1964b. Blood group antigens and cross-reacting antibodies in primates including man. I. Production of antisera for agglutinogen M by immunization with blood other than human type M blood. *J. Immunol.* **92**: 391–396

Wiener, A.S. and H.R. Peters. 1940. Hemolytic reactions following transfusions of blood of the homologous group, with three cases in which the same agglutinogen was responsible. *Ann. Int. Med.* **13**: 2306–2322

Wiener, A.S., A.A. Samwick, M. Morrison and L. Cohen. 1953c. Studies on immunization in man. II. The blood factor C. *Exp. Med. Surg.* **11**: 276–285

Wiener, A.S. and W.W. Socha. 1974. Macro- and microdifferences in blood group antigens and antibodies. II. Subgroups of A, an example of graded microdifferences. *Int. Arch. Allergy Appl. Immunol.* **47**: 946–950

Wiener, A.S., W.W. Socha and E.B. Gordon. 1973. Further observations on the serological specificity C of the A-B-O blood group system. *Br. J. Haematol.* **24**: 195–203

Wiener, A.S. and E.B. Sonn. 1943. Additional variants of the Rh type demonstrable with a special human anti-Rh serum. *J. Immunol.* **47:** 461–465

Wiener, A.S. and E.B. Sonn Gordon. 1947. Réaction transfusionnelle hémolytique intra-groupe due a un hémagglutinogène jusqu'ici décrit. *Rev. Hematol.* **2:** 1–10

Wiener, A.S., E.B. Sonn and R.B. Belkin. 1944. Heredity of the Rh blood types. *J. Exp. Med.* **79:** 235–253

Wiener, A.S. and L.J. Unger. 1959. Rh factors related to the Rh_0 factor as a source of clinical problems. Diagrammatic representation of their reactions and predictions of still undiscovered Rh factors. *J. Am. Med. Assoc.* **169:** 696–699 and see Wiener, 1961

Wiener, A.S., L.J. Unger and L. Cohen. 1954. Distribution and heredity of blood factor U. *Science* **119:** 734–735

Wiener, A.S., L.J. Unger, L. Cohen and J. Feldman. 1956. Type-specific cold auto-antibodies as a cause of acquired hemolytic anemia and hemolytic transfusion reactions: biologic test with bovine red cells. *Ann. Int. Med.* **44:** 221–240

Wiener, A.S., L.J. Unger and E.B. Gordon. 1953d. Fatal hemolytic transfusion reaction caused by sensitization to a new blood factor U. *J. Am. Med. Assoc.* **153:** 1444–1446

Wiener, A.S. and M. Vaisberg. 1931. Heredity of the agglutinogens M and N of Landsteiner and Levine. *J. Immunol.* **20:** 371–388

Wiener, A.S. and I.B. Wexler. 1958. *Heredity of the Blood Groups,* Grune and Stratton, New York & London

Wilkie, M.H. and E.L. Becker. 1955. Quantitative studies in hemagglutination. I. Assay of anti-B isohemagglutinins. II. Effect of certain variables upon the isohemagglutinin assay. *J. Immunol.* **74:** 192–198 and 199–204

Williamson, P. and G.F. Springer. 1959. Blood-group B active somatic antigen of *E. coli* O_{16}:B7. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **18:** 604

Windhorst, D.B. 1969. Inheritance of chronic granulomatous disease. *Lancet* **2:** 543

Witebsky, E. 1927. Zur Methodik der Gruppenbestimmung in menschlichen Blutflecken. *Muench. Med. Wochenschr.* **74**(37): 1581–1582

Witebsky, E. and N.C. Klendshoj. 1941. The isolation of an O specific substance from gastric juice of secretors and carbohydrate-like substances from gastric juice of non-secretors. *J. Exp. Med.* **73:** 655–667

Wolf, R.O. and L.L. Taylor. 1964. The concentration of blood-group substance in the parotid, sublingual and submaxillary salivas. *J. Dent. Res.* **43:** 272–275

Wolff, E. and B. Jonsson. 1933. Studien über die Untergruppen A_1 und A_2 mit besonderer Berücksichtigung der Paternitätsuntersuchungen. *Dtsch. Z. Gesamte Gerichtl. Med.* **22:** 65–85

Wolff, I. and G.F. Springer. 1965. Observations on the Rh substances, *in:* Proc. 10th Congr. Int. Soc. Blood Transf., *Bibliotheca Haematol.,* Fasc. **23:** 510–514, S. Karger, Basel and N.Y.

Wrobel, D.M., B.P.L. Moore, S. Cornwall, E. Wray, R. Øyen and W.L. Marsh. 1972. A second example of Lu(-6) in the Lutheran system. *Vox Sang.* **23:** 205–207

Wurmser, R. and S. Filitti-Wurmser. 1957. Thermodynamic study of the isohaemagglutinins. *Progr. Biophys. Biophys. Chem.* **7:** 88–113

Wurzel, H.A. and W.E. Haesler. 1968. The Yt blood groups in American Negroes. *Vox Sang.* **15:** 304–305

Wynbrandt, F. and W.J. Chisum. 1971. Determination of the ABO blood group in hair. *J. Forensic Sci. Soc.* **11:** 201–204

Yada, S. 1961. Mixed agglutination on smears. *Jpn. J. Leg. Med.* **15:** 98–103 §[1]

Yada, S. 1962. Determination of the ABO groups of blood stains by means of elution test. *Jpn. J. Leg. Med.* **16:** 290–294 §[1]

Yada, S., G. Ishimoto and M. Okane. 1968a. Blood grouping of scalp hairs and fingernails derived from ABO variant persons. *Acta Criminol. Med. Leg. Jpn.* **34:** 55–56 §[6]

Yada, S., G. Ishimoto and M. Okane. 1968c. Experience of grouping Caucasian hair specimens. *Acta Criminol. Med. Leg. Jpn.* **34:** 90–92 §[6]

Yada, S., G. Ishimoto and M. Okane. 1968d. Blood grouping of an 88 year old hair rope. *Acta Criminol. Med. Leg. Jpn.* **34:** 149–151 §[6]

Yada, S., G. Ishimoto and M. Okane. 1968e. Use of a "hair roller" in the practice of blood-grouping human hairs. *Acta Criminol. Med. Leg. Jpn.* **34:** 152–154 §[6]

Yada, S., M. Mori and M. Okane. 1968b. Photographic illustration of the technique of grouping single human hairs. *Acta Criminol. Med. Leg. Jpn.* **34:** 87–89 §[6]

Yada, S., M. Okane and Y. Sano. 1966a. Blood grouping of a single human hair by means of elution technique. *Acta Criminol. Med. Leg. Jpn.* **32:** 7–8 §[6]

Yada, S., M. Okane and Y. Sano. 1966b. Blood grouping of human hairs derived from various parts of the body by elution. *Acta Criminol. Med. Leg. Jpn.* **32:** 52–55 §[6]

Yada, S., M. Okane and Y. Sano. 1966c. Blood grouping of aged and formalin-fixed human hairs. *Acta Criminol. Med. Leg. Jpn.* **32:** 92–95 §[6]

Yada, S., M. Okane and Y. Sano. 1966d. A simple method for blood-grouping fingernails. *Acta Criminol. Med. Leg. Jpn.* **32:** 96–98 §[6]

Yada, S., M. Okane and Y. Sano. 1966e. A simple method for blood-grouping bone fragments. *Acta Criminol. Med. Leg. Jpn.* **32:** 99–101 §[6]

Yada, S., M. Okane and Y. Sano. 1966g. ABO blood group antigens demonstrated in the rabbit hair. *Jpn. J. Leg. Med.* **20:** 204–206 §[1]

Yada, S., M. Okane and Y. Sano. 1966h. Blood grouping of human cerumens by means of elution technique. *Jpn. J. Leg. Med.* **20:** 220–222 §[1]

Yada, S., M. Okane, Y. Sano and Y. Fukumori. 1966f. Blood grouping of eyebrows, eyelashes and vibrissae by means of elution technique. *Acta Criminol. Med. Leg. Jpn.* **32:** 173–175 §[6]

Yada, S., K. Yamasawa and S. Mori. 1962. The ABO group antigens in various tissue cells of *Rana catesbiana* shown by mixed agglutination. *Jpn. J. Leg. Med.* **16:** 233–237 §[1]

Yamaguchi, H., Y. Okubo and F. Hazama. 1965. An A_2B_3 phenotype blood showing atypical mode of inheritance. *Proc. Japan Acad.* **41**(4): 316–320

Yamaguchi, H., Y. Okubo and F. Hazama. 1966. Another Japanese A_2B_3 blood-group family with propositus having group O father. *Proc. Japan Acad.* **42**(5): 517–520

Yamaguchi, H., Y. Okubo, T. Seno, K. Matsushita and G.L. Daniels. 1979. A 'new' allele, Kp^c, at the *Kell* complex locus. *Vox Sang.* **36:** 29–30

Yamaguchi, H., Y. Okubo and M. Tanaka. 1970. "Cis AB" bloods found in Japanese families. *Jpn. J. Hum. Genet.* **15:** 198–215 §[7]

Yamakami, K. 1926. The individuality of semen, with reference to its property of inhibiting specifically isohemagglutination. *J. Immunol.* **12:** 185–189

Yamasawa, K. 1962. Blood grouping of saliva and semen stains by the use of fluorescein-labelled antibodies. *Jpn. J. Leg. Med.* **16:** 238–241 §[1]

Yokoyama, M. and H.H. Fudenberg. 1964. Studies on "cross-reacting" isoagglutinins. *J. Immunol.* **92:** 966–976

Yokoyama, M., M.E. Mermod and A. Stegmaier. 1967. Further examples of Jk(a–b–) blood in Hawaii. *Vox Sang.* **12:** 154–156

Yoshida, A. 1980. Identification of genotypes of blood group A and B. *Blood* **55:** 119–123

Yoshida, A., Y.F. Yamaguchi and V. Davé. 1979. Immunologic homology of human blood group glycosyltransferases and genetic backgrounds of blood group (ABO) determination. *Blood* **54:** 344–350

Yosida, Kan-Iti (Kan-Itiyosida). 1928a. Contribution a l'étude des isohemagglutinines au point de vue médico-légale. *Ann. Med. Leg. Criminol. Police Scientif.* **8:** 249–254

Yosida, K-I. 1928b. Über die gruppenspezifischen Unterschiede der Transsudate, Exsudate, Sekrete, Exkrete, Organextrakte und Organzellen des Menschen und ihre rechtsmedizinischen Anwendung. *Z. Gesamte Exp. Med.* **63:** 331–339

Yudina, G.S. 1972. Reaktsiya absorbtsii-elyutsii primenitel'no k vyyavleniyu antigenov M i N pyatnakh krovi. *Sud. Med. Ekspert.* (Moscow) **15**(3) 37–40

Yudina, G.S. 1974. Vyyavlenie antigenov Lewis v pyatnakh krovi reaktsiyey absorbtsii-elyutsii. *Sud. Med. Ekspert.* (Moscow) **17**(1): 35–37

Zinsser, H. and A.F. Coca. 1931. Remarks concerning Landsteiner's

discovery of isoagglutination and the blood groups, with special reference to a paper by J.A. Kennedy. *J. Immunol.* **20:** 259–267

Zipp, W.N. 1931. Über den Einfluss von Gewebsstoffen auf den Verlauf der Isohämagglutination. *Dtsch. Z. Gesamte Gerichtl. Med.* **18:** 66–72

Zmijewski, C.M. and J.L. Fletcher. 1972. *Immunohematology,* 2nd ed., Appleton-Century-Crofts, New York

Zoutendyk, A. and P. Levine. 1952. A second example of the rare serum anti-Jay (Tj^a). *Am. J. Clin. Pathol.* **22:** 630–633

Bibliographic Notes to References for Unit V

§[1] *Japanese Journal of Legal Medicine* (*Jpn. J. Leg. Med.*) has Japanese title *Nippon Hoigaku Zasshi*

§[2] *Vox Sanguinis* began publication in 1951 as *Bulletin van het Centraal Laboratorium van de Bloedtransfusiendienst van het Nederlandse Rode Kruis.* Title changed to *Vox Sanguinis* (*Vox Sang.*) in 1953 but volume numbering continued with issues of 1953 as vol. 3. In 1956, the present series (new series) began with volume 1, and continued to the present. Those references to volumes 3 through 5 for 1953–1955 are designated "O.S." for "old series".

§[3] *Ochanomizu Medical Journal* (*Ochanomizu Med. J.*) has Japanese title *Ochanomizu Igaku Zasshi*

§[4] *Sbornik Klinický* had alternate French title *Archives Bohème de Médécine Clinique*

§[5] *Journal of the Tokyo Medical College* (*J. Tokyo Med. College*) has Japanese title *Tokyo Ika Daigaku Zasshi*

§[6] *Acta Criminologiae et Medicinae Legalis Japonica* (*Acta Criminol. Med. Leg. Jpn.*) has Japanese title *Hanzaigaku Zasshi*

§[7] *Japanese Journal of Human Genetics* (*Jpn. J. Hum. Genet.*) has Japanese title *Jinrui Idengaku Zasshi*

§[8] *Forensic Serology News* (*Forensic Serol. News*)—see Note §[1] to the References for Unit VI

¶ References marked with this symbol have been translated and appear in Unit IX