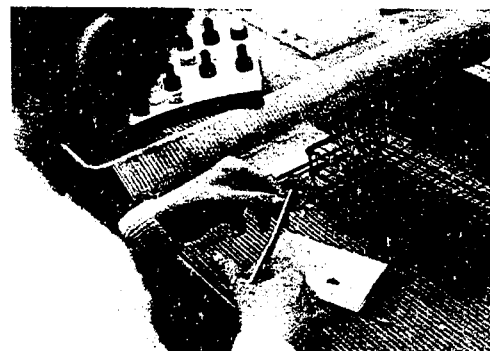
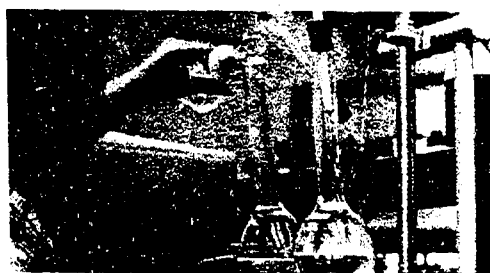


Sourcebook in Forensic Serology, Immunology, and Biochemistry



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U.S. Department of Justice
National Institute of Justice

Sourcebook in Forensic Serology, Immunology, and Biochemistry

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with 1989 Update

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Sourcebook Errata

On page 17, the equation following line 11, after the words "The above equation may be rewritten:" should read as follows:

$$[ES] = \frac{k_1}{k_2 + k_3} [E][S]$$

The equation following lines 16 and 17, after the words "Solving for ES and substituting in the previous equation yields" should read as follows:

$$\frac{v_0}{V_{\max}} = \frac{k_1}{k_2 + k_3} \frac{[E] [S]}{[E_t]}$$

On page 426, lines 29 and 30 giving the correspondences of different PGM subtype nomenclatures should read as follows:

1- = a3 = 1F; 1+ = a1 = 1S; 2- = a4 = 2F; and 2+ = a2 = 2S.

PREFACE TO THE REPRINT EDITION

In the six years or so since this *Sourcebook* appeared, the stock of copies was exhausted. There were indications from a number of sources, however, that there was still a demand for copies and that the book still has a valuable place as a reference work in forensic biology. Accordingly, the National Institute of Justice is to be commended for its decision to reprint the book on a demand basis through the National Criminal Justice Reference Service. In this respect, I am grateful to Mr. James K. Stewart, Director of the Institute, and to Dr. Richard M. Rau, manager of the Forensic Science and Criminal Justice Technology Program. As a result of their continued support, this book will continue to be available to those interested in it.

It was not realistic to consider undertaking a complete and systematic update and review of all the literature that has appeared since the book's publication. I was asked, however, to provide a brief summary of some of the more recent information in the field in this new Preface, and that is its major purpose. Because the literature grows with such rapidity, a number of more recent reviews as well as some specific papers are cited here and an effort has been made to related these to specific subject areas covered in the book. In addition, an introduction to the rapidly developing field of molecular biology and DNA analysis that has recently become a part of forensic biology is given, and some references provided. DNA analysis has become a part of forensic serology in the relatively few years since the book was written.

Selected Recent Material on Sourcebook Subjects

Several more recent reviews cover blood and body fluid identification in stains, species determination and blood and body fluid stain grouping [1-3], and the application of genetic markers including HLA to parentage testing has also been reviewed [4-6]. Volume 1 of *Advances in Forensic Science* [7] contains a number of review chapters by noted authorities on various subjects: Divall, on menstrual blood identification (cf. §8.1); Katsumata and Oya, on feta and neonatal blood identification (cf. §§8.2 and 8.3); Suzuki and Oya on semen identification in stains (cf. §10); Fiori, on body fluid grouping (cf. §19.10.5); Benciolini and Cortivo, on ABO grouping of human hair (cf. §19.10.7.1); Carracedo, as well as Pascali, on isoelectric focusing and its applications in serum group protein typing (cf. §§ 40-43 and 45); Tumosa, on the occurrence of ABH antigens in infrahuman species; Newall, on typing HLA antigens in bloodstains (cf. §46.7.2.); and Smith, on detecting drugs in bloodstains (cf. §50.2.2).

In the area of semen identification, there have been a number of newer developments. The original description of and earlier papers on γ -seminoprotein (γ -Sm) are discussed in §10.10. Similarly, the original work on seminal protein p30 may be found in §10.14. It is highly likely (though perhaps not proven) that γ -Sm and p30 are identical to one another, as well as to "human prostate antigen" (sometimes abbreviated PA) [8]. The γ -Sm protein has been further characterized biochemically and its amino acid sequence determined [9-11]. An ELISA assay using anti- γ -Sm has been developed for use with seminal stains [12]. Similarly, an ELISA has been developed for p30 and shown to be applicable to the investigation of seminal stains and vaginal swabs in sexual assault cases [13].

Another smaller seminal protein of prostatic origin, called β -microseminoprotein (β -MSP) has been isolated and extensively characterized by Hara and his collaborators in Japan [14-17]. I am indebted to Prof. Dr. Mitsuwo Hara at the Kurume University School of Medicine for making copies of his more recent work available. Another human seminal protein of seminal vesicle origin, known as MHS-5, has been purified and a monoclonal antibody prepared against it [18]. The monoclonal anti-MHS-5 has been used as the basis for an ELISA test for human semen identification.

The theory underlying absorption-inhibition testing as well as a novel two-dimensional A-I method are discussed in a paper by Lee and collaborators [19]. More recent material on the biochemical genetics of and relationships between ABO, Lewis, Secretor and related antigens (cf. § 19.9) may be found in reviews by Watkins and by Oriol and coworkers [20-22]. Extensive reviews of the application of the polymorphic isoenzyme (and other) systems in forensic serology (cf. Unit VI) have been published by Sensabaugh [23-26].

The U.S. population data for various genetic marker systems that are included in the book have been updated and analyzed in a series of three papers [27-29]. In addition, several papers have discussed the application of population genetic marker data to stain typing information as might be obtained in particular case situations [24, 30-32].

The forthcoming third volume of *Advances in Forensic Science* [33] offers reviews of several important subjects, in addition to its extensive coverage of DNA typing (about which more below). Schanfield extensively reviews immunoglobulin allotyping (cf. §44), Bütler reviews and updates the Ag system (cf. §45.1.2), and Mayr reviews the application of HLA typing in disputed parentage cases (cf. §46.7.1).

In addition, Fletcher reviews enzyme-linked immunosorbent assay (ELISA) as applied to forensic blood and body fluid identification and grouping problems. ELISA applications have come along sufficiently recently that there is nothing about them in the book.

Molecular Biology and DNA Typing

Evidence that DNA is in fact the genetic material, the structure of the nucleic acids, and the manner in which DNA controls protein synthesis are briefly reviewed in §1.2.2 of this book. In the past decade or so, extraordinary advances have been made in the field of molecular biology. These advances have enabled the development of what is often called genetic engineering. Perhaps the most significant advances in molecular biology from the point of view of forensic biology have been: (1) the discovery and characterization of a large variety of restriction endonucleases and their widespread availability; (2) the discovery and refinement of techniques for cloning manageable-sized fragments of DNA into vectors; (3) the discovery of restriction fragment length polymorphisms and the availability of human DNA probes for their detection; and (4) the description and refinement of polymerase chain reaction techniques, and their use in connection with allele specific oligonucleotide probes.

The large array of restriction endonucleases (restriction enzymes; RE) allows very large DNA to be cleaved into smaller, manageable fragments for subsequent characterization and/or manipulation. Knowledge of the RE cleavage recognition sequences in DNA has meant that sequence information is available about the ends of the fragments produced.

Some of the REs produce blunt ends, but many others produce "jagged" cuts in double stranded DNA producing fragments in which a few bases from one strand protrude as a single strand beyond the terminus of the other strand. These few base single stranded ends are sometimes called "sticky," because if another piece of double stranded DNA with a complementary single stranded sticky end is produced, the two fragments of DNA can be recombined into a single double stranded molecule using appropriate ligases. Variations of this procedure form the basis of genetic engineering. Using these techniques, fragments of human DNA can be introduced into vectors (usually plasmids or cosmids). Then, by subsequent cloning, these human DNA fragments can be reproduced in any desirable quantity in perpetuity, and in addition they can be isolated and recovered from the vectors.

In recent years it has been recognized that the human genome contains substantial segments of repetitive sequence DNA [34,35]. Some repetitive DNA occurs in the form of relatively short, highly repeated sequences that have been called 'minisatellites.' Certain minisatellite loci have been found in the human genome at which there is substantial variation between individuals in the number of times the core sequence is repeated. If a RE is used to cleave DNA outside the repeat region, fragments of differing size are produced according to the number of repeats occurring in the region. Human DNA loci of this kind are termed "variable number of tandem repeat" or "VNTR" loci. Separation of the RE-digested VNTR fragments according to size by electrophoresis, transfer of the fragments to a nitrocellulose or nylon membrane, and hybridization with a labeled human DNA probe that recognizes the core sequence produces banding patterns that are characteristic of the individual from whom the DNA came.

This phenomenon is called "restriction fragment length polymorphism" or "RFLP" and is the basis of most current "DNA typing" as it is applied in forensic serology.

In 1980, Botstein, White, Skolnik and Davis [36] recognized that RFLP could be used as a basis for genetic mapping, and this approach has indeed yielded considerable information [37]. Wyman and White [38] soon described a highly polymorphic VNTR locus, and a large number of other such loci are now known. Not much notice of these developments was taken by the forensic science community until Jeffreys described several multilocus probes [39] that could be employed to produce what were described as DNA 'fingerprints' [40]. The importance of these findings in terms of their applications both to disputed parentage problems and to individual identification problems was quickly recognized [41,42].

In just the past few years, Jeffreys has further characterized the multilocus probes [43] and cloned a series of single locus probes recognizing several of the loci detected by the original probes [44-46]. These probes are used exclusively by Cellmark Diagnostics in their DNA typing work in the U.S. Other DNA probes are used by the Lifecodes Corp. in their DNA typing work, and some information about their probes and procedures has been published [47-49]. A number of DNA probes from GenMark are available through Promega, and still other probes are available from Collaborative Research, Inc. Recently, the FBI Laboratory initiated DNA typing in casework after a lengthy research and development effort aimed at selecting a typing system, appropriate probes, and validating the procedures that are to be used [50-51].

Another DNA analysis procedure that has already found limited application in forensic serology, and is certain to be significant in the future, is the polymerase chain reaction (PCR) technique. PCR was developed by Erlich and collaborators at the Cetus Corp. in California [52-53]. With PCR, specific sequences of DNA can be replicated to produce hundreds of thousands to millions of copies provided specific primers are available. The primers are constructed from knowledge of the sequences flanking the region of interest. PCR has been applied to the diagnosis of genetic disorders [54] and to the analysis of polymorphism at specific subregions of the *HLA* locus [55]. DNA analysis of the *HLA-DQ α* polymorphism has been described in single human hair roots [56]. Many samples of forensic interest are limited in quantity and may also have been subjected to environmental conditions that degrade the DNA. As a result, RFLP analysis may not be possible. PCR techniques are attractive for forensic analysis because so little DNA is required for analysis, and experience has shown that some samples which were unsuitable for RFLP analysis could be analyzed using PCR procedures. At the present time, PCR procedures are used in conjunction with allele-specific oligonucleotide (ASO) probes. The loci currently detected do not show the degree of polymorphism exhibited by RFLP loci. The information obtained at present from PCR analysis is thus very valuable as an exclusionary tool, but less valuable in inclusionary cases. Efforts are underway in many laboratories, however, to develop primers that will enable PCR amplification of VNTR loci [57]. Further research and development will be necessary to evaluate the forensic applications of PCR techniques, as very few forensic laboratories have had much experience with PCR at the present time.

Further information about DNA typing and its forensic applications may be obtained from a number of currently available sources. Volume 3 of *Advances in Forensic Science* [33] will have ~~eight~~^{seven} chapters on DNA polymorphisms and their forensic applications. The Banbury Conference on forensic applications of DNA has papers by a number of authorities in the field [58]. The FBI Forensic Science Research and Training Center at Quantico has sponsored two major symposia on the forensic applications of DNA [59,60]. The proceedings of the first of these symposia are available on videotape, and the proceedings of the second will be published. An extensive report on forensic applications of DNA typing is currently in preparation by the Office of Technology Assessment of the U.S. Congress, and should be delivered sometime in 1989.

DNA typing is undoubtedly the most exciting development in forensic serology in many years, and arguably the most exciting development ever. It will take some time for techniques and procedures to be worked out and tested on a relatively wide scale. The need for standardization of methodology and for some general agreement on procedures for the interpretation of RFLP typing results has recently been discussed [58,61]. In the next few years, molecular and forensic biologists working together will undoubtedly establish guidelines and standards for reliable and reproducible DNA typing procedures that can be widely employed in the analysis of both disputed parentage and identification cases.

West Haven, CT
July, 1989

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FOREWORD

The National Institute of Justice is pleased to publish this important reference work for forensic serologists. The late John O. Sullivan, manager of the Institute's forensic science program from 1975 to 1981, played a key role in encouraging and supporting development of this publication. It is a particularly fitting legacy of Mr. Sullivan's contributions to advancing the state of the art in the forensic sciences.

James K. Stewart
Director

PREFACE

For a number of years, I have thought it would be desirable to have available a comprehensive review of the literature of the many subjects that now comprise forensic serology, immunology and biochemistry. My appointment as a Visiting Fellow in the National Institute of Law Enforcement and Criminal Justice (now the National Institute of Justice) in 1976 afforded me the opportunity to prepare this review. I trust that the product may be a useful reference work for forensic serologists working in various laboratories, particularly in this country.

I have taken a more or less historical approach to each of the major subjects, in part because I thought it would provide continuity, and in part because I thought it would be more interesting. Accordingly, the different subject areas are discussed from the time of their origins in the published literature up to the present time. Much of the material is now of purely historical interest, and does not represent the current understanding of the subjects. I hope that the distinctions between older notions of purely historical interest, and current ones, have been clearly made.

There are many excellent reviews of the subjects covered here by specialists in those fields. They treat the various topics more comprehensively and better than I have been able to do, and I have cited them in the reference lists. In this work, I have attempted to treat all the subjects of interest in present-day forensic serology, and to combine the historical developments, the essential background information, and the forensic applications under the same cover.

This work has been entitled a "sourcebook", because it is quite simply a narrative review of the scientific literature. Because I regard this book primarily as a guide to the published literature, careful attention has been paid to the accuracy of the reference lists which appear at the end of each unit.

The book is divided into a total of nine units. The first unit consists of background material in serology, immunology, biochemistry, genetics and methods that are employed in the field. I was persuaded that this material should be included, and that it might serve a useful purpose. Units II and III have to do with the identification of blood and body fluids, respectively, and Unit IV has to do with species determination. These make up most of the identification sections. Units V, VI and VII have to do with the different classes of genetic markers in blood and body fluids, and make up most of the individualization sections. Unit VIII is concerned with the sexing of bloodstains, and with efforts to individualize blood using non-genetic markers. Unit IX consists of a set of translations of original papers of historical interest in the field. The rationale for the translations set is discussed in the Preface to that unit, which is self-contained. The eight units of the sourcebook are further divided into sections and subsections.

References are compiled at the end of each unit. Because of the large number of references, some consistent bibliographic style had to be selected, and in arriving at these conventions, I have made an effort to provide as much information as possible for readers who wish to find particular references. An effort was made to consult every reference which is cited here. References which could not be examined have a notation of the source that was used. These are indicated as "cited by" or "through". If another reference contains similar information to the one cited, or an abstract of it, I have indicated this fact with the words "and see".

The A.I.B.S. convention has been followed in citing all the references [Council of Biological Editors, Committee on Form and Style: *CBE Style Manual*, 3rd ed., American Institute of Biological Sciences, Washington, D.C., 1972]. References are cited in the text by the name(s) of the author(s) and the year the paper was published. The use of the name(s) and year as part of a sentence constitutes a citation. Papers written by more than two authors are cited in the text by the last name of the first author, and "*et al.*", followed by the year. In cases where the same author(s) wrote

several papers in the same year, they are distinguished in the text and in the reference lists by lower case arabic letters, e.g. 1971a, 1971b, etc. In some cases, a senior author with two or more coauthors, not always the same people, wrote more than one paper in a given year. The year and lower case letter convention is used to distinguish these, even though the full list of names on the papers is not the same. Thus, for example, if A. Smith, B. Jones and C. Williams wrote a paper in 1960, and A. Smith, B. Jones, C. Johnson and D. Williams had another paper in the same year, the former would be cited in the text as "Smith *et al.*, 1960a", the latter as "Smith *et al.*, 1960b". The arabic letters are used in the reference list as well as in the text in these cases. The reference lists are in strict alphabetical order by first letter of last name of first author, including institutional authors. Editorials are cited as "Editorial", unless they were signed, and it was clear who wrote them. In the older literature, first name(s) or initial(s) of authors were not always given. There was a tendency to use titles. Authors' initials which are given in parentheses in the reference lists were supplied, and did not appear in the original article. Titles of articles are given in full in the original language, except in cases where the original language does not use the Latinic alphabet. I have tried to retain accent and diacritical marks in citing authors' names and article titles. Russian and Japanese journals generally provide an English translation of the names of authors and the title of the article. I have usually given these in English. Transliteration of author names and article titles from sources in languages using Cyrillic alphabets follow the *U.S. Government Printing Office Style Manual* (1973). Abbreviations of journal titles have been taken from *Bibliographic Guide for Editors and Authors*, American Chemical Society, Washington, D.C., 1974, or from *BIOSIS List of Serials*, BioSciences Information Service of Biological Abstracts, Philadelphia, PA, 1976. In cases where these sources did not provide a standard abbreviation, I have followed the guidelines given in ANSI Standard Z39.5-1969 (R1974) of the American National Standards Institute in arriving at the usage which appears.

In some libraries, foreign journals are catalogued according to their foreign titles. Where I encountered this practice, footnotes were added to the reference lists giving the appropriate information. Similarly, many journals have undergone title changes over the years, many have been superseded by other journals, and some have been divided up into a number of separate parts, and so forth. In cases where I thought these changes might cause difficulty in locating an article in a library, I have added explanatory bibliographic footnotes. The principal Russian medicolegal journal Судебно-медицинская Экспертиза is uniformly cited in the reference lists as "Sud. Med. Ekspert.". Journal title abbreviations are set in italic type, and volume numbers are in boldface type. In many cases, journals have been issued in several series over the years. Sometimes, the original volume numbering was dropped when a new series was issued, but in other cases it was retained. The series in which the cited volume number appeared is given in parentheses following the volume number. "N.S." means "new series" and this series is always the second one. In German language journals, the word "Folge" indicates a series; thus, "N.F." means "neue Folge", "3F" means third "Folge", and so on. If the original volume number was retained in the journal, even though a subsequent series designation was being used, both designations are given. For example, "21 (2 ser. 6)" means that the piece is the 21st volume of the journal, and is also the 6th volume of the second series. An arabic numeral in parentheses following the volume number is the *number* of the journal within the particular volume (or over-all). Thus, "14(12)" indicates volume 14, number 12. I included this in some cases because it was common in the older literature to cite references by number only, rather than by volume and page number. Thus an author might cite "*Berl. Klin. Wochenschr.*, 1906, No. 6". I would cite this reference as "*Berl. Klin. Wochenschr.* 43(6): pages". In this way, a reader could verify that the two papers were the same, though cited differently. Full pagination for each article has been given as called for by the A.I.B.S. convention. A single page number indicates that the reference occupies only one page. Deviations from these conventions are in the direction of giving more information about the reference. I hope that the use of well defined conventions, and explanatory footnotes where they seem to be necessary, will help readers to find references in which they are interested more easily than I was able to do in many cases.

Papers in the reference lists marked with the symbol ¶ have been translated into English as part of the translations set, which appears as Unit IX.

The term "substrate" is sometimes used in forensic serology to mean the object or material upon which a stain was deposited. The term also has the technical biochemical meaning of the reactant(s) in enzyme-catalyzed reactions. I have restricted the use of "substrate" to the biochemical meaning. Objects or materials upon which stains have been deposited are "substrata" (singular: "substratum").

In many of the respective sections dealing with genetic marker systems, I have compiled as much U.S. population data as I could find with a reasonable amount of effort. Some criteria had to be used in selecting and presenting this data. Since this book was prepared with forensic serologists in this country in mind, I have included only U.S. population data. I also decided, arbitrarily, not to include any data published before 1950. The data are presented in tables in essentially the same form as given by the original author(s). The only additions I have made are percentages of individuals representing various phenotypes, in cases where the author(s) gave only numbers. I have not tried to calculate numbers if the author(s) presented percentages. The population sampled is described in the terms used by the original author(s). At the present time, the single, most comprehensive reference work on population data ever compiled is the 1976 edition of *The Distribution of the Human Blood Groups and Other Polymorphisms*, by Mourant, Kopeć and Domaniewska-Sobczak (cited in the text as Mourant *et al.*, 1976). No one seriously interested in human blood group population data can do without this reference. In the older literature, a comprehensive tabulation was prepared by W.C. Boyd in 1939. ABO and MN frequencies for many of the world's populations which had been studied were given.

Because this book took considerably longer to complete than was originally anticipated, some more recent references may be cited in later units, and not in earlier ones, even though they contain information on the subjects covered in both places. I have made some effort to remedy this problem in revision, but may not have succeeded entirely.

A large number of people have been helpful to me in many different ways in the course of this project. I take pleasure in acknowledging their help and assistance in the remainder of the preface. I am grateful to the following for granting their kind permission to use material from figures and tables in published sources: American Association for the Advancement of Science [publishers of *Science*]; Dr. V. A. McKusick; W. B. Saunders & Co.; Elsevier Sequoia, S.A.; Prof. Dr. Hiroshi Hirose in Japan; Interscience Publishers, Division of John Wiley & Sons; and Rutgers University Press.

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As the project was largely bibliographic, I could not have managed it without the assistance of many people associated with various libraries I used. Morton Goren and Lavonne Wienke of the LEAA Library were very helpful in obtaining interlibrary loan materials. I owe a special debt of thanks to Mr. Albert Berkowitz and his staff at the National Library of Medicine in Bethesda. They provided me with space to work, and a most congenial environment in which to do so, for more than two years time. The NLM staff treated me as a colleague during my stay in the library. I would particularly thank Doralee Agayoff, Jeanne Crosier, Edith Blair, Paula Strain, Maxine Henke, Peggy Beavers, Richard Mumford, John Broadwyn, Dr. Stephen Kim, Charlotte Kenton, and Dorothy Hanks. All of them went out of their way to assist me in

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I reserve very special praise and gratitude for Maureen Swift and for Danice Gomien. Ms. Swift typed the entire manuscript, in some cases more than once. Ms. Gomien prepared all the figures and tables. Both of them navigated hundreds of pages of difficult material skillfully and well, somehow managing to make sense out of the many curious symbols and usages that are found in this field. There is no doubt that the work could not have been completed but for their continuing cooperation and perseverance.

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CONTENTS

PREFACE	v
UNIT I. BACKGROUND MATERIAL	
SECTION 1. BACKGROUND MATERIAL IN BIOCHEMISTRY, GENETICS AND IMMUNOLOGY	
1.1 Biochemistry	1
1.1.1 Classes of Biologically Important Organic Compounds	1
1.1.1.1 Carbohydrates	1
1.1.1.2 Lipids	1
1.1.1.3 Amino Acids and Proteins	11
1.1.1.4 Nucleotides and Nucleic Acids	12
1.1.2 Proteins	12
1.1.2.1 Protein Structure	12
1.1.2.2 Protein Purification	14
1.1.2.3 Estimation of Protein	15
1.1.2.4 Criteria of Purity and Molecular Weight Determination	16
1.1.3 Enzymes	16
1.1.3.1 Introduction	16
1.1.3.2 Enzyme Nomenclature	16
1.1.3.3 Kinetics of Enzyme Catalyzed Reactions	16
1.1.3.4 Enzyme Catalyzed Reactions and Cofactors	19
1.1.4 Metabolism	20
1.2 Genetics	20
1.2.1 Introduction	20
1.2.2 Gene Action at the Biochemical Level	20
1.2.2.1 Development of the One Gene-One Enzyme Hypothesis— The Beginning of Present Day Understanding	20
1.2.2.2 Evidence that DNA is the Genetic Material	21
1.2.2.3 Structure of DNA and RNA	21
1.2.2.4 Replication of DNA	23
1.2.2.5 RNA, Protein Synthesis and the Genetic Code	23
1.2.3 Chromosomes	25
1.2.3.1 Mitosis	26
1.2.3.2 Meiosis	26
1.2.3.3 Human Chromosomes and Sex Determination	26
1.2.4 Patterns of Inheritance	27
1.2.4.1 Simple Patterns	27
1.2.4.2 Variable Expressivity, Codominance and Multiple Allelic Systems	30
1.2.4.3 Linkage, Crossing Over and Genetic Mapping	32
1.2.4.4 Sex-Related Inheritance	33
1.2.5 Mutation	38
1.2.6 Polymorphism	38
1.2.7 Methods in Human Genetics	39
1.2.8 Population Genetics [written with Dr. Peter Shenkin, Dept. of Mathematics, John Jay College of C.U.N.Y.]	39
1.2.8.1 Hardy-Weinberg Equilibrium	39
1.2.8.2 Significance of Marker Systems	41
1.3 Immunology and Serology	43
1.3.1 Introduction	43
1.3.2 Antigens	43

1.3.2.1 Nature of Antigens	43
1.3.2.2 Conditions of Antigenicity	43
1.3.2.3 Types of Antigens	44
1.3.2.4 Antigen Specificity and the Nature of the Antigenic Determinant	44
1.3.3 Antibodies	45
1.3.3.1 Formation of Antibodies—The Immune Response	45
1.3.3.2 Types and Structure of Antibody Molecules	45
1.3.4 Antigen-Antibody Reactions	48
1.3.4.1 Agglutination	49
1.3.4.2 Precipitation	53
1.3.4.3 Radioimmunoassay	54
1.3.5 Complement and Complement-Mediated Reactions	54
1.3.5.1 Introduction	54
1.3.5.2 Nature and Properties of Complement	54
1.3.5.3 Complement Fixation	55
1.3.6 Hypersensitivity	55
1.3.6.1 Immediate Hypersensitivity	56
1.3.6.2 Delayed Hypersensitivity	56
SECTION 2. SURVEY OF SELECTED METHODS	
2.1 Introduction	57
2.2 Immunodiffusion	57
2.2.1 Single Immunodiffusion	57
2.2.2 Double Immunodiffusion	57
2.3 Electrophoresis	58
2.3.1 Introduction	58
2.3.2 Factors Influencing Migration and Separation	58
2.3.3 Paper Electrophoresis	59
2.3.4 Starch Gel Electrophoresis	59
2.3.5 Agar Gel Electrophoresis	60
2.3.6 Cellulose Acetate Electrophoresis	60
2.3.7 Polyacrylamide Gel Electrophoresis	61
2.3.7.1 Polyacrylamide Disc Gel Electrophoresis	61
2.3.7.2 Polyacrylamide Flat Gel Electrophoresis	62
2.4 Immunoelectrophoresis	62
2.4.1 Simple Immunoelectrophoresis	62
2.4.2 Some Variations of Immunoelectrophoresis	62
2.4.3 Quantitative Immunoelectrophoresis	63
2.4.3.1 Rocket Electrophoresis	63
2.4.3.2 Crossed Immunoelectrophoresis	63
2.4.3.3 Other Methods of Quantitative Immunoelectrophoresis	65
2.5 Isoelectric Focusing and Isotachopheresis	65
2.5.1 Isoelectric Focusing	65
2.5.2 Isotachopheresis	65
UNIT II. IDENTIFICATION OF BLOOD	
SECTION 3. HISTORY AND DEVELOPMENT OF MEDICOLEGAL EXAMINATION OF BLOOD	
SECTION 4. CRYSTAL TESTS	
4.1 Structure and Nomenclature of Porphyrins and Hematin Compounds	77
4.2 Crystal Tests	79
4.2.1 Introduction	79
4.2.2 Hematin Crystal Tests	80
4.2.3 Acetone Chlorhemin Crystal Test	85
4.2.4 Hemochromogen Crystal Tests	85

SECTION 5. SPECTRAL AND MICROSCOPICAL METHODS	
5.1 Spectroscopic and Spectrophotometric Methods	89
5.2 Spectrofluorimetric Methods	94
5.3 Microscopical Methods	94
5.3.1 Blood Identification by Microscopical Techniques	94
5.3.2 Biological Stains and Dyes	97
SECTION 6. CATALYTIC TESTS	
6.1 Guaiacum Test	101
6.2 Aloin Test	103
6.3 Phenolphthalin Test	103
6.4 Benzidine Test	105
6.5 Leucomalachite Green and Leucocrystal Violet Tests	108
6.6 Other Catalytic Tests	109
6.6.1 Peroxide	109
6.6.2 Eosin	109
6.6.3 2,7-Diaminofluorene	109
6.6.4 Rhodamine B	109
6.6.5 p-Phenylenediamine	110
6.6.6 o-Tolidine and o-Toluidine	110
6.6.7 o-Dianisidine	110
6.6.8 Amidopyrine	110
6.6.9 Benzyldine Dimethylaniline	111
6.6.10 3,3',5,5'-Tetramethylbenzidine	111
6.6.11 Chlorpromazine	111
6.6.12 Diphenylamine	112
6.6.13 Fluorescein	112
6.7 Luminol Test	112
6.8 Catalytic Tests—General Consideration	114
SECTION 7. OTHER TESTS	
7.1 Immunological Tests with Anti-human Hemoglobin Sera	117
7.2 Chromatographic Methods	119
7.3 Electrophoretic Methods	120
7.4 Heating Test	120
SECTION 8. IDENTIFICATION OF BLOOD FROM PARTICULAR SOURCES	
8.1 Identification of Menstrual Blood	121
8.1.1 Microscopical and Histological Methods	121
8.1.2 Methods Based on Fibrinolytic Properties	122
8.1.3 Immunological Methods	124
8.1.4 Methods Based on Menstrual Blood Toxicity	124
8.1.5 Determination of LDH Isoenzymes	125
8.2 Identification of Retroplacental Blood, Blood Shed at Parturition and in Abortion, and the Forensic Diagnosis of Pregnancy	126
8.2.1 Methods Based on Pregnancy Hormones	126
8.2.2 Methods Based on Pregnancy-Associated Proteins	127
8.2.3 Methods Based on Leucine Aminopeptidase and Cystine Aminopeptidase	128
8.2.4 Methods Based on Alkaline Phosphatase	128
8.3 Identification of Fetal Blood and Blood from Children	129
8.3.1 Fetal Hemoglobin	129
8.3.2 Methods Based on α -Fetoprotein	129
8.3.3 Miscellaneous Methods	130
SECTION 9. DETERMINATION OF THE AGE OF BLOODSTAINS	
	131

UNIT III. IDENTIFICATION OF BODY FLUIDS

SECTION 10. IDENTIFICATION OF SEMEN

10.1 Introduction	149
10.2 Detection and Identification of Spermatozoa	150
10.2.1 Isolation and Identification of Spermatozoa from Seminal Stains	150
10.2.2 Survival of Spermatozoa in the Vagina	152
10.2.3 Spermatozoan Morphology—Medicolegal Implications	154
10.3 Seminal (Prostatic) Acid Phosphatase and Vaginal Acid Phosphatase	155
10.3.1 Introduction	155
10.3.2 Seminal Acid Phosphatase Detection for Medicolegal Semen Identification	155
10.3.3 Persistence of Acid Phosphatase	160
10.3.4 Acid Phosphatase Assay Techniques and Activity Units	162
10.3.5 Specificity of the Acid Phosphatase Test—The Problem of Vaginal Acid Phosphatase	163
10.3.6 Purification, Properties and Molecular Heterogeneity of Prostatic Acid Phosphatase	166
10.3.7 Identification of Vaginal Secretions	168
10.4 Immunological Methods	169
10.4.1 Precipitin Tests	169
10.4.2 Other Immunological Methods	171
10.5 Crystal Tests	172
10.5.1 Florence Test	172
10.5.2 Barberio Test	173
10.5.3 Puranen's Test	174
10.5.4 Other Crystal Tests	174
10.6 Chromatographic and Electrophoretic Methods	174
10.7 Creatine Phosphokinase	175
10.8 Lactic Dehydrogenase-X Isoenzyme	176
10.9 Sperm and Seminal Fluid Esterases	176
10.10 γ -Seminoprotein	177
10.11 Other Methods	177
10.12 Seminal Stain Fluorescence	178
10.13 The Composition of Semen	178
10.13.1 Sperm Cell Antigens	179
10.13.2 Seminal Plasma Proteins and Antigens	179
10.13.3 Enzymes and Low Molecular Weight Components	180
10.14 Seminal Protein p30	180

SECTION 11. IDENTIFICATION OF SALIVA

11.1 Identification of Inorganic Ions	183
11.1.1 Thiocyanate	183
11.1.2 Nitrite	183
11.2 Alkaline Phosphatase	183
11.3 Amylase	184
11.3.1 Application of Amylase Detection to Saliva Stain Identification	184
11.3.2 Some Properties of Amylase and Starch	187
11.4 Immunological Methods	187
11.5 Microscopical Methods	189
11.6 Fluorescence of Saliva Stains under Ultraviolet Light	189

SECTION 12. IDENTIFICATION OF URINE

12.1 Microscopical Methods, Ultraviolet Light and Odor	191
12.2 Inorganic Ions	191
12.3 Urea	191

12.4 Creatinine	193
12.5 Indican	193
12.6 Chromatographic Methods.....	194
12.7 Immunological Methods	194
SECTION 13. IDENTIFICATION OF FECAL MATTER	197
SECTION 14. IDENTIFICATION OF OTHER BODY FLUIDS AND SECRETIONS	199
UNIT IV. DETERMINATION OF SPECIES OF ORIGIN	
SECTION 15. OLDER METHODS	
15.1 Introduction	215
15.2 Chemical Methods.....	215
15.3 Micrometric Methods	215
SECTION 16. IMMUNOLOGICAL METHODS FOR BLOOD AND BLOODSTAINS	
16.1 The Precipitin Test	221
16.1.1 Development of the Precipitin Test and Its Medicolegal Application	221
16.1.2 More Recent Developments—Gel Methods	224
16.1.3 Effects of Some External Influences.....	225
16.1.4 Tests with Anti-human Hemoglobin Sera.....	227
16.1.5 The Antigen-Antibody Reaction—Optimization of Reactant Concentrations	227
16.2 Anti-human Globulin Serum Inhibition Technique.....	227
16.3 Passive Hemagglutination Techniques	229
16.4 Mixed Antiglobulin Technique	231
16.5 Sensitized Particle Techniques.....	231
16.5.1 Sensitized Colloidon Particles.....	231
16.5.2 Sensitized Latex Particles	233
16.6 Other Immunological Methods	233
16.6.1 Complement Fixation Tests.....	233
16.6.2 Anaphylaxis (Hypersensitivity) Tests.....	234
16.6.3 Hemolysins	235
16.6.4 Agglutinins	236
16.6.5 Serum-Hemoglobin Precipitation.....	236
16.6.6 Phytoprecipitation and Phyttagglutination Methods.....	237
16.6.7 Gamma-globulin Deviation	237
16.6.8 Fluorescent Antibody Techniques.....	237
16.7 Immunoelectrophoresis	238
16.8 Cross Reactions of Antisera and the Problem of Closely Related Species Differentiation	238
16.9 Serum Protein Structure and Phylogeny—Taxonomic Serology and Immunology	241
SECTION 17. OTHER METHODS FOR SPECIES DETERMINATION OF BLOOD AND BLOODSTAINS, BODY FLUIDS AND TISSUES	
17.1 Differential Denaturation of Hemoglobin with Alkali	243
17.2 The Fibrin Plate Method	243
17.3 Hemoglobin Separation by Chromatography or Electrophoresis.....	244
17.4 Isoenzyme Patterns	244
17.5 Species Diagnosis in Other Body Fluids and in Tissues.....	244
UNIT V. BLOOD GROUPS	
SECTION 18. INTRODUCTION TO THE FORENSIC APPLICATION OF GENETIC MARKER SYSTEMS TO IDENTIFICATION AND DISPUTED PARENTAGE PROBLEMS	
	257

SECTION 19. THE ABO AND SECRETOR SYSTEMS

19.1	Origins and Earlier Studies	261
19.2	Inheritance of the ABO Blood Groups	264
19.3	Subgroups in the ABO System	266
19.3.1	Subgroups of A	266
19.3.1.1	A ₁ and A ₂ Subgroups	266
19.3.1.2	Subgroup A ₃	268
19.3.1.3	Further Subgroups of A	268
19.3.1.4	So-called Intermediate A (A _{int} ; A _i)	269
19.3.1.5	Additional Subgroups of A	269
19.3.1.6	Quantitative Approaches	269
19.3.2	Variants of B	270
19.4	Antibodies of the ABO System	270
19.4.1	Anti-A and Anti-B	270
19.4.2	Anti-H and "Anti-O"	272
19.4.3	Isoagglutinins in Body Fluids Other Than Serum	273
19.5	Quantitative and Physicochemical Approaches	274
19.6	The Bombay Phenotype	275
19.7	Some Other Complexities in the ABO System	276
19.7.1	So-called "Cis-AB"	276
19.7.2	Cross Reacting Anti-A-Like and Anti-B-Like Antibodies in Group O Serum—Blood Group C	276
19.7.3	Acquired B	279
19.8	The Secretor System	280
19.8.1	Group Specific Substances in Body Fluids	280
19.8.2	Inheritance of the Secretor Characteristic	281
19.8.3	Further Studies on Group Substances in Body Fluids	282
19.8.3.1	Saliva	282
19.8.3.2	Seminal Plasma and Spermatozoa	284
19.8.4	Inhibition Tests for Group Substances in Body Fluids	285
19.9	Biochemical Studies on the ABO System	286
19.9.1	Early Studies	286
19.9.2	Chemical Nature of the Blood Group Substances	287
19.9.3	Biochemical Genetics of the ABO, Secretor and Lewis Systems	290
19.10	Medicolegal Applications	293
19.10.1	Introduction and Disputed Parentage Testing	293
19.10.1.1	General Introduction	293
19.10.1.2	Early Developments in the Medicolegal Application of Blood Groups	296
19.10.1.3	Disputed Parentage	296
19.10.2	Early Studies on Grouping Bloodstains	297
19.10.3	Further Developments—Bloodstain Grouping Methods	298
19.10.3.1	Detection of Isoagglutinins—Lattes Test	298
19.10.3.2	Detection of Agglutinogens in Bloodstains by Absorption Technique—Absorption-Inhibition or Agglutinin Binding	300
	• Development of the Technique	300
	• Sensitivity	301
	• The Use of O (Anti-A,B) Sera	301
	• Interpretation	302
	• Inhibition With a Doubling Dilution Titration Series of Antiserum	302
	• The Problem of Nonspecific Absorption and Interference Due to Contamination	302
	• Lectins	304
	• Testing of the Immunoglobulins in Antisera	305
	• Gamma Globulin Deviation Procedure	305

19.10.3.3	Detection of Agglutinogens in Bloodstains by Inhibition of Hemolysis	305
19.10.3.4	Detection of Agglutinogens in Bloodstains by Absorption-Elution Technique.....	305
	• Development of the Technique.....	305
	• Further Modifications	307
	• Sensitivity	307
	• Interference by Adventitious Substances	308
	• Selection and Evaluation of Antisera for Elution	308
	• Reliability and Specificity.....	310
	• Other Methods of Eluting Antibodies.....	310
19.10.3.5	Detection of Agglutinogens in Bloodstains by Mixed Agglutination Technique	310
19.10.3.6	Detection of Agglutinogens in Bloodstains by Fluorescent and Ferritin Labelled Antibody Technique	312
19.10.3.7	Use of Formalin Treated Red Cells	312
19.10.3.8	Reversible Agglomeration Technique.....	312
19.10.4	Determination of A Subgroups in Bloodstains	312
19.10.5	ABO Grouping in Body Fluids and Tissues	313
19.10.5.1	Introduction	313
19.10.5.2	Methods of ABO Grouping in Body Fluids and Tissues	313
	• Inhibition.....	313
	• Mixed Agglutination	314
	• Elution.....	314
	• Other Techniques.....	315
19.10.5.3	ABO Grouping of Different Body Fluids and Tissues	315
19.10.5.4	Problems in the Grouping of Body Fluids and Tissues.....	316
19.10.5.5	Fractionation of Soluble ABH Substances from Secretor Fluids (and Red Cells)	319
19.10.6	Factors Affecting the Success of Medicolegal Blood Group Determinations	320
19.10.7	ABO Grouping of Hair Nails and Teeth.....	320
19.10.7.1	ABO Grouping of Hair.....	320
19.10.7.2	ABO Grouping in Nails and Teeth.....	321
19.11	The Distribution of ABO Groups and Secretors in U.S. Populations	322
SECTION 20. THE LEWIS SYSTEM		
20.1	Introduction	329
20.2	Discovery and Development	329
20.3	Lewis Antigens on the Red Cell	330
20.4	Lewis Substances in Saliva and Other Body Fluids	330
20.5	Some Complexities of the Lewis System	330
20.5.1	Antigens of Le(a-b-) Red Cells.....	330
20.5.2	Le ^a	331
20.5.3	Anti-A ₁ Le ^b	331
20.6	Lewis Antisera	331
20.6.1	Anti-Le ^a	331
20.6.2	Anti-Le ^b	331
20.6.3	Other Lewis Antisera.....	333
20.7	Theories About the Lewis System.....	333
20.8	Biochemical Studies.....	333
20.9	Medicolegal Applications.....	333
20.10	Distribution of Lewis Phenotypes in U.S. Populations	333
SECTION 21. THE MNSs SYSTEM		
21.1	Discovery and Mode of Inheritance of the MN Blood Groups	335
21.2	The S and s Factors	335

21.3 Recombination and Mutation in the MNSs System.....	335
21.4 The Variant S ^u —The Problem of U	337
21.5 Complexities of the MN System	337
21.5.1 Variants of M and N	337
21.5.2 Other Associated Antigens	338
21.6 Antisera to MNSs Antigens.....	338
21.7 Heterozygous Advantage and MN	338
21.8 Biochemical Studies on the MN System	338
21.8.1 Introduction.....	338
21.8.2 The Thomsen Phenomenon	339
21.8.3 The Nature of the MN Receptors	339
21.9 Medicolegal Applications	342
21.9.1 Introduction—Disputed Parentage Applications	342
21.9.2 Detection of MN in Bloodstains.....	343
21.9.3 More Recent Developments in MN Grouping in Bloodstains	344
21.9.4 The Problem of Anti-N Cross Reactivity	344
21.9.5 The Detection of Ss and Other Antigens in Bloodstains.....	345
21.10 Distribution of MNSs Phenotypes in U.S. Populations	346
SECTION 22. THE Rh SYSTEM	
22.1 Introduction	349
22.2 Discovery and Development of the Rh System	349
22.3 Rh Nomenclature	350
22.4 The Incomplete Rh Antibody.....	352
22.5 Complexities of the Rh System—Further Rh Factors	353
22.5.1 Subdivisions of D or Rh ₀	353
22.5.2 Variations in C or rh'.....	353
22.5.3 Variations in E or rh'' and e or hr''	354
22.5.4 Compound or Complex Antigens and/or Antisera.....	354
22.5.5 The LW Antigen	355
22.5.6Suppressions, Deletions and Modifiers.....	356
22.6 Inheritance of the Rh Factors and Further Nomenclature	
Considerations	357
22.6.1 Rh Inheritance	357
22.6.2 The Numerical System of Nomenclature	357
22.7 Biochemical Studies on the Receptors—Biochemical Genetics.....	358
22.7.1 The Number of Rh Antigenic Sites on Red Cells	358
22.7.2 Relationship of the Rh Antigens to the Erythrocyte Membrane	359
22.7.3 Isolation and Purification of the Rh Antigens	359
22.7.4 Biochemical Genetics of Rh	361
22.8 Medicolegal Applications of Rh.....	361
22.9 Distribution of Rh Phenotypes in U.S. Populations	364
SECTION 23. THE KELL, DUFFY AND KIDD BLOOD GROUP SYSTEMS	
23.1 Kell System	369
23.1.1 The K and k Antigens	369
23.1.2 Complexities of the Kell System.....	369
23.1.3 Numerical Notation and Nomenclature for the Kell System	370
23.1.4 Genetics of the Kell System	370
23.1.5 Kell Antibodies	371
23.1.6 Medicolegal Applications of Kell.....	371
23.2 Duffy System	372
23.2.1 Discovery and Development	372
23.2.2 Extension of the Duffy System	373
23.2.3 Other Aspects of the Duffy System	373
23.2.4 Medicolegal Applications.....	373
23.3 Kidd System.....	373

23.3.1	Discovery and Development	373
23.3.2	The Jk(a—b—) Phenotype	374
23.3.3	Kidd Antibodies	374
23.3.4	Medicolegal Applications.....	375
23.4	Distribution of Kell, Duffy and Kidd Phenotypes in U.S. Populations	375
SECTION 24. THE P AND LUTHERAN SYSTEMS		
24.1	P System.....	381
24.1.1	Discovery and Development	381
24.1.2	Extension of the P System.....	381
24.1.3	Blood Factor Q.....	383
24.1.4	Additional Notes about the P System	383
24.1.5	Biochemical Studies on the P System	383
24.1.6	Medicolegal Applications.....	385
24.2	Lutheran System	386
24.2.1	Discovery and Development	386
24.2.2	The Lu(a—b—) Phenotype.....	387
24.2.3	Extension of the Lutheran System	387
24.2.4	Development of Lutheran Antigens	387
24.2.5	Medicolegal Applications.....	387
SECTION 25. SOME OTHER BLOOD GROUP SYSTEMS		
25.1	Introduction	389
25.2	The Ii System.....	389
25.3	Diego	389
25.4	Yt.....	390
25.5	Auberger.....	390
25.6	Dombrock.....	390
25.7	Colton	390
25.8	Sid and Cad	390
25.9	Some General Considerations on Blood Groups	391
UNIT VI. ISOENZYMES		
SECTION 26. INTRODUCTION TO ISOENZYMES.....		423
SECTION 27. PHOSPHOGLUCOMUTASE		
27.1	Recognition of PGM.....	425
27.2	PGM Polymorphism	425
27.2.1	PGM ₁	425
27.2.2	PGM ₂	426
27.2.3	PGM ₃	427
27.2.4	Linkage Relationships of the PGM Loci.....	427
27.3	Biochemical Studies on PGM Isoenzymes	427
27.3.1	Properties of the PGM Enzyme	427
27.3.2	Studies on the Enzymes Produced by the Different PGM Loci	429
27.4	Medicolegal Applications	430
27.4.1	Disputed Parentage	430
27.4.2	PGM Grouping in Bloodstains	431
27.4.2.1	Development of Methods	431
27.4.2.2	Survival of PGM Isoenzymes in Blood.....	432
27.4.2.3	Problems in PGM Grouping of Blood	433
27.4.3	PGM Grouping of Semen, Vaginal Secretions and Other Tissues	433
27.5	Distribution of PGM Phenotypes in U.S. Populations	435
SECTION 28. ADENYLATE KINASE		
28.1	Recognition of Adenylate Kinase	439
28.2	AK Polymorphism	439

28.2.1	<i>AK</i> ₁	439
28.2.2	Additional AK Loci—Linkage Relations	440
28.3	Biochemical Studies on AK	440
28.4	Medicolegal Applications	441
28.4.1	Disputed Parentage	441
28.4.2	AK Phenotyping in Bloodstains	441
28.4.3	Survival of AK in Blood and Bloodstains	442
28.4.4	AK Phenotyping in Other Tissues	442
28.5	Distribution of AK Phenotypes in U.S. Populations.....	442
SECTION 29. ERYTHROCYTE ACID PHOSPHATASE		
29.1	Recognition of Acid Phosphatase in Blood.....	445
29.2	ACP Polymorphism.....	445
29.3	Additional Genetic Loci Determining Acid Phosphatase	
	Enzymes—Tissue Acid Phosphatases	446
29.3.1	Human Tissue Acid Phosphatase Isoenzymes.....	446
29.3.2	Some Comparisons of the Various Acid Phosphatases	448
29.3.3	Linkage Relations of the ACP Loci.....	449
29.4	Studies on the <i>ACP</i> ₁ Isoenzymes (EAP Isoenzymes).....	449
29.5	Medicolegal Applications	450
29.5.1	Disputed Parentage	450
29.5.2	ACP Phenotyping in Dried Bloodstains.....	450
29.5.3	Methods of Phenotyping ACP Isoenzymes	452
29.5.4	Red Cell ACP Phenotyping in Aged Whole Blood Samples.....	453
29.6	Distribution of <i>ACP</i> ₁ Phenotypes in U.S. Populations	453
SECTION 30. ADENOSINE DEAMINASE		
30.1	Recognition of Adenosine Deaminase.....	457
30.2	ADA Polymorphism in Human Red Cells.....	457
30.3	ADA Isoenzymes in Other Tissues	458
30.4	Purification and Properties of Red Cell ADA	460
30.4.1	Detection and Assay of ADA	460
30.4.2	Studies on Red Cell ADA.....	460
30.5	Medicolegal Applications	461
30.5.1	Disputed Parentage	461
30.5.2	ADA Phenotyping in Dried Bloodstains	461
30.5.3	ADA Phenotyping in Other Tissues.....	462
30.6	Distribution of ADA Phenotypes in U.S. Populations	462
SECTION 31. ESTERASES		
31.1	Introduction to Esterases	463
31.2	Cholinesterases.....	463
31.2.1	Recognition and Classification of Cholinesterases	463
31.2.2	Red Cell Acetylcholinesterase (E.C. 3.1.1.7)	463
31.2.3	Plasma (Serum) Cholinesterase (ChE; Pseudocholinesterase; PCE; E.C. 3.1.1.8; acylcholine acyl-hydrolase)	464
31.2.3.1	Early Studies on Plasma ChE	464
31.2.3.2	Genetically Controlled Variation in Plasma ChE	464
31.2.3.3	Further Genetic Variation in Plasma ChE.....	465
31.2.3.4	Electrophoretically Detectable Genetic Variation in Plasma ChE—A Second Plasma ChE Locus	465
31.2.3.5	Silent Alleles at <i>E</i> ₁	466
31.2.3.6	Molecular Heterogeneity of Plasma ChE— Biochemical Studies	467
31.2.3.7	Assay Methods for ChE—Detection of <i>E</i> ₁ Phenotype and Screening Techniques.....	468
31.3	Carboxylesterases.....	469
31.3.1	Classification of Carboxylesterases.....	469
31.3.2	Red Cell Carboxylesterases.....	469

31.3.3 Genetic Variation of the Red Cell A-Esterases	471
31.3.4 Esterase D and Its Polymorphism	471
31.3.5 Biochemical Studies on Esterases.....	471
31.3.6 Tissue Esterases—Carboxyl Esterase Classification and a Genetic Interpretation.....	472
31.4 Medicolegal Applications of the Esterases.....	473
31.4.1 Disputed Parentage	473
31.4.2 Plasma Cholinesterase Phenotyping in Bloodstains	473
31.4.3 ESD Phenotyping in Bloodstains	475
31.4.4 ESD Phenotyping in Other Tissues	475
31.5 Distribution of Cholinesterase and ESD Phenotypes in U.S. Populations	475
SECTION 32. CARBONIC ANHYDRASE	
32.1 Recognition of Carbonic Anhydrase	479
32.2 Multiple Forms of Carbonic Anhydrase.....	479
32.2.1 Recognition of CA Isoenzymes.....	479
32.2.2 Genetics and Nomenclature of CA Isoenzymes	480
32.3 Genetic Variation of CA.....	480
32.3.1 Genetic Variation at the CA_I Locus.....	480
32.3.2 Genetic Variation at the CA_{II} Locus	481
32.4 Biochemical Studies on the CA Isoenzymes	481
32.5 Red Cell CA Variation in Infrahuman Species—Phylogenetic Relationships	482
32.6 Medicolegal Applications	482
32.7 Distribution of CA_{II} Phenotypes in U.S. Populations	482
SECTION 33. GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND 6-PHOSPHOGLUCONATE DEHYDROGENASE	
33.1 Glucose-6-Phosphate Dehydrogenase (Glc-6PD; Gd; Zwischenferment; D-Glc-6P:NADP ⁺ 1-oxidoreductase; E.C. 1.1.1.49).....	483
33.1.1 Recognition of Glc-6PD.....	483
33.1.2 The Relationship of Glc-6PD and Hemolytic Anemia— Recognition of Genetic Variants in Glc-6PD	483
33.1.3 The Genetic Variants of Glc-6PD	484
33.1.4 X-linkage of the <i>Gd</i> Locus—X-Chromosome Inactivation	486
33.1.5 Biochemical Studies on Glc-6PD	486
33.1.6 Medicolegal Applications.....	487
33.1.7 Distribution of Common <i>Gd</i> Phenotypes in U.S. Black Populations...	487
33.2 6-Phosphogluconate Dehydrogenase	488
33.2.1 Recognition of PGD.....	488
33.2.2 Genetic Variants of PGD	488
33.2.2.1 Variants Exhibiting Normal Activity	488
33.2.2.2 Variants Exhibiting Deficiencies in Activity— <i>PGD</i> ^o and <i>PGD</i> [*] ..	490
33.2.2.3 The Nomenclature of PGD Variants	490
33.2.2.4 Linkage Relations and Chromosomal Localization of <i>PGD</i>	492
33.2.3 Biochemical Studies on PGD	492
33.2.4 Medicolegal Applications.....	492
33.2.5 Distribution of PGD Phenotypes in U.S. Populations	493
SECTION 34. GLYOXALASE I	
34.1 Recognition of Glyoxalase	495
34.2 Genetic Variation of Glyoxalase I.....	495
34.3 Assay and Detection Methods for GLO.....	495
34.4 Biochemical Studies on Glyoxalase I	496
34.5 Medicolegal Applications	496
34.5.1 Disputed Parentage	497
34.5.2 Electrophoretic Methods and Bloodstain Typing	497
34.6 Distribution of GLO Phenotypes in U.S. Populations	497

SECTION 35. GLUTAMIC-PYRUVIC TRANSAMINASE	
35.1 Recognition of GPT.....	499
35.2 Genetic Variation of GPT	499
35.3 Procedures for GPT Phenotyping	500
35.4 Biochemical Studies on GPT	501
35.5 Medicolegal Applications	502
35.5.1 Disputed Parentage	502
35.5.2 GPT Phenotyping in Bloodstains	502
35.5.3 GPT in Other Tissues	502
35.6 Distribution of GPT Phenotypes in U.S. Populations.....	502
SECTION 36. PEPTIDASES	
36.1 Introduction to Peptidases	503
36.2 Peptidases of Human Red Cells and Tissues—Multiple Genetic Loci Determining Peptidases.....	503
36.3 Detection of Peptidase Activity	504
36.4 Genetic Variation of the Peptidases	506
36.4.1 PEPA	506
36.4.2 PEPB	507
36.4.3 PEPC	507
36.4.4 PEPD	507
36.4.5 Linkage Relation of the <i>PEP</i> Loci.....	507
36.5 Biochemical Studies on the Peptidases	507
36.6 Medicolegal Applications	507
SECTION 37. OTHER ISOENZYME SYSTEMS	
37.1 Introduction	509
37.2 Alkaline Phosphatase	509
37.2.1 Alkaline Phosphatases of Serum and Tissues	509
37.2.2 Placental Alkaline Phosphatase (<i>PL</i>)	509
37.2.3 Other Notes on Alkaline Phosphatases	510
37.3 α -Amylase.....	510
37.3.1 Activity and Occurrence of α -Amylase	510
37.3.2 Genetics of Salivary and Pancreatic Amylases (<i>AMY</i> ₁ and <i>AMY</i> ₂) ...	510
37.3.3 Biochemical Studies on the Amylase Isoenzymes.....	511
37.4 Superoxide Dismutase.....	512
37.5 Galactose-1-Phosphate Uridyl Transferase	512
37.5.1 Metabolic Role of the Enzyme.....	512
37.5.2 Genetic Variation of GALT	513
37.5.2.1 Galactosemia	513
37.5.2.2 Further Genetic Variants of GALT	513
37.5.3 Population Studies on the GALT Variants—A Further Type of “Galactosemia” and a GALK Variant.....	514
37.5.4 Other Notes on GALT	515
37.6 Glucose Phosphate Isomerase.....	515
37.7 Glutathione Reductase	516
37.8 Glutathione Peroxidase	516
37.9 Hexokinase	516
37.10 Lactate Dehydrogenase.....	517
37.11 Pepsinogen (Pg).....	517
37.12 Uridine Monophosphate Kinase	518
37.13 Diaphorases	518
37.13.1 <i>DIA</i> ₁ and <i>DIA</i> ₂	518
37.13.2 <i>DIA</i> ₃ (Sperm Diaphorase).....	519
37.14 Phosphoglycolate Phosphatase	520

UNIT VII. HEMOGLOBIN, SERUM GROUP SYSTEMS, HLA AND OTHER GENETIC MARKERS

SECTION 38. HEMOGLOBIN

38.1 Introduction	545
38.2 Hemoglobin Structure	545
38.2.1 Normal Adult Hemoglobins	545
38.2.2 Normal Embryonic and Fetal Hemoglobins	547
38.2.3 Genetic Variants of Hemoglobin	549
38.2.3.1 Introduction	549
38.2.3.2 α Chain Variants	550
38.2.3.3 β Chain Variants	550
38.2.3.4 γ Chain Variants	550
38.2.3.5 Other Variants and Other Hemoglobins	552
38.2.3.6 Hemoglobin Nomenclature	553
38.3 Biochemical Genetics of Hemoglobin	554
38.4 Methods of Separating and Characterizing Hemoglobins	554
38.5 Medicolegal Applications	555
38.5.1 Disputed Parentage	555
38.5.2 Hb Typing in Bloodstains	555
38.6 Distribution of Common Hemoglobin Variants in U.S. Populations	558

SECTION 39. INTRODUCTION TO SERUM (PLASMA) PROTEINS..... 563

SECTION 40. HAPTOGLOBIN

40.1 Recognition of Haptoglobin (Hp)	569
40.2 Haptoglobin Physiology	569
40.3 Genetics and Biochemistry of Haptoglobins	569
40.3.1 Genetic Variation in Haptoglobin	569
40.3.2 Additional Genetic Variation at the <i>Hp</i> ¹ Locus—Haptoglobin “Subtypes”	570
40.3.3 Other Hp Variants and Hp 0	570
40.3.3.1 Quantitative Variants	570
40.3.3.2 Qualitative Variants	572
40.3.4 Structure of the Haptoglobins	573
40.3.5 Subunit and Polypeptide Chain Structure	575
40.3.6 Biochemical Genetics	575
40.4 Medicolegal Applications	577
40.4.1 Disputed Parentage	577
40.4.2 Haptoglobin Typing in Dried Bloodstains	577
40.5 Distribution of Hp Phenotypes in U.S. Populations	579

SECTION 41. GROUP SPECIFIC COMPONENT

41.1 Recognition—Genetic Variation	581
41.2 Further Gc Phenotypes	581
41.3 Additional Genetic Variation at the <i>Gc</i> ¹ Locus—Gc “Subtypes”	583
41.4 Methods of Phenotyping Gc	583
41.5 Physiological and Biochemical Studies on Gc	584
41.5.1 The Function and Properties of Gc Protein	584
41.5.2 Biochemical Studies	584
41.6 Medicolegal Applications	584
41.6.1 Disputed Parentage	584
41.6.2 Gc Phenotyping in Bloodstains	585
41.7 Distribution of Gc Phenotypes in U.S. Populations	586

SECTION 42. TRANSFERRIN (SIDEROPHILIN)

42.1 Introduction	589
42.2 Genetic Variation	589
42.3 Further Genetic Heterogeneity in Tf—Tf C Subtypes	590

42.4 Biochemical Studies on Transferrin	592
42.4.1 Structure of Transferrin	592
42.4.2 Structural Differences Among the Tf Variants	592
42.4.3 Metal Binding Properties	592
42.5 Medicolegal Applications	593
42.6 Distribution of Tf Phenotypes in U.S. Populations	593
SECTION 43. α_1 -ANTITRYPSIN (Pi)	
43.1 Introduction	595
43.2 Genetic Variation	595
43.2.1 Multiple Alleles Controlling α_1 -Antitrypsin	595
43.2.2 Quantitative Variation and Complete Deficiency	596
43.2.3 Refinements of Pi Typing Methods, Isoelectric Focusing, Pi M Subtypes and Other Variants	596
43.2.4 Standardization of Nomenclature and Techniques	597
43.3 Relationship of α_1 -Antitrypsin Deficiency and Disease	597
43.4 Biochemical Studies	597
43.5 Medicolegal Applications	598
43.6 Distribution of Pi Phenotypes	598
SECTION 44. GENETIC MARKERS OF THE IMMUNOGLOBULINS	
44.1 Introduction	601
44.2 Genetic Variation in the γ Chains of IgG—The Gm System	601
44.2.1 The Gm Factors	601
44.2.2 Assignment of Gm Factors to IgG Subclasses	604
44.2.3 Isoallotypic Markers of Immunoglobulins—The Nonmarkers	604
44.2.4 Gm Genetics	605
44.3 Light Chain Markers—The Km System	607
44.4 Other Immunoglobulin Markers	607
44.5 Determination of Immunoglobulin Types	607
44.6 Medicolegal Applications	608
44.6.1 Disputed Parentage	608
44.6.2 Gm and Km Typing in Bloodstains	608
44.6.3 Gm and Km Typing in Body Fluids and Tissues	609
44.7 Distribution of Gm and Km Phenotypes in Populations	610
SECTION 45. OTHER SERUM PROTEIN GENETIC MARKERS	
45.1 Serum Lipoproteins—The Ag, Lp and Ld Systems	611
45.1.1 Introduction	611
45.1.2 The Ag System	611
45.1.3 The Lp System	613
45.1.4 The Ld System	614
45.2 Complement Components	614
45.2.1 Introduction	614
45.2.2 C2 Component	614
45.2.3 C3 Component	615
45.2.4 C4 Component	615
45.2.5 C6 Component	616
45.2.6 C7 Component	616
45.2.7 C8 Component	616
45.2.8 Properdin Factor B (Bf)	617
45.3 α_2 -Macroglobulin (α_2 M)—Xm, AL-M and α_2 M	617
45.4 α_1 -Acid Glycoprotein (Orosomucoid)	617
45.5 Ceruloplasmin (Cp)	618
45.6 Transcobalamin II (TcII)	619
45.7 Thyroxine Binding α -Globulin (TBG)	619
45.8 Albumin	620
45.9 β_2 -Glycoprotein I (Bg)	621

SECTION 46. THE HLA SYSTEM AND THE HUMAN MAJOR HISTOCOMPATIBILITY COMPLEX	
46.1 Introduction	623
46.2 Definition and Function of HLA Specificities	624
46.3 Genetics and Nomenclature of the Serologically Defined HLA Loci	624
46.3.1 HLA-A and HLA-B Antigens	624
46.3.2 Cross Reactivity of Antisera and "Splits"	625
46.3.3 Formal Genetics and Chromosomal Localization	625
46.3.4 The HLA-C Locus	626
46.4 Lymphocyte Defined Antigens—A Further HLA Locus	627
46.5 HLA Testing and Typing Procedures	628
46.6 Biochemical Studies on HLA Antigens	628
46.7 Medicolegal Applications	629
46.7.1 Disputed Parentage	629
46.7.2 HLA Antigen Typing in Dried Blood	630
SECTION 47. POLYMORPHIC PROTEINS OF HUMAN SALIVA	
47.1 Introduction	631
47.2 Polymorphic Salivary Protein Systems	631
47.2.1 Acidic Protein (Pa System)	631
47.2.2 The Pr and Db Systems and their Relation to Pa	632
47.2.3 Basic Protein (Pb)	632
47.2.4 Other Polymorphic Salivary Proteins	633
47.2.4.1 The Major Parotid Salivary Glycoprotein (Gl)	633
47.2.4.2 Pm	633
47.2.4.3 Ph	633
47.2.4.4 Sal I and Sal II	633
47.3 Polymorphic Salivary Enzyme Systems	634
47.4 Typing Salivary Polymorphic Proteins	634
47.5 Medicolegal Applications	635
SECTION 48. POLYMORPHIC PROTEINS IN HAIR	
637	
UNIT VIII. DETERMINATION OF SEX OF ORIGIN, NONGENETIC MARKERS AND BLOOD COMPONENT PROFILING	
SECTION 49. DETERMINATION OF SEX OF ORIGIN	
49.1 Introduction	665
49.2 Determination of Sex Chromatin	665
49.2.1 Sex Chromatin Determination in Blood and Bloodstains	665
49.2.2 Sex Chromatin Determination in Epithelial Cells and Hair	666
49.3 Y-Body (F-Body) Determinations	666
49.3.1 F-Body Determinations in Dried Blood and Bloodstains	666
49.3.2 F-Body Determinations in Other Tissues	667
49.4 Sex Hormone Level Determinations	668
SECTION 50. NON-GENETIC MARKERS AND BLOOD COMPONENT PROFILING	
50.1 Introduction	669
50.2 Detection of Specific Components of Blood	669
50.2.1 Antibodies	669
50.2.2 Drugs and Plasma Metabolites	670
50.2.3 Hepatitis Antigens	670
50.3 Serum Protein Profiling	671

FIGURES

1.1	D-Series of Aldoses	8
1.2	Haworth Formulas for the Anomers of Glucose	9
1.3	Maltose	9
1.4	Cellobiose	9
1.5	A Triglyceride	9
1.6	A Phosphatidic Acid	9
1.7	A Phosphatidyl Derivative	10
1.8	Sphingosine	10
1.9	A Sphingomyelin	10
1.10	N-Acetyl Neuraminic Acid	10
1.11	Perhydrocyclopentanophenanthrene	10
1.12	Cholesterol	11
1.13	Testosterone	11
1.14	Estradiol	12
1.15	Isoprene	12
1.16	β -Carotene	12
1.17	Ionic Forms of an Amino Acid	14
1.18	Initial Velocity of Enzyme Catalyzed Reaction as a Function of Substrate Concentration	17
1.19	Lineweaver-Burk Plot Showing Hypothetical Curves for Enzyme Catalyzed Reaction and Behavior with Competitive and Noncompetitive Inhibitors	18
1.20	Purine and Pyrimidine Bases	22
1.21	Ribose and Deoxyribose Sugars	23
1.22	Nucleosides	23
1.23	Nucleotides	24
1.24	A Deoxyribose Polynucleotide Chain	25
1.25	Double Stranded DNA	26
1.26	Double Helical DNA	27
1.27	DNA Replication	28
1.28	Structure of Alanine-t-RNA	28
1.29	Overall Scheme of Protein Synthesis	29
1.30	Mitosis	31
1.31	Meiosis	31
1.32	Illustration of Segregation	32
1.33	Illustration of Independent Assortment	33
1.34	Illustration of Linkage	34
1.35	Illustration of Crossing Over	35
1.36	Diagrammatic Summary of the Gene Map of Human Chromosomes	37
1.37	Hypothetical Pedigree for a Simple Mendelian Recessive Trait	40
1.38	Hypothetical Pedigree for an X-linked Recessive	41
1.39	Prediction of Outcome of Crosses in a Population with 50% AA and 50% Aa Individuals Under Random Mating	42
1.40	Prediction of Outcome of Crosses in a Population with 25% AA : 50% Aa : 25% aa Individuals Under Random Mating	42
1.41	Diagrammatic Structure of Immunoglobulin Molecule	47
1.42	Proteolytic Cleavage of IgG	49
1.43	The Complement Cascade	56
2.1	Precipitin Patterns in Double Diffusion	59
2.2	Rocket Electrophoresis	64
2.3	Schematic Representation of Pattern of Normal Serum Examined by Crossed Electrophoresis	64

4.1	Porphin	77
4.2	Porphin—Shorthand Notation	78
4.3	Structural Isomers of Etioporphyrin	78
4.4	Equivalent Representations of Protoporphyrin IX	80
4.5	Iron Protoporphyrin	80
4.6	Interrelationships Among Hemoglobin Derivatives	82
5.1	Absorption Spectra of Some Hemoglobin Derivatives (after Lemberg and Legge, 1949)	91
5.2	Absorption Bands for Hemoglobin and Some Derivatives	92
5.3	Biebrich Scarlet	100
5.4	Hematoxylin (A) and Hematein (B)	100
5.5	Eosin	100
5.6	Methylene Blue	100
6.1	Barbaloin	104
6.2	Phenolphthalin Oxidation and Phenolphthalein	104
6.3	Benzidine	106
6.4	Course of Benzidine Oxidation by Peroxide	106
6.5	Crystal Violet	108
6.6	Malachite Green	108
6.7	2,7-Diaminofluorene	109
6.8	Rhodamine B	110
6.9	o-Tolidine	110
6.10	o-Toluidine	111
6.11	o-Dianisidine	111
6.12	Amidopyrine	112
6.13	3,3',5,5'-Tetramethylbenzidine	112
6.14	Luminol	113
6.15	Luminol Structures in Solution	114
6.16	Albrecht Mechanism for Luminol Light Reaction	115
6.17	Shevlin and Neufeld Mechanism for Luminol Light Reaction	116
8.1	Fibrinolytic System	123
8.2	Fibrinogen Degradation by Plasmin	125
9.1	Scheme for Determination of α -Ratio	132
9.2	Scheme for Determination of α -Ratio	132
10.1	Reaction of β -Naphthol with Stabilized Diazonium Salt	158
10.2	Reaction of α -Naphthol with Stabilized Diazonium Salt	158
10.3	ZnCl ₂ Double Salt of Tetrazolium Chloride of Diazo Blue B (Fast Blue B)	159
10.4	ZnCl ₂ Double Salt of Diazo Red RC (Fast Red RC)	159
10.5	Spermine	174
10.6	Choline	174
10.7	Naphthol Yellow S (A) and Flavianic Acid (B)	174
11.1	Amylose	188
11.2	Amylopectin	188
12.1	Reaction of Xanthidrol with Urea	192
12.2	Jaffe Reaction Product (after Blass <i>et al.</i> , 1974)	193
15.1	Relationships of Red Cell Sizes of Many Species (after Gulliver, 1875)	218
16.1	Scheme of Agglutination of Sensitized Rh ⁺ Cells by AHG Serum	228
16.2	Scheme of Testing Bloodstains for Human Origin by AHG Inhibition	230
16.3	Scheme of Testing Bloodstains for Human Origin by Mixed Antiglobulin Technique	232
16.4	Quantitative Precipitin Analysis by Reflectometry (after Boyden, 1957)	239
19.1	Determination of Inhibitive Titer for an Antiserum with Secretor Saliva Containing the Corresponding Group Substance	285
19.2	α -L-Fucose	287
19.3	N-Acetyl-Galactosamine	287
19.4	Lacto-N-Fucopentaose II	288
19.5	Lacto-N-Fucohexaose I	288

19.6	Melibiose.....	289
19.7	Composition of the Carbohydrate Fragments Responsible for Blood Group Activities A (a), B (b), and H (c)	289
19.8	Composition of the Carbohydrate Fragments Responsible for Le ^a Specificity (a) and Le ^b Specificity (b)	290
19.9	Overall Scheme for the Genetically Controlled Synthesis of A, B, H and Le ^a	292
19.10	Conversion of Type 1 Chain to Group Substances	293
19.11	Conversion of Type 2 Chain to Group Substances	294
19.12	Comparison of Results on Inhibition Test by Holzer vs. Hirsfeld and Amzel-Kind Techniques	303
21.1	Terminal Oligosaccharide Structure of M, N, T and Tn Immunodominant Groups (according to Springer and Desai, 1974)	341
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 <i>et al.</i> , 1978).....	343
24.1	Biosynthetic Scheme for the P System Antigens.....	386
27.1	Electrophoretic Patterns of PGM Types.....	428
27.2	Scheme for Phosphoglucomutase Reaction	430
27.3	Detection Reaction Sequence for PGM	431
28.1	Detection Reaction Sequences for AK	439
28.2	Electrophoretic Patterns of AK Phenotypes at pH 7	440
29.1	Electrophoretic Patterns of ACP ₁ Phenotypes in pH 6 Buffers Containing Citrate.....	447
30.1	Electrophoretic Patterns of ADA Phenotypes	458
30.2	Segregation of a Silent ADA Allele (after Chen <i>et al.</i> , 1974)	459
30.3	Detection Reaction Sequence for ADA	461
31.1	Succinyl Dicholine.....	464
31.2	Dibucaine	464
31.3	Solanine.....	465
31.4	RO2-0683.....	469
31.5	Relative Electrophoretic Mobilities of the Red Cell Carboxylesterases	472
31.6	Electrophoretic Patterns of ESD Phenotypes.....	473
32.1	Designations of Minor Isozymes in Relation to the Major Isozymes of CA (after Tashian, 1969)	480
32.3	Electrophoretic Patterns of CA II Isoenzymes. Electrophoresis in Gels at pH 8.6 and Staining with Fluorescein Diacetate.....	482
33.1	Simplified Representation of Major Red Cell Pathways of Glucose Metabolism.....	485
33.2	Basis of Detection Reaction Sequences for Glc-6-PD and PGD.....	487
33.3	Electrophoretic Patterns of Glc-6-PD Common Variant Isoenzymes.....	488
34.1	Electrophoretic Patterns of GLO Phenotypes	496
34.2	Detection Reaction Sequences for GLO.....	497
35.1	Electrophoretic Patterns of GPT Phenotypes.....	500
35.2	Detection Reaction Sequences for GPT	501
36.1	Relative Electrophoretic Mobilities of Peptidases A through F and S (after Harris and Hopkinson, 1976).....	504
36.2	Schemes for the Detection of Peptidase Activity.....	506
36.3	Electrophoretic Patterns of PEPA Phenotypes in Black Populations	508
37.1	Electrophoretic Patterns of AMY ₁ and AMY ₂ Phenotypes Following Vertical Polyacrylamide Gel Electrophoresis (after Merritt <i>et al.</i> , 1973a).....	511
37.2	Principal Reactions of Galactose Metabolism	513
37.3	Electrophoretic Patterns of GALT Variants (after Ng <i>et al.</i> , 1973)	514
38.1	α and β Chain Sequences in Hemoglobin	546
38.2	Sequences of the α , β , γ and δ Chains of Human Hemoglobins.....	548
38.3	Tetrameric Formulas of Adult, Fetal and Embryonic Hemoglobins.....	549

38.4	Relative Electrophoretic Mobility of Some Hemoglobins at pH 8.6	556
38.5	Relative Electrophoretic Mobility of Some Hemoglobins at pH 6	557
39.1	Plasma (Serum) Protein Profiles by Various Techniques.....	567
40.1	Electrophoretic Patterns of Hp Types and Subtypes	571
40.2	Electrophoretic Patterns of Some Hp Variants	573
40.3	Diagrammatic Structure of Monomeric Haptoglobin.....	574
40.4	Diagrammatic Representation of Possible Unequal Crossovers in Hp α Chains	576
41.1	Electrophoretic Patterns of Some Gc Phenotypes (after Immunofixation Ag- arose Gel Electrophoresis at pH 8.6)	582
42.1	Relative Electrophoretic Mobility of Some Tf Variants at Alkaline pH	590
42.2	Arrangements of Transferrin Variants According to Relative Electrophoretic Mobility on (A) Polyacrylamide Gels (Sutton and Jamieson, 1972), and (B) Agarose Gels after High Voltage Runs (Rittner and Rittner, 1975).....	591
44.1	Distribution of the Genetic Markers on the IgG Subclasses	606

TABLES

1.1	The Amino Acids.....	13
1.2	Coenzymes	19
1.3	Naming of Bases, Nucleosides and Nucleotides	25
1.4	The Genetic Code	30
1.5	Comparison of Some Properties of the Classes of Immunoglobulin Molecules	48
4.1	Side Chain Structures of Some Porphyrins	79
4.2	Comparison of Nomenclature of Hematin Compounds	81
4.3	Some Modifications of the Hematin Crystal Test.....	83
5.1	Summary of Absorption Maxima of Hemoglobin and Some Derivatives ...	90
5.2	Pyridine Hemochromogen Spectrum Survival After Aging Blood-Iron Powder Mixtures (after Hirose, 1976)	95
5.3	Biological Stains and Dyes.....	98
10.1	Recommended Concentration of Acid Phosphatase for Diagnosis of Seminal Stains.....	160
10.2	Some Units of Phosphatase Activity.....	164
10.3	Concentrations of Some Components of Human Seminal Plasma	181
11.1	Amylase Concentrations in Several Body Fluids.....	186
12.1	Concentrations of Some Components of Urine and Other Body Fluids.....	195
15.1	Comparative Table of Red Cell Measurements by Several Authorities (after Bell, 1892)	220
16.1	Results of the Application of the Coetzee Method to Human and Chimpanzee Sera (after Coetzee, 1955)	240
18.1	Genetic Marker Systems in Human Blood.....	259
19.1	Historical and Present ABO Nomenclature.....	263
19.2	Blood Group Genetics According to von Dungern and Hirszfeld	265
19.3	ABO System Genetics with A ₁ and A ₂ Subgroups	267
19.4	Summary of Some Weak B Types	271
19.5	ABO System as Conceived by Wiener	278
19.6	Additional Blood Groups Predicted by the C/Anti-C Hypothesis	279
19.7	Some Enzymes Acting on Blood Group Specific Structures	291
19.8	Relationships of Genotypes to Red Cell and Secretor Phenotypes	295
19.9	Reactions of Several Acquired B Samples with Anti-B Antisera, Variously Treated to Remove the Acquired B Reactions (after Jenkins <i>et al.</i> , 1972) ..	318
19.10	Frequency of ABO Groups in U.S. Populations	325
19.11	Frequency of Secretors in U.S. Populations (Caucasian).....	328
20.1	Saliva and Red Cell Relationships for the Four Lewis Factors.....	332
20.2	Distribution of Lewis Phenotypes in U.S. Populations.....	334
21.1	MNSs types Using All Four Antisera	336
21.2	Frequency of MN Groups in U.S. Populations	346
21.3	Frequency of MNSs Groups in U.S. Populations	347
22.1	Genes, Gene Products and Reactions in the Rh System With the Five Common Antisera.....	351
22.2	Rh Phenotypes and Genotypes.....	352
22.3	Numerical Designations of Rh Factors and Their Equivalents.....	359
22.4	Rh Phenotypes in U.S. Populations.....	366
23.1	Kell System Numerical Nomenclature.....	371
23.2	Kell Phenotypes in U.S. Populations.....	376
23.3	Duffy and Kidd Phenotypes in U.S. Populations.....	378
24.1	P System Relationships.....	382
24.2	P System Relationships According to Wiener (1968).....	383

24.3	Names and Structures of Glycosphingolipids of Erythrocytes (after Schwarting <i>et al.</i> , 1977)	385
27.1	Distribution of <i>PGM</i> ₁ Phenotypes in U.S. Populations	436
28.1	Distribution of AK Phenotypes in U.S. Populations	443
29.1	Distribution of <i>ACP</i> ₁ Phenotypes in U.S. Populations	454
30.1	Distribution of ADA Phenotypes in U.S. Populations	462
31.1	Nomenclature and Properties of the Plasma Cholinesterase Variants	466
31.2	Classification of Carboxylesterases	470
31.3	Nomenclature and Properties of Various Tissue Esterases (after Coates <i>et al.</i> , 1975)	474
31.4	Distribution of Cholinesterase and ESD Phenotypes in U.S. Populations ..	476
32.1	<i>CA</i> ₁ Locus Variants	481
33.1	Distribution of Common <i>Gd</i> Phenotypes in U.S. Black Populations	489
33.2	Nomenclature and Properties of PGD Variants	491
33.3	Distribution of PGD Phenotypes in U.S. Populations	494
34.1	Distribution of GLO Phenotypes in U.S. Populations	498
36.1	Activities of Various Peptidases with a Number of Substrates (after Rapley <i>et al.</i> , 1971)	505
38.1	Some Hemoglobin Variants	551
38.2	Distribution of Common Hb Variants in U.S. Populations	560
39.1	Properties of Some Serum (Plasma) Proteins	565
40.1	Distribution of Hp Phenotypes in U.S. Populations	580
41.1	Gc Variant Nomenclature Equivalents	583
41.2	Distribution of Gc Phenotypes in U.S. Populations	587
42.1	Distribution of Tf Phenotypes in U.S. Populations	594
44.1	Genetic Markers of the Immunoglobulins	602