Section 3. Determination of Species of Origin

The oldest systematic species test is that of J.-P. Barruel. He said that concentrated sulfuric acid evoked an odiferous principle from blood and bloodstains, which could identify the source of the blood. The method was taken seriously for quite a number of years, although it seems absurd by present day standards. Casanti's proposal was not much more enlightened.

After the microscope gained popularity, there was considerable interest in, and controversy over, the micrometric method of determining species of origin. Even the illustrious Rudolf Virchow wrote a paper on the subject. The papers of Roussin, Masson and Vibert all deal with micrometry, and Masson gives an historical review as well.

The modern period began in 1901 with the first paper by Paul Uhlenhuth. There is no question that other fundamental immunological findings of the 1890's provided the groundwork for the immunological species tests, but Uhlenhuth's paper was the first to apply the immunological test to medico-legal species determination. Uhlenhuth (1870–1957) was an immunologist all his life. His obituary appeared in the *Zeitschrift für Immunitätsforschung und Experimentelle Therapie* **115** (4) for 1958, along with a list of his numerous publications. The 1900 paper on ovalbumin has been included, since the serum work so clearly evolved from it. A representative set of the 1901 papers has been included, as has an interesting account of his personal recollections of the early years which he was persuaded to write, and which appeared in 1949.

Wassermann and Schütze published the precipitin method almost simultaneously—about a week after Uhlenhuth's paper—and they are clearly to be regarded as joint originators of the technique. Neisser and Sachs introduced the complement fixation procedure for detecting the species antigen-antibody reaction in the medico-legal test.

Marx and Ehrnrooth's method was based on the agglutination of human cells by animal sera. It was actually a method for demonstrating the absence of human blood. Lattes in 1913 (see in Section 4) devoted a section of his paper to this procedure, which is closely related to isoagglutination, and hence, to blood grouping.

Memoir on the Existence of a Principle Peculiar to Blood to Characterize the Blood of Man and of Various Animal Species*

J. P. Barruel

267 Blood has been the object of the meditations of the most ancient philosophers; the imporant functions it fulfills, and the changes it undergoes in nutrition, have not ceased to occupy physiologists; finally, chemists make it the daily subject of their research. If it were left to me to express an opinion on the whole of these works, I would say that, numerous and important as they might be, they aren't sufficient for establishing satisfactory theories on "sanguification" or hematosis, the nutrition or organic composition, the warmth of or the secretions in blood. But it is not my object here to discuss, or even to recall the different theories, which try to explain the role blood plays in the phenomena of life. I find it sufficient to recall that chemists have already found a large number of principles in this liquid which participate not only in the composition of organs but also of the secreted products. Thus, it has long been known that the purified fibrin of blood is similar to muscle fiber, which differs only in its
68 organization; that *serum* is of the same nature as certain secretions destined for the mechanism and activity of various organs. We are all aware of the results of the research of the celebrated Vauquelin, who reported the existence of fat in blood, and of the experiments of Chevreul, who found in fibrin a fatty matter analogous to that found in cerebral matter. Finally, we have lately seen that, when the kidneys of an animal are removed, the blood contains urea a short time afterward. Nor are we ignorant of the existence in blood of phosphate and sodium carbonate, the bases of all bones.

Chyme, the immediate product of digestion, contains the elements of all known animal matter. Couldn't it be possible that, by the single act of "sanguification," the elements of air absorbed by respiration, in the course of circulation and under the influence of life, determine the reaction of these elements, their combinations in different proportions, from which would result the formation, in necessary quantity, of all the materials specific for constituting and renewing organs and furnishing the secreted fluids? I leave to the physiologists the difficult task of clarifying this scientific point. I should and will limit myself to what is related to the single fact, which is the subject of this memoir, and to the consequences derived from it.

Blood is divided into red or arterial blood and black or venous blood. The former blackens in a few hours when completely deprived of atmospheric air; the second reddens in a few seconds on immediate contact with oxygenated gas or atmospheric air. Hydrocarbon and carbon monoxide gas do not give venous blood a ruby color, as has been supposed. The action of oxygen gas on black blood demonstrated to me 269/ a phenomenon worthy of note, and which strongly merits, I believe, the attention of physiologists; it happened that this liquid, preserved for several weeks, still possessed the property of becoming ruby colored even though some of its elements, especially fibrin and albumin, had already submitted to the immutable law attracting all things of which decomposition is only the result. It would appear that the coloring matter of blood, on which oxygen is preferentially carried, is endowed with a great assimilating or vital force, which is extinguished only a long time after the complete death of all the other immediate principles of the same liquid.

Moreover, whatever opinion can be expressed on the functions and nature of the coloring matter of blood, whether it comes from arterial blood, or whether it comes from venous blood a few minutes after extraction from the vessels where it circulates, I maintain that this substance has the same physical characteristics and that it is the only principle which distinguishes blood from all the other animal fluids. For the property of coagulating at rest and of dividing into a solid mass, or clot, and a liquid, or serum, is not exclusive to blood, but belongs also to chyme; likewise the property of hardening by the action of heat, acid or alcohol is not exclusive to it either, for all types of albumin possess it.

Brande, in England, and *Vauquelin,* in France, have already tried obtaining this coloring principle of blood separate from all other substances of this fluid. But with the procedures indicated by these chemists, it is never obtained 270/ pure; it is always accompanied by a rather considerable proportion of albumin. Filtration does not give any better results because the coloring matter of blood is so fine it sifts through the tightest filters. It is also accompanied by serum of blood, from which it results that albumin cannot be entirely separated from it for the study of its chemical characteristics, but that is of little import.

It is sufficient to acknowledge that, either this coloring substance still enjoys the faculty of becoming ruby-colored on contact with air, or, deprived of this property, the manner in which it behaves under the influence of heat is the same, differing in one or the other of these conditions only in the following property: 1) in the first case, when blood is diluted with water, the solution takes on a ruby-red color and in the second it has a red-wine color; 2) blood dried in air takes on

* Translation of: "Mémoire sur l'existence d'un principe propre a caractériser le sang de l'homme et celui des diverses espèces d'animaux." in *Annales d'Hygiène Publique et de Mêdecine Lêgale* **1**: 267-277 (1829).

a red-wine color when treated with water, because the very act of dessication suffices to extinguish in blood the faculty of the coloring substance in changing to ruby red on contact with air.

It is in the very action of heat on the coloring substance of blood that resides the truly distinctive characteristic of this principle, which, as I have pointed out, is always accompanied by albumin.

I do not believe it necessary to recall here the details relative to this action because they have been faultlessly presented in the memoir published by Professor Orfila, in response to a work by a distinguished scientist, who alleged
/271 it impossible, with our present level of knowledge, to decide in medico-legal cases if stains on linen were stains of blood or some other coloring substance. In this work, the scientist claimed to have composed a fluid which, even though containing no blood, possessed, however, all its properties. Orfila demonstrated beyond doubt that, up to the present, a liquid could not be composed, whose coloring matter exhibited the same chemical characteristics as those of the coloring matter of blood. It is important to acknowledge also that these characteristics are preserved in their integrity in blood dried in air, even after several years, fortunately permitting after a long lapse of time the immediate confirmation that stains are due to blood or another substance altogether. I will add that these characteristics are the same in the coloring principle of blood of all animal species.

Though chemists have been able for a long time now to pronounce and affirm before the magistrates in all tranquility of conscience that stains, provided they are extensive enough or at least numerous enough, are due to blood or some other coloring matter (three or four drops are sufficient to obtain this result), it is quite different when the authorities ask them if they can likewise say if these stains are formed by human blood, or the blood of another animal. I well know that already, in a few cases of this nature, fortunately very rare, some authorities have confirmed that the blood stains, which they were charged with examining, were produced by human blood, but they gave none of the grounds on which their opinion was based. It seems to me that when the life of
/272 an innocent or the punishment of a guilty person might depend on the opinion of an expert, one cannot be too conservative, and that nothing should ever be affirmed in cases of this type without the support of positive proof, not hypothetical proof. One must never lose sight of the wise old adage: *When in doubt, refrain.*

As for myself, in a great number of instances, I have been charged by magistrates with determining if stains, perceived on clothing of people suspected of having committed homicide, were blood stains or stains of another nature. I never hesitated to pronounce the affirmative when I could find the principal characteristic of the coloring matter of blood in these stains, because I know of no other material possessing it; but when I was asked if these stains were of human blood, I never hesitated to reply it was impossible for me to express an opinion in this regard, because I knew of no trait in the blood of each animal species serving to characterize it.

Actually, the brilliant research with the microscope by Prévost and Dumas have demonstrated that blood is composed of serum in which float globules of form and dimension different in man and in animals. But besides the fact that these differences are only slightly marked, if not entirely non-existent, between individuals belonging to neighboring species, not everyone is familiar with observations with a microscope, an instrument not very widespread, and consequently at the disposal of a small number of people. Besides, the form of various globules can only be recognized inasmuch as the blood has not lost its liquid form. For as soon as it has been dried on any object whatever, if this blood is diluted in water, the resulting solution presents nothing distinctive, and it is almost always dried blood stains that chemists are called upon to test. Thus, the discovery of Prévost and Dumas can be only very rarely applied to cases of homicide and legal medicine.

For many years, in seeking to obtain the coloring matter of blood by the procedure given to us by Vauquelin, which consists of boiling the blood clot for a while in fairly concentrated sulfuric acid, and having employed in this context a clot of beef blood, I was struck by the strong odor of a cattle barn which emanated from it. This fact remained engraved in my memory, without my looking to derive any consequences from it until, lately, a very peculiar circumstance permitted me to observe an analogous fact: an individual decided to commit suicide after a considerable gambling loss and swallowed for this purpose a considerable quantity of opium. This deadly design was known about almost as soon as executed, Orfila was called on, arriving just in time to save the patient; and because among the means employed in fighting the effect of the poison was a profuse bleeding, Orfila profited from this circumstance to look into whether blood from persons under the influence of a large quantity of opium didn't contain traces of morphine. With this intention in mind, he brought me this blood and invited me to do the necessary research.

I began by coagulating the blood in a water-bath, to be able to more easily divide it by crushing, which I did without perceiving any odor. I then heated the divided blood to the boiling point with an ample quantity of sulfuric acid diluted with water, and there immediately escaped from the round-bottom flask which I was using an odor of human sweat so intense that it permeated the laboratory to the point where I was forced to abandon it for a few moments. This reminded me of the odor which manifested itself when I was extracting the coloring principle of blood by the procedure of Vauquelin, and from that moment I imagined the possibility of arriving at distinguishing the blood of various animals from that of man. It was with this in mind that I took up numerous experiments, of which the principal results are:

1) That blood of each animal species contains a principle peculiar to each of them.

2) That this principle, which is very volatile, has an odor similar to that of sweat, or cutaneous and pulmonary ex-

halation, of the animal from which the blood comes.

3) That this volatile principle is bound in the blood and, inasmuch as this combination exists, it is not discernible.

4) That when this combination is ruptured, the principle of blood which gives off the odor volatilizes and, from then on, it is not only possible but even rather easy to recognize the animal to whom it belongs.

5) That in each animal species the principle of odor of blood is much more pronounced, or, in other terms, has more of an intensity in the blood of the male than that of the female, and that in man hair color brings nuances to the odor of this principle.

/275 6) That the binding of this principle of odor is in a state of solution in the blood, which permits its development, either in whole blood, or in blood deprived of fibrin, or in blood serum.

7) Lastly, that of all the means I employed to liberate the principle of odor of blood, concentrated sulfuric acid gave best results.

It suffices, to obtain these results, to pour a few drops of blood or blood serum in a glass; then to pour a slight excess of concentrated sulfuric acid into it, about a third or a half of the volume of blood, and to stir with a glass rod: the aromatic principle immediately manifests itself. It is by these means that I easily distinguish all the bloods which I am going to name in designating the odor peculiar to each of them.

1) That of man releases a strong odor of the sweat of man, which is impossible to confuse with any other.

2) That of woman, an analogous odor, but much less strong, in short, that of the sweat of woman.

3) That of beef, a strong odor of cattle barn or of beef manure.

4) That of horse, a strong odor of horse sweat or of horse droppings.

5) That of sheep, a vivid odor of wool impregnated with its sweat.

6) That of ewe, an odor analogous to that of sheep mixed with a strong odor of billy goat.

/276 7) That of dog, the odor of perspiration of dog.

8) That of hog, a disagreeable odor of a pig sty.

9) That of rat diffuses a disagreeable odor of rat.

Analogous results are obtained with the blood of various birds: thus the blood of hen, of turkey, of duck and of pigeon release a particular odor peculiar to each of them. Finally, I just recently experimented on frog blood. It released a strongly pronounced odor of marsh reeds, and the blood of carp furnished an odor principle similar to that of mucus covering the body of fresh-water fish.

It was important to experiment to see if it were still possible to distinguish the aromatic principle of each blood with blood stains applied to linen and dried. I assured myself by direct experiments that, provided the stain was of a certain size, it was easy to recognize with what blood it had been produced, even after more than two weeks. For this, it suffices to cut out a portion of the stained linen, to put it in a watch glass, to pour a small amount of water on it and to leave it to rest for a while. When the stain is well-moistened, concentrated sulfuric acid is poured on it, it is stirred with a rod and sniffed. I don't know if after a more considerable lapse of time the species of blood on the linen might still be characterized. When in doubt, I believe it necessary to recommend to the examining magistrates, when they are charged with investigating a person accused of homicide, to delay as little as possible the experiments which the experts must do to determine not only if the stains observed on the 2
clothing are due to blood, but particularly to designate their species.

I believe it necessary to urge the physicians and pharmacists who, by their status, are ordinarily requested by the magistrates in these instances, to repeat my experiments to educate their sense of smell, so to speak. For, if the odor of the aromatic principle of certain blood is so strong that it suffices to have smelled it once never to forget it; if it is, so to speak, impossible to confuse human blood with that of other animals, it is only after having experimented a certain number of times with human blood that the blood of man can be differentiated from that of woman, and important services might then be rendered to the magistracy in the case of a suspicion of homicide, in certain cases of actual and alleged rape, and especially cases of pretended defloration.

I will stop myself here. What I have said here suffices, I believe, for everything in relation to legal medicine. But I have not yet satisfied science, for she will ask me of what nature is the aromatic principle of blood. I reply that this will be the subject of the continuation of my research; but that, at this moment, I have strong reasons to think it a very peculiar acid substance and that it exists in blood as a salt.

A New Way of Distinguishing Human Blood from that of Other Mammals*

Casanti

/673 The attempts by means of sulfuric acid with this purpose in mind are known. Casanti employed phosphoric acid of a density of 1.18, but following principles and with the intention of what actually constituted a new method.

A first necessity was that of finding means of distinguishing blood of a mammal from that of another vertebrate, of *Gallinaceae,* for example. For this, after collection of the blood and its reduction by evaporation into a dry substance, it is treated with excess phosphoric acid. It is noted that mammalian blood enjoys the property of agglutinating in a brilliant, homogeneous, coherent, somewhat stiff mass, whereas that of the Gallinaceae is entirely lacking in this characteristic. This state of agglutination is distinct from coagulation in that in the first case the accumulated blood not only does not soften and no longer liquefies when left under the same conditions, but on the contrary, contracts, hardens and becomes almost tough, does not adhere at all to solid bodies, and does not change its characteristics even when heated up to 100°.

This having been determined, the author sought a more specific differentiation for the blood of man. Six grains of this blood, reduced to a fine powder, then 9 grains of phosphoric acid were placed in a glass. On stirring with a glass rod, the blood was observed to swell and soften; its particles attracted each other and adhered together, then united in a very brilliant mass of the color of liver, of the consistency of a very dense, non-glutinous extract, very coherent and having a lot of plasticity. Compression with the glass rod causes it to yield to the pressure without dividing, becoming, on the
/674 contrary, more homogeneous, more coherent as it is pressed for a longer time. Left to itself, it becomes harder, more difficult to break, without losing its brilliance.

Performing the same experiment with horse blood, the phenomena were entirely different. Blood molecules penetrated by the acid at first swelled and softened. But, instead of uniting to form a single homogeneous mass, they formed diverse lumps the color of liver, very hard and brilliant, obstinately refusing to adhere to each other. Pressed by the glass rod, they did not appear very coherent or very hard and were almost entirely lacking in plasticity, explaining their division into several parts, and of these into successively smaller parts; the more one tries to unite them, the more they separate into fine particles which lose their sheen quite rapidly.

Casanti experimented on blood of ox, calf, mule, mare, pig, roebuck and waterhog, and the results were always the same as those of horse. The blood of cat presents a few differences. It becomes a single homogeneous mass at first, like that of man; but it shows a lesser density, coherence and toughness, and it suffices to compress or fold it to see it instantly divide into several parts.

The author repeated these experiments numerous times, always with identical results. He also remarked that human blood presents the same properties despite differences in age, sex, health or various diseases.

The applications of this discovery in legal medicine, and especially in those cases where the purpose is to shed light on the investigations of criminal justice, are self-evident. However, human blood presents a different aspect in a particular case: that of menstruation. The author has twice seen the reaction of menstrual blood. Addition of phosphoric acid provoked a homogeneous mass, yielding to pressure; but it was so lacking in coherence that it sufficed to compress it for an instant or to fold it, to reduce it to a mass of dry, swollen particles no longer able to be united into a *whole.* These characteristics will surely differentiate menstrual blood from that coming from any other part of the vacular system.

Editors note. We are contemplating, along with Lassaigne, doing the experiments to discover the value of the procedure reported by Casanti.

* Translation of: "Nouvelle Manière de Distinguer le Sang Humain de Celui des Autres Mammifères."

in *Journal de Chimie Médicale de Pharmacie et de Toxicologie* **4** (3rd series): 673-675 (1848).

On the Forensic Investigation of Dried Bloodstains*

Rudolf Virchow

/334 The various methods of testing dried blood stains were recently submitted to earnest criticism by Brücke (*Wiener medic. Wochenschrift,* 1857, no. 23), and they were, at the same time, substantially expanded by this perceptive observer. By chance I was recently in a position to conduct several such examinations for forensic purposes. In these two cases, following closely upon one another, a large wooden pole and two very dirty coats were handed over to me to determine whether there were stains of human blood on these objects. I took this opportunity to make a few different examinations to test the usefulness of these various methods.

As has been recognized, the first question which the researcher must solve is always whether blood is even involved; then come the more specific questions whether mammal, and finally, whether human blood is present. For the first question, we can find an approximately certain solution by means of a strictly chemical method; for an answer to both of the other questions, I believe, as do all the more sober research-
/335 ers, that we can rely only on the microscope. Even for determining whether blood is present at all, the microscope can provide a much more dependable decision than the purely chemical examination.

Here I believe I must point out from the outset that we have totally neglected one morphological component of the blood, namely, the colorless blood corpuscles. It was not really the case that I came to this idea after first having busied myself with these elements, but rather I came to it through simple experience. As I treated dried blood drops with the media usually suggested (water, salt water, iodine water, sulphuric acid and acetic acid-containing water), it turned out that I obtained every time very clear bodies which resembled completely the colorless corpuscles in form, size, content, and nucleus, and which, more than any other part of the blood, resisted the various effects of being dried and then dissolved again in solution. I was able to measure very easily not just the whole corpuscles but also the nuclei, and was able to compare them with other known, colorless corpuscles. The value of this experience is obvious. The colorless blood corpuscles do not have any characteristics specific enough that their discovery would be sufficient, in itself, to prove that any organic substance whatsoever is blood or contains blood, but their discovery, along with the other determining signs, strengthens substantially the probability of the diagnosis; indeed, one could say that their absence very much lowers the probability that one is dealing with blood. Although stains from pus could contain the same corpuscles, this objection has not much validity, since pus often is mixed with enough blood that the usual blood tests will also not be correct in this case. Here, however, is a fact of very great importance, namely, the determination of the number of colorless corpuscles in comparision to the size of the stain under examination. If a great many such corpuscles are present, then it is likely that one is dealing with pus, a purulent mucus, or some similar, pathological product. If relatively few are present, then it is probable that these are colorless blood corpuscles. The possibility of leukemia should be kept in mind, but in view of the rarity of the disease, this problem recedes into the background. In one forensic case I counted in a particle of dried blood of $\frac{1}{400}$ inch (Par.)† 7, in one of $\frac{1}{200}$ inch (Par.)† 5 of such corpuscles which on the average measured 0.004 to 0.006 lines across. Not all of these, however, were located in the same place; some were found in different layers of the drop.

It would be incomparably more important if one could find red corpuscles in a suspicious stain and could also measure these corpuscles. If these proved not to have nuclei, one could declare these the corpuscles of mammals or humans, and measuring would finally determine whether one was dealing with one or the other species. The latter is now in fact recommended and maintained by C. Schmidt. I know, however, of only one single case—and that from an uncertain description (*Med. Times and Gaz.* 1857, April, No. 354, p. 365)—in which such an assertion, based on measurement, 33
was involved in a judicial decision; namely a case at Taunton in which Herapath had used the microscopical examination. In evaluating this method, I can only agree with the damning judgment of Brüke, and I do not believe that any microscopist would consider himself justified in placing a man's life in question as the result of the uncertain estimation of the drying coefficient of a blood corpuscle. Blood, of course, at times dries so that one can still clearly recognize the individual corpuscles, if one moistens the dried blood with oil. In some cases turpentine oil is still better suited for this task, while glycerine has almost always failed in my tests. The drying process, alone, is subject to so many conditions, and, after drying, the blood can be exposed to so many unfavorable influences, that a judgment concerning the size of individual components can make no claim to be reliable.

* Translation of: "Ueber die forensische Untersuchung von trockenen Blutflecken."

in *Archiv für pathologische Anatomie und Physiologie und für Klinische Medizin* [Virchow's Archiv] **12:** 334-338 (1857).

† [Note: The term in the original article was "Zoll Par.," and may be a reference to an old micrometric measure called a Paris Line, which was equal to 0.0888 inch. In present day German, "Zoll" would be translated as "inch" in this context.]

In the cases which I examined, apparently moisture had had an effect; mold had formed, and neither fatty nor volatile oil enabled me to perceive anything of the blood corpuscles. Nevertheless, it is certainly justified in every case to submit the substances to testing.

Among the substances for moistening the blood and separating the individual blood corpuscles, most researchers use only a very limited number in those cases where the blood has been preserved under unfavorable circumstances. I, however, have found one substance to be very valuable, a substance which Donders mentioned casually some time ago, namely, concentrated potassium hydroxide. Kölliker also mentions this special characteristic, that the blood corpuscles preserve well in potassium hydroxide, if that chemical is concentrated, while they break up when it is diluted. If, to the dried blood which has been divided into smaller fragments, one adds directly the concentrated reagent, one can see after a short time the individual red-colored little globules outlined clearly on the surface of the fragment; not infrequently, one sees individual corpuscles separate themselves from the main body, bodies which reveal their nature as red blood corpuscles by their mobility, their more flatly-rounded shape, and their gold-green hue. Thus, the dichroism of the hemoglobin, emphasized by Brücke, is validated in this fashion. At times, it is also possible to carry out specific measurements.

There now remains a third morphological component of the blood, namely, fibrin, the identification of which completes the microscopical diagnosis. One recognizes it clearly as the binding agent of blood fragments if one treats these fragments for some time with water. Its character, now fibrous and now more pleated and homogeneous, makes it stand out. The most likely source of error in identifying fibrin is mucus, but mucus has a much greater swelling capacity. It also has the characteristic that it coagulates with acetic acid, while fibrin contracts greatly at first but then swells up and becomes transparent. These differences make distinguishing the two easy. Moreover, while examining the above-mentioned pole which had blood-red, gleaming spots on it, marks very similar to blood stains, I happened to meet with a colorless substance which very much resembled fibrin. This, however, produced with iodine very beautiful blue colorations, and it seemed that a pasty substance, apparently of plant amylases, formed all of the efflorescence, whose apparent coloration was caused only by the underlying brown bark of the wood.

/337

Although it was possible to identify successfully the three morphological components of the blood (red and colorless corpuscles along with fibrin) both with the microscope (and with microchemistry), it is still obvious that the chemical identification is not very successful with albumin, salt, and extractive substances, especially when one has only very small and impure particles to test. The chief task is the identification of hematin. The older methods are well-known, although one also knows that they were not very dependable, and that many iron substances produced a positive reaction, which were, however, not hematin. Even the Brücke method, which tests the behavior of solutions of the pigment with alkali, proved unreliable as far as I was concerned in the two cases in which I had to examine coats made of dyed fabric. On the other hand, I was perfectly successful in carrying out the method, first recommended by Teichmann (*Zeitschr. für rat. Med.* N. F. Bd. III, p. 375) and in producing the hemin crystals which he discovered. In this work, I adhered closely to the process he described, while I was not satisfied with the modification suggested by Brücke. I would like to recommend the first method all the more, because very small drops of blood (for example, drops from ⅓ to ½ lines across were sufficient) were capable of producing a more certain result. I collected carefully the quantity of dried blood on a slide; in this process it is of no concern whether individual foreign particles (e.g., vegetable fiber) are mixed in. Then, I added dry, finely pulverized salt, amounting to about half of the mass of blood, and covered the whole with a cover slide in such a way that this rested loosely on the lower slide. Then I put as much acetic acid on the slide as it takes to fill the entire space under the cover slide, and then evaporated this over an alcohol lamp by slowly heating it. After cooling, one adds to the dried mass some distilled water; now one looks through a microscope at the place where the blood fragments were before, and he sees everything completely filled with hemin crystallizations, easily recognized by their black-brown or gold-brown color, their rhombic crystal form, and their indifference to reagents.

During one experiment, it happened that I obtained through this treatment blue crystals from a fiber, apparently dyed with indigo, crystals which displayed a distant similarity to hemin crystals. Besides the facts that the color was very different and that the crystal form also showed a recognizably different structure, I obtained these blue crystals by treating the fiber simply with acetic acid, without needing to add the salt, an ingredient which was absolutely indispensable for the production of hemin crystals.

In this way, I believe the forensic blood test has been made significantly more certain than was previously the case. To determine whether human blood is present seems to me to be a demand that can scarcely be met. On the other hand, if the presence of blood has been established, one can state with certainty that either mammal or human blood must be present if in the fibrin mass, extracted with water and acetic acid, no other nuclei can be seen besides those of the colorless corpuscles. If one has enough material, he may try also the older methods of testing, but he should always begin with the ones presented here.

3.

Medico-legal Examination of Blood Stains*

Z. Roussin

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139 The law discovers that a murder has just been committed and eagerly gathers information about it. Following information of every kind procured for itself or spontaneously furnished by public opinion, a man is arrested. A meticulous house search, performed as much on the accused himself as on his home, uncovers some clothing or various objects soiled
140 with reddish stains suspected of being blood. The accused denies this or claims that these stains come from some other source and particularly from the blood of various domestic animals. Due to the impossiblility of discovering the truth by investigation by ordinary interrogation, the examining magistrates commit one or several experts to the examination of the above-mentioned stains.

Apart from secondary questions which are variables according to the details of the affair itself, the magistrate generally asks the experts to express clearly their opinion on the two following points.

1) Are the observed stains produced by blood?

2) In the case of the affirmative, is the blood human blood?

Such is, in a few words, the summary of the usual course in this type of inquiry. For the past several years, since the Public Prosecutor's Office on the Seine has been confiding these assessments to us, we have never seen another course of procedure.

Is it always possible for the experts to express an opinion as explicitly as the case demands? This report has precisely as its purpose to find out if our present level of knowledge permits a response to these two questions in every case, and what degree of certainty the various means recommended and used up to the present entail. These means are of two kinds: 1) Examination of stains exclusively by chemical procedures; 2) examination by microscope.

§I. Chemical reactions. To appreciate the value of these reactions, bearing on the various elements of blood, it is fitting to recall in a few words the composition of this liquid.

Human blood can be considered as a solution of albumin and fibrin, in which float two types of blood cells. The first
141 cells, red and very small, compose a considerable proportion; they are called red blood cells. They are formed essentially of a special albuminous matter; iron is contained among their elements. The other blood cells are much larger, not very numerous, uncolored and of a singular transparence; they are called white blood cells. Let us add that blood also contains sea salt, sodium phosphate, etc., etc., and undoubtedly special diverse elements still little known, for they are present only in small quantity and they vary in composition according to the part of the body from which they are drawn.

None of the organic substances enumerated above possess well-defined chemical characteristics such that they might at least permit the certain identification of *traces*.

Blood albumin resembles albumin of egg or any other origin. It presents the property of coagulation upon heating or the addition of nitric acid when in solution. Other than the oft-encountered difficulty, when confronted with only one droplet of blood dried on the surface of a knifeblade or buried in the thickness of a fabric, of determining coagulation by heat or nitric acid, it is certainly impossible for the expert to affirm if the coagulum belongs to albumin of blood or some other animal or vegetable matter containing this last substance. Besides, a solution of albumin too diluted with water no longer precipitates either by heat or nitric acid, and the very nature of the stains to be examined does not often permit testing under the best conditions for the expert. Let us even allow that with great care and ability, all the soluble portions of a suspicious red stain can be concentrated in four or five drops of clear liquid. Introduced into a small tube closed at one end and heated to boiling, the reddish liquid precipitates a small coagulum. How to prove that this co- 142
agulum is due to albumin and, *a fortiori*, comes from a bloodstain?

The search for fibrin is also illusory. Deposited on the surface of cloth, as is the case in general, a drop of blood dries by evaporation and very rapidly coagulates. The fibrin, rendered insoluble, becomes entangled in the thousands of fibrils of wool, cotton, or hemp, strongly adheres to each anfractuosity, and is detached only with the greatest difficulty. In this last case, the proportion of fibrin is infinitely small, often hardly visible, completely amorphous, sometimes mixed with debris of the fabric which it imprisons, and does not lend itself to chemical determination. Moreover, insoluble fibrin presents none of the special properties sufficient to characterize a substance when present in only small amount; it exhibits only the properties common to all the nitrogenous matters called proteinaceous matters.

We will say nothing of the many special chemical reactions devised to reveal blood stains. They have all been successively abandoned by those seriously interested in toxicology.

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* Translation of: "Examen Médico-légal des Taches de Sang".

in *Annales d'Hygiène Publique et de Médecine Légale* **23** (2nd series): 139-157 (1865).

We will not exclude from this rightful proscription those crystals called *blood crystals*, whose formation some chemists and physiologists have observed in several instances and with certain blood samples. Other than that the composition of these microscopic crystalline corpuscles is still problematic, and that their form is neither precise nor consistent, it is admitted today that they cannot be produced in every case, especially with human blood, that the appearance of these crystals is, instead, an accident, an almost fortuitous case of a delicate reaction, and a fortunate evaporation, rather than a consistent fact, easy to observe and to reproduce on every occasion.This method of investigation
/143 lacks, then, the two qualities indispensable to every scientific procedure, rigor and consistency. Although it can undoubtedly provide useful information in certain instances, it would be more than imprudent, in our opinion, to apply it exclusively to the legal investigation of blood stains.

The successive elimination of each of the preceding properties, which are specifically chemical, naturally leads us to the examination of the value of observation by microscope in medico-legal investigation of blood stains.

§ II. Observation by microscope. A fresh droplet of human blood, deposited between two glass slides, and examined with a good microscope, offering a magnification of at least 350 diameters, presents in the field of the instrument the following objects which we will describe with care.

In the middle of a uniformly lighted circular space a considerable number of small red discs, of a perfectly circular form and a uniform diameter, are seen floating in an uncolored or scarcely rose liquid. Their form was not actually known until a few years ago. It was, on the one hand, by very prolonged observation by microscope, and notably by the differences in focusing according to the different parts of the surface of the blood cells, and on the other, by observation by the binocular microscope, producing the usual stereoscopic effect, permitting the appreciation of the reliefs and depressions there where they exist, that the exact form of red blood cells was definitively determined.

From these observations, these last-mentioned discs are none other than small water-skins closed on all sides, extremely flat, formed by a very thin, elastic, transparent
/144 membrane, containing a red liquid in its interior. Their exact form is a circular disc concave on both sides. To give a better idea, imagine a checker piece, slightly hollow on each of its large surfaces in such a way as to determine two large concavities, but rounded at the angles. It results that red blood cells are thicker toward the circumference than at the center, and are quite similar to small biconcave lenses with rounded edges. When one of these blood cells being examined by microscope presents itself by chance in a three-quarter view, the concavities are splendidly apparent. This biconcave lenticular form permits explanation of the two following facts: 1) if, after focusing on a red blood cell lying flat on one of its surfaces, the body of the microscope is slightly raised, a shadow imperceptibly forms at the center of the blood cell, increases, and soon takes on the form of a concentric, round spot; 2) if, on the contrary, the same blood cell is in focus and one lowers the body of the microscope, the periphery gradually dims, whereas the center appears more luminous. These two phenomena, easy to observe and reproduce on human blood cells, if not deformed, in every type of circumstance, constitute, along with the form itself, the color and especially the invariable diameter of the blood cells, the best criterion for identification of blood cells today.

The diameter of blood cells of man and woman is $\frac{1}{126}$ of a millimeter. Though the determination of this diameter presents no difficulties, we feel it useful to discuss a few practical details in this regard.

There exist several methods for measuring microscopic objects. We will mention only the following for it is the simplest and most rigorous.

Good microscopes, those of Nachet in particular, are furnished with two types of micrometers, i.e. two glass slides divided into equal parts by lines engraved with diamonds. The first is the ocular micrometer (thus called because it is introduced into the ocular itself), in which the millimeter is divided into ten parts; the second is the objective micrometer (placed below the objective and on the stage itself of the microscope) in which the millimeter is generally divided into a hundred parts.

If the objective micrometer is used as the true microscopic object, the divisions engraved on the glass slide can be exactly focused and, after introduction of the ocular micrometer into the ocular, a little trial and error will bring any two of the divisions of these two micrometers to coincide exactly. This done, another similar coincidence, either to the right or to the left can easily be found. From here, it is easy to count with precision how many divisions of the ocular micrometer between the two coincidences are needed to cover the subjacent divisions of the objective micrometer.

Let us suppose that for these observations we are using objective no. 3 and ocular no. 2, a combination which gives an average magnification of 390 diameters (we are intentionally choosing these numbers considering that they are perfectly suitable for medico-legal research of blood stains). After focusing of the divisions of the objective micrometer (the millimeter divided into a hundred parts), the ocular micrometer (millimeter divided into ten parts) is introduced into the body of the ocular. After the two superimposed divisions coincide, it is determined by scrupulous counting that twenty divisions of the objective micrometer are exactly covered by sixty-six divisions of the ocular micrometer.

The divisions of the objective micrometer equaling $\frac{1}{100}$ of a millimeter, it results that $\frac{20}{100}$ of a millimeter equal sixty-six divisions of the ocular micrometer, or what comes to the same thing, $\frac{1}{100}$ of a millimeter corresponds to three and three-tenths divisions of the ocular micrometer. Any object of $\frac{1}{100}$ of a millimeter in diameter, seen with a microscope furnished with an objective no. 3 and ocualr no. 2, will necessarily occupy three and three-tenths divisions of the ocular micrometer; reciprocally, any object of unknown diameter,

seen in the same microscope, occupying three and three-tenths of a division of the ocular micrometer will necessarily have a diameter of $\frac{1}{100}$ of a millimeter.

Since three and three-tenths divisions of the ocular micrometer represent $\frac{1}{100}$ of a millimeter, one of the divisions of this micrometer represents $\frac{1}{330}$ of a millimeter. For the measurement of microscopic objects, only this last figure is suitable for retention. Let us measure the diameter of a red blood cell with its help.

After arrangement of the preparation intended for microscopical examination in a suitable manner (we will indicate the procedure further on), the various blood cells observed are meticulously focused. The above-mentioned ocular micrometer is then introduced into ocular no. 2, and the number of divisions and fractions of divisions occupied by a blood cell lying flat is determined. In measuring various blood cells in different sites, it is found that a blood cell occupies on the average two and six-tenths divisions of the ocular micrometer.

/147 Each division of the ocular micrometer representing $\frac{1}{330}$ of a millimeter, two and six-tenths divisions represent $\frac{2.6}{330}$ millimeters. This value is simplified in dividing 330 into 2.6 and $\frac{1}{126}$ of a millimeter is obtained as the exact measurement of the blood cell of man.

The natural conclusion of these observations is self-evident: "To measure the diameter of any microscopic object whatever, it suffices to furnish the microscope with objective no. 3 and ocular no. 2, to focus it exactly, to see how many divisions of the ocular micrometer the diameter of the object being examined occupies. The number of these divisions substituted for the numerator of the fraction $\frac{1}{330}$ will give in fractions of a millimeter the exact diameter of the object in question".

The diameter of the normal blood cell is almost invariable. The maximum it varies is between $\frac{1}{124}$ and $\frac{1}{128}$ of a millimeter. It is undertandable from this of what importance is the exactness with which this measurement is performed, from a medico-legal viewpoint.

It is fitting here to present some explanation concerning endosmosis in blood corpuscles and the difficulty often encountered in observing them intact. Each of these little water-skins, called blood cells or blood corpuscles, is filled with a reddish liquid denser than pure water. As soon as water is added to a droplet of blood a rapid endosmosis is established between the contents of the blood cell and the external liquid. The biconcave disc gradually deforms under the continual influx of liquid into its interior; it swells, takes the form of a small sphere, pales considerably, breaks up and disappears. There remains only some formless, scarcely visible debris of the external transluscent memberane. If the blood cells are placed in contact with a liquid denser than the

/148 contents of the blood cell, the inverse phenomenon is produced; each blood cell gradually empties itself of liquid it contains, its surface wrinkles and shortens. After a little while, if the difference in density is rather considerable, the blood cell finds itself reduced to a small corpuscle, externally crenated, and greatly diminished in volume.

The biconcave form and the diameter of $\frac{1}{126}$ of a millimeter can only be found, then, in blood cells which have not been subjected to either of these two phenomena, and consequently have not undergone any deformation.

If the blood is fresh, nothing is simpler than to determine the presence, form and diameter of blood cells. It is otherwise for blood dried on the surface of a fabric or of any other object. This case must be recognized as the most frequent and most difficult for the expert. In the small red stain submitted for examination, blood cells exist; they can regain their form and their diameter. The only difficulty lies in dilution of solid blood such that there is no appreciable endosmosis or exosmosis for the blood cells, and consequently, no deformation to be feared.

The best liquid which could be used would be the serum itself of a blood-letting, used after filtration, if it were not often a bit difficult to procure and conserve it, and if the origin of this liquid itself didn't tend, regardless of what is done, to cast doubt on medico-legal experiments. It is preferable to employ artificial liquids whose density is intentionally brought to approximate that of serum, so as to avoid all endosmosis of liquids; for example, a solution of sodium sulfate, gum or sugar made in proportions such that the density is about 1.028. These liquids have only one inconvenience, which is often serious; they spontaneously concentrate by evaporation, and taking on a higher density, deforming and 14
shrinking the blood cells they are supposed to conserve.

As well as many micrographs, we have had in our possession a liquid remarkable for the facility with which it conserves blood cells[1]. It concentrates very little by spontaneous evaporation at the surface of the bottom slide, and keeps indefinitely, with no clouding or any alteration whatever.

The following mixture, which we have been using for the past five years, and whose formula we present, offers the same advantages:

Liquid specific for the preservation of blood cells

Ordinary glycerin of pharmacies . . . 3 parts by weight
Concentrated, pure sulfuric acid . . . 1 part
Distilled water in sufficient quantity to obtain a solution which gives a density of 1.028 at a temperature of +15°.

The presence of sulfuric acid does not alter the form or color of the red corpuscles in any way. The mixture of this acid and glycerin with water largely delays the evaporation and concentration of the liquid. It is necessary to avoid touching it with a metallic instrument and to restrict drawing from the flask to glass tubes.

Operating procedure. After a long and meticulous examination in broad daylight, a single stain is chosen, clearly limited and very distinct and having escaped, as much as is possible, any serious traction or rubbing. A fragment of stained material, with a surface area the size of a 20-centime piece

[1] This liquid is sold by a manufacturer of microscopical objects.

is removed with sharp scissors, or the point of a scalpel, and deposited on the bottom slide. A few drops of preserving liquid
/150 are drawn from the flask by means of a tapered tube and dropped on the material, and imbibition is allowed to occur for about three hours. At the end of this time, the fragment of material is rubbed, turned over several times, and finally unravelled on the surface of the glass slide by means of two small solid tubes tapered at their extremities so that the insoluble substances are detached and placed in suspension. After removal of the fabric, there remains on the slide a droplet of liquid somewhat clear, somewhat colored, which is immediately covered by a very thin glass slide and placed on the stage of the microscope. As we pointed out previously, the magnification which appears most favorable to these observations is obtained by the combination of objective no. 3 and ocular no. 2 (Nachet microscope). Apart from the red blood cells which can be discovered in this examination, a certain number of foreign bodies are generally seen whose origin is self-explanatory: 1) debris of cotton, hemp or wool fibers immediately recognizable by their considerable size and length; 2) cells and debris of epithelial cells if the fabric being examined comes from a shirt, trousers, handkerchief or any other article of clothing in contact with skin or mucus; 3) amorphous bodies of very diverse origin, which are instinctively ignored, because there are never two of the same form and they are extraneous to the purpose of the research. If, on the contrary, the preparation contains red blood cells, they are immdiately perceived in considerable number, sometimes several hundred at once, presenting a uniformity of diameter and color. It is then that the exact measurement of a few of the least deformed of these blood cells is taken. The average of these various observations approximating the
/151 true diameter of these blood cells ($\frac{1}{126}$ of a millimeter) suffices to settle the medico-legal question. It often happens that endosmosis or exosmosis cannot be completely avoided, and the bloodcells do not present a diameter of exactly $\frac{1}{126}$ of a millimeter. The divergence in this case is not very considerable, and the general form, the color, as well as the large number of blood cells observed, suffice to demonstrate that the stains are of blood.

It is unnecessary to add that despite all imaginable precautions, blood cells of this kind never present the sharpness of unaltered blood cells. Experience in observation by microscope and in this sort of examination are indispensable for an expert charged with these determinations.

It is good in these assessments to have constantly on hand and to observe from time to time a glass slide covered with some blood. This slide is easily prepared in the following manner: a fine droplet of blood is placed on a very clean glass slide, and immediately spread over a large area by a small tube or the feather of a plume. This blood dries in a few moments and constitutes a very convenient, unalterable preparation in which blood cells preserve their form, their color, and their true diameter.

In the observation of white blood cells many micrographers have looked for a method surer than the preceding to determine the presence of blood stains. We can not agree with this opinion and here are our reasons: it is without doubt that white blood cells resist washings and various deformations accompanying the action of aqueous liquid on dried blood more than red blood cells. We readily add that this latter character should tend to render them preferable to red blood cells themselves in many cases in medico-legal research, except for the following fact which just about equals an absolute rejection. In the opinion of every micrographer, white blood cells completely resemble mucus and pus corpuscles in their form, color and diameter. It would suffice to have some nasal, urethral, or other mucus, some pus from a pimple, boil or superficial abcess present to lead an expert into error. Simple enunciation of this fact condemns this method of investigation. It is suitable to add here that white blood cells, in relation to red blood cells, are present in an extremely small proportion, and that they easily escape observation, due to their singular transparency. Although the determination by itself of these white blood cells does not prove very much, we are obliged to add that the simultaneous presence in the same stain of red blood cells and white blood cells, however, constitutes an additional proof in favor of the existence of blood. The expert will not be neglecting anything in observing it and pointing it out when the occasion presents itself.

To sum up, there exists today in science only one sure means of expressing an opinion on the presence of a blood stain on the surface of a fabric or any object whatever. This means is observation by microscope of the form, color and diameter of red blood cells. In all the cases where this observation does not reveal anything positive, we conclude in the negative, no matter what the external appearance of the stains submitted to our examination.

If it is of importance in the preliminary examination to determine the presence of a blood stain; it is sometimes just as imporant to know if the blood is human blood. This second part of the expert's task is always the trickier.

The solution of this problem is still to be found: no one today takes seriously the indications furnished by a Parisian chemist, who formerly claimed that by only the odor developed by contact of such and such a stain with sulfuric acid, or the series of bizarre chemical reactions devised by an Italian chemist, it could be determined if blood comes from such and such an animal.

At our present level of knowledge, human blood differs from other mammalian blood only in the diameter of its red blood cells. It is only micrometry, then, which would be able to furnish the solution of this desideratum, if the diameter of red blood cells of the principal mammals did not, unfortunately, closely approximate the diameter of blood cells of man. The following table presents the results:

Animals	*Diameter of red blood cells*
Man	$\frac{1}{126}$
Dog	$\frac{1}{139}$
Hare	$\frac{1}{142}$
Pig	$\frac{1}{166}$

Ox	$\frac{1}{168}$
Horse	$\frac{1}{184}$
Sheep	$\frac{1}{209}$

If the red blood cells of man are compared to those of the other animals presented in the preceding table, it is evident that the diameter of the former are larger. *A priori,* it would seem easy, then, to express an opinion on the origin of blood stains submitted to assessment by an exact measurement. This is not entirely so. Other than the fact that it is often difficult in micrometric measurements performed by microscope to be answerable for an error of $\frac{1}{500}$ or $\frac{1}{600}$ of a millimeter, the changes of dryness and humidity, to which the blood could have been exposed, the more or less rapid endosmosis produced during moistening, and the deformation which can result, are so many incentives for hesitation on the part of the /154 expert. Such a circumstance is possible giving in the observation, and the micrometric measurement, a diameter a bit larger or smaller than human blood cells and consequently, cause them to resemble blood cells of another animal. The inverse would be more serious. When the difference in diameter with the blood cells observed is considerable, if, for example, a series of executed measurements gives an average of $\frac{1}{200}$ of a millimeter, if the blood cells do not present any appreciable deformations, tears, folding or crenation, so that it appears evident that their external volume has not been appreciably modified by dessication or moistening, the expert can claim that the examined stain does not appear to come from human blood.

The most delicate case is the following—given that the expert has determined in the most evident manner the presence of red blood cells in considerable number, and that the average of all the measurements is precisely $\frac{1}{126}$ of a millimeter, should he conclude in the affirmative as to the presence of human blood? Enlightened today by the experience of several years, especially dominated by the fear of a chance coincidence and the awesome responsibility of a conclusion which sometimes draws capital punishment, we do not hesitate to reply: *Even in this case, the expert should remain doubtful and be wary of affirming that blood is human blood.*

Not all red-blooded animals have circular blood cells. Without citing the few exceptions in the mammals, it has been demonstrated that all fish, birds, batrachians, ophidians, etc. have elliptical blood cells and an interior nucleus. It is superfluous to add that the single determination of this type of blood cell suffices for rejection of the possibility of human blood. This is the most favorable case for the accused, given that, as a result, there can be no doubt in the mind of the jury of the exact nature and origin of the blood stains, if the expert correctly exposes these facts.

/155 Observation I. In the month of October, 1860, a man was found murdered in the neighborhood of B . . . , stabbed twice with a knife, which must have caused a rapid death. There were traces of a struggle around the corpse. Legal machinations immediately intervened and directed the chase in several directions. Two days after the crime was discovered, a man was arrested and his premises meticulously searched. Among other objects were seized a blue smock, as well as a cotton handkerchief, both covered with blood stains. The defendant denied this, but could not find an explanation for the above-mentioned stains, which he attributed sometimes to a nosebleed, sometimes to an old wound incurred on his hand and of which there remained a slight scar. He is, moreover, of a very limited intelligence and does not seem to understand the importance of the questions very well. On the execution of a rogatory commission of the Public Prosecutor's Office of B . . . . , these two stained objects are submitted for our examination. We have to answer the following questions:

1) *Have the red stains soiling the smock and the handkerchief of Mr. X . . . been produced by blood?*

2) *In the case of the affirmative, is this blood human?*

A careful inspection of these two objects reveals at first the following facts: 1) the smock was stained at the opening and in the interior of one of the two pockets; 2) the handkerchief was stained in two places, the stains being large and very stiff.

Examination by microscope shows us we are dealing with elliptical blood cells. The largest diameter was $\frac{1}{69}$ and the smallest $\frac{1}{116}$ of a millimeter. We were quite happy, in addition, to discover, buried in the middle of one of the large stains of the handkerchief, three shiny scales, whose form and sparkling color, as well as the presence of sinuous parellel striations sufficently characterized them as scales of fish. As a result of our research, which we present here only in summary, we adopted the following conclusions:

"1) The red stains soiling the smock and handkerchief of Mr. X . . . have certainly been produced by blood.

2) The red blood cells observed in the preceding stains, being of elliptical form, can only belong to blood of fish, bird or reptile. Due to the presence of three fish scales found by us in the middle of one of these stains, it is highly probable that the blood soiling the smock and handkerchief of Mr. X . . . is blood of fish. It is certain, in any case, that these stains were not produced by human blood."

As a result of our report, there was a dismissal of the charge.

Forty days after these events, the real murderer was discovered and convicted as a result of thorough confessions to 156/ this last crime and several others.

It was hardly a couple of days ago (November 1864) that Ambroise Tardieu and myself received the following rogatory commission:

Observation II. "We, Louis-August Parmentier, examining magistrate of the district of Sancerre,

"Owing to the proceedings conducted at the request of the Public Prosecutor against Marie D. . ., wife of Louis F. . ., proprietor and farmer, residing at Garigny, accused of the double crime of castration and poisoning:

disclose the following facts:

§I. — Marie D. . ., thirty-five years old, married to the said F. . ., well-to-do farmer, but of limited intelligence.

Given to profligacy these past several years, this woman was most recently the lover of the man named Simon J. . ., who customarily worked at her home as a thresher in the barn; this liaison, however, was terminated last June as a result of the marriage of Simon J. . . . The woman F. . ., seeing herself abandoned by her lover, gave rein to an intense resentment and resolved to exact from him a terrible vengeance. She entices him to her home on the evening of October 23 last, under the pretext of remitting to him a sum of money she owed him, and makes toward him the most provocative advances and overwhelms him with caresses which were received with utmost indifference. She went so far as to unbutton his trousers and take his sexual parts in her hands. Finally, at the moment when J. . . made a movement to escape her grip, she severed his member with a razor. Despite the damning testimony of the injured, who survived this hideous mutilation, despite other charges useless to mention here, the accused persists in denying the charges. A petticoat spotted with red stains was seized at her home. Those stains, presumed to be the blood of her victim, were, according to her, the blood of a goose which had fallen on her petticoat while she was bleeding a fowl. It would be useful, then, to submit these stains to a serious examination and to verify if they were produced by human blood or the blood of a fowl."

§II. This second chapter of the rogatory commission deals with a poisoning executed by the woman F. . . on the person of her child of ten months. It is useless to relate it here.

Following this rogatory commission, Tardieu and myself
/157 were designated by Chopin, examining magistrate attached to the court of first instance of the Seine, to proceed with the various experiments indicated above.

It is understandable of what importance it is in this serious affair to determine precisely if the petticoat stains are formed by the blood of a mammal or the blood of a bird. Now, at this very moment when we were writing these lines, this determination has just been accomplished. Here is how we have proceeded.

We cut out a strip of material from the petticoat with scissors, in a part completely free of all stains, and we dropped several droplets of goose blood which we have expressly pricked in the neck. Four days later, when the stains were completely dried and brittle, we proceeded to examine them by microscope comparatively with the stains under suspicion on the petticoat itself. The two types of stains were simultaneously treated according to the procedure indicated above, submitted to the same time of imbibition, examined under the same conditions of time and temperature, and finally, submitted to the same magnification. The two results are as dissimilar as possible. The stains produced by the blood of goose yield to the preserving liquid, and permit the observation in the field of the instrument of a considerable number of elliptical red blood cells, with an evident central nucleus and with the following average for microscopic measurements: largest diameter, $\frac{1}{80}$ of a millimeter; smallest diameter, $\frac{1}{146}$ of a millimeter. The observation by microscope of the stains under suspicion present nothing comparable; as a matter of fact, a considerable number of reddish corpuscles are indeed discovered, but *all of them* are perfectly circular, and despite the most sustained attention, we could not discover any elliptical forms. The average of twelve measurements executed on these circular red blood cells, chosen, of course, from the most intact and the least deformed, gave $\frac{1}{131}$ of a millimeter in diameter.

The conclusion of these facts is self-evident:

1) It is incontestable that the stains observed on the petticoat of the woman F. . . are stains of blood.

2) Although the red blood cells observed and measured by microscope approximate by their dimensions human blood cells more than the blood of any other domestic animal, it is *impossible for us to affirm* that the stains of the petticoat are produced by human blood.

3) It is quite certain, in any case, that the stains of the petticoat do not and could not have as their origin the blood of a goose or any other bird.

The Source of Blood in Legal Medicine*

Dr. Charles Masson

Pharmacist-Medical Officer of the Army

/385 During our stay in Algeria, we were called upon many times to give an opinion on this serious question of the source of blood.

Our research, with imperfect equipment and a very limited library, had no other result than to inspire in us a great uncertainty and a judicious reserve: sentiments which agreed poorly with the affirmative character of certain model reports, and instilled an intense desire in us to better enlighten ourselves on this important question.

At Lyon, where the scientific resources are so generously put at the disposal of everyone, the opportunity presented itself and we eagerly seized upon it.

Historical Review

The history of the methods used to characterize blood stains is divided into two periods. During the first, recapitulated by the works of Orfila around 1848, the chemical /386 characteristics of certain immediate principles formed the basis of every assessment whose purpose was the identification of blood. Albumin, fibrin, a particular coloring substance, nitrogen and iron were sought: isolated, these characteristics were without value; together, they were diagnostic.

Until 1829, no one was occupied with finding if it were possible to distinguish human blood from that of other animals. At that time, Barruel published an interesting memoir on this question whose conclusions, very coldly received by chemists, in particular, Raspail and Orfila, are unconditionally rejected today. Barruel wanted to identify the source of blood by appreciation of the odor of a particular principle liberated by sulfuric acid. Every nose cannot serve as a reagent, said Raspail; a comparable proof, we will say, does not have sufficient scientific character for legitimately winning a conviction.

The discovery of Prevost and Dumas, of blood cells of different form and dimension, and the work of Mandl[1] who demonstrated the possibility of distinguishing oviparous from mammalian blood, made little impression on Orfila, who preferred chemical experiments to the microscope, and wrote on this subject: "After having repeatedly examined by means of excellent microscopes human blood and pigeon blood, detached from fabric, not only was it impossible to distinguish them, but sometimes even to recognize that it was blood".

In 1857 the second period abruptly arrived; from the most complete obscurity came a most intense enlightenment, without transition. Ch. Robin, the eminent histologist, on the occasion of a medico-legal assessment, published a remarkable memoir[2] in which he presented his research, his oper- 387/ ating procedures and his results. The chemical experiments of Orfila were, from this moment, relegated to second place. The microscope reigned as master: only the morphologic elements of blood were invoked to decide the nature and source of the stain.

Let us quote from this work, the passages most relevant to our investigations:

> After maceration of small superficial crusts for twelve hours in liquid of Bourgogne.
>
> Each blood cell [says Robin] had just about recaptured its flat, biconcave, circular form. All were 6 to 7 μ, rarely a bit bigger.
>
> After twelve hours of immersion in the same liquid, the dissociated filaments of fabric impregnated with blood. . . .
>
> It was easier yet, he claims, here than in the first series of operations, to isolate the blood cells, to separate them from the cotton filaments. . . .
>
> Conclusions: 5) the elements of blood forming the stains on the smock are elements of blood belonging to the human species. The red blood cells found here have the volume, etc. . . . but at the present stage of science it is impossible . . . to determine either the sex or age of the individual from whom it comes.

This was indeed a big step forward! Such a brilliant beginning inspired the greatest hopes for the next solution of desiderata, which the master seemed grudgingly to confess. This, however, was not to be the case: this intense light would soon be dimmed.

This same year, Virchow wrote[3]:

> I don't believe that a micrograph can ever be authorized to determine that the life of a man depend on the yet so uncertain appreciation of the coefficient of dessication of blood cells. Blood undoubtedly dessicates sometimes in such a way that isolated globules can clearly be identified; but the dessication is under the control of so many varied conditions. And blood, once dried, can be exposed to such unfavorable circumstances, that a judgment on the importance of its constituent parts cannot be formulated with certainty.

* Translation of: "De l'Origine du Sang en Médecine Légale."

in *Annales d'Hygiène Publique et de Médecine Légale* **13** (3rd series) 385-402 and 530-549 (1885).

In 1865 Roussin, a military pharmacist, and one of the
/388 greatest authorities, ended one of the most knowledgeable and wise communications done on this subject:

> In addition to the difficulty of being responsible for an error of $\frac{1}{500}$ to $\frac{1}{600}$ of a millimeter in micrometric measurements at the microscope, the alternations between dryness and humidity to which blood cells have been exposed and the more or less rapid endosmosis produced during moistening are just as much incentives for hesitation on the part of the expert: even in the case where the average of all the measurements give $\frac{1}{126}$, the expert must be in doubt and keep himself from affirming that the blood is human blood[4].

For Blondlot, then professor at Nancy:

> If the blood has been dried, the corpuscles change, deform, and according to the density of the liquid used to dilute them, diminish or swell in such a way as to be most often unrecognizable even for the most practiced eye.[5]

In 1873 a report of the Society of Legal Medicine, drawn up by a committee composed of Mayet, Mialhe, Cornil and Lefort[6], returned us to affirmative ground:

> When the dessication is not carried too far and the stain not washed in water, especially hot water, red blood cells in a sufficient state of preservation are always found after after careful, lengthy search.
>
> Conclusion: the expert measures the blood cells and can thereby determine if it consists of human blood or not.

The following year, Rabuteau replied[7]:

> In our opinion the authors of the report of the Society of Legal Medicine go beyond present-day science, saying that measurement of blood cells permits determination of whether blood is human or from another mammal. The diameter of human blood cells is 0.0075, that of the dog 0.0073; moreover, blood cells are always more or less distorted; the conclusion that blood can be diagnosed as being human or not is inadmissible.

/389 In 1876, Malinin, of Tiflis, in a remarkable work where measurement of blood cells was the object of a profound study, does not find it possible at this present stage of science, to distinguish blood of man from blood of dog, rabbit, or pig.[8]

In 1877, Professor Cauvet said in a report on blood stains:[9]

> Research on blood cells is easy. It consists of cutting out from one of the points of the stain a piece of material, which is impregnated with a few drops of Roussin preserving liquid.
>
> Conclusions: the stains are due to blood. The size of the corpuscles observed determines whether the blood can be regarded as human blood.

In 1879 Malassez published a very interesting report from two points of view. He had been able to find characteristic blood cells where it was impossible to obtain hemin crystals; in addition, the blood cells being altered, he tried to evaluate what Virchow calls the coefficient of dessication, without, however, arriving at any affirmative conclusion,

> The circular form of the blood cells, [he points out] the absence of nuclei, show they are mammalian blood cells. Their dimensions give less certain results. These blood cells have all become spherical and lost their diameter. In addition, they have dried and their volume diminished. Taking these facts into consideration, it is seen that in their primitive state they should have had dimensions very near those of human blood cells, and of those of several of our domestic animals. Conclusions: it is mammalian blood. It is impossible to identify the blood as human or of one of our domestic animals.

In 1880, Professor Morache, principal physician, in a most instructive medico-legal report[10] presents meticulous research, to which he must have devoted himself, to assert that blood submitted for his examination was blood from a bird. Some very interesting plates accompany this work, 390/
which is stamped with the most sensible reserve.

Finally, in 1882 Vibert published a study of the possibility of distinguishing blood of man from that of mammals[11]. The study was essentially practical, of great originality, where certain points are brought out and elaborated on, they being the most delicate points of this difficult question. Here are his conclusions:

> It is always impossible to assert that a stain is formed by human blood. It can only be said, in certain cases, that it could have come from human blood.

Such are the most noteworthy original works published on this question in the last sixty years: the classical works are the most often more or less faithful reflections of this. In Briand and Chaudé, the memoir of Robin is reviewed more or less favorably. The ideas arising from particular facts are sometimes generalized without experimental control. The author, for example, formulates the erroneous proposition that blood stains on flax, hemp and cotton fabrics are most favorable for the investigation of blood cells. Furthermore, no conclusions can be drawn unless certain restrictive notes are considered, which singularly diminish the affirmative character of the exemplary report which follows them.

In Dragendorff, there is a summary of the work of Roussin.

Taylor[12] does not concede that it is possible to distinguish the blood of man from that of dog, hare or rabbit.

Hoffman[13] concludes: "It is difficult to decide if blood cells, contracted by dessication and later rendered visible by use of liquids, come from the blood of man or other mammals."

For Clément[14], the data furnished by measurement of elements of blood can be considered only indicative, except 391
in the case where it's a question of distinguishing blood of man from that of animals with elliptical blood cells.

On the subject of the species of blood, Tourdes says[15]:

The diagnostic signs are furnished by the form and dimensions of blood cells. The measurements have great value with fresh blood; but with blood cells altered by dessication and by the liquid used for extraction, the diagnosis is difficult.

Vibert[16] ends an excellent article, entirely his own work, an article we have frequently consulted, and whose essential data have been most often confirmed by our personal experiments:

To conform with scientific data, the expert must conclude thusly: Such and such a stain is not constituted by blood from such and such an animal (beef, mutton, goat, according to the accused), it comes from man or a mammal of blood cells of similar dimensions (dog, rabbit).

In bringing to light opinions of the most authoritative experts, so contradictory, this review proves how uncertain the question of the source of blood, with regard to species, still is. Studies being conducted most often during a case assessment, each experimenter conceptualized from the point of view of the particular case submitted to him, supporting his conclusions on experiments similarly performed, under conditions as identical as possible, much more than from scientific principles.

The composition of liquids used varies with each expert: sometimes neutral, sometimes acidic, they are sometimes of an alkalinity making it at first difficult to understand how blood cells resist their destructive action. Some of these liquids seem to have no action on the blood cell itself, others dilating or contracting it.

This variability in reagents as in the methods, besides, /392 explains a bit the comparable variability in the results. Some attribute to certain liquids the property of rendering to blood cells their natural softness, with their circular, flat, biconcave form: others recognize only the property of dissociating, isolating the blood cells as they are. Some regenerate blood cells in great number; others find two or three in one preparation, and this, with difficulty. For some, the blood cells regain their previous form, presenting for some a preparation like that of fresh blood; for others, they remain irregular, deformed, unrecognizable. To recognize human blood and to affirm its presence is an easy thing for some authors, impossible for the greater number of authors.

Most often, the nature of the stain substratum, wood, various fabrics, paper, iron, etc., seem without importance: if their influence is appreciated, it is different for different authors. Little is known of the causes of alteration of blood cells, what Virchow called the coefficient of dessication; there is no definition of the consequences. As for measurements, the difficulty of which Roussin had already shown in 1865, not everyone seemed to concede that the diameter of blood cells, variable even in the same animal, as well as the imperfection of our apparatus and senses, necessarily limit the scope of our evaluations.

How much this uncertainty must weigh on the expert, called on to give an opinion on a question so serious, who seeks in vain for a guide to provide him with limits in which he can and must enlighten justice!

What previous work could not provide, we looked for in experimentation. Completely immersed in the reading of these works, but operating, however, without preconceived ideas, confirming the brutal facts and reporting, as they gradually passed under our eyes, the different methods of operation which we will summarize further on, we were led to formulate certain conclusions. Without resolving a problem out of reach of our resources, it should at least result in rendering the task of the expert easier and, especially more 393/ certain, in defining better the principle elements of this very complex question.

Our research essentially tends to clear up the three following points, which seem to us to sum up the desiderata of the question;

1) To determine under the conditions which present themselves to the expert, the liquid which gives the best preparations with human blood, the most favorable to a good measurement;

2) To study the causes of the alterations of blood cells and to define the consequences, as much as possible;

3) To conduct experiments on the blood of animals in the same way as on that of man and to find out within what limits the expert can give an opinion as to the source of blood.

FIRST PART

A. Action of the Principal Liquids on Fresh Human Blood

Liquid of Virchow: *Composition*. Solution of potassium hydroxide of 30 parts per 100; potassium hydroxide 30, distilled water 70.

Microscopical Examination [hereinafter: "M.E."]: Blood cells quite regular, but immediately contracted to about $\frac{1}{176}$ of a millimeter; variable destruction after twenty-four to thrity-six hours.

Liquid of Bourgogne: *Composition*. Unknown.

M.E. The discoid form of blood cells is exaggerated. The very thick circular brim often shows traces of tears on its internal border at the limit of the center which appears more depressed. The blood cell, thickened and dilated on the whole, has, however, a lesser diameter, about $\frac{1}{139}$ millimeter.

Liquid of Roussin: *Composition*. Glycerine, 3; Sulfuric acid, 1; water, quantity sufficient for obtaining a liquid of 1.028 at +15°.

M.E. Blood cells are discolored, spherical and dilated to $\frac{1}{97}$ of a millimeter.

Iodated Serum of Ranvier: *Composition.* Potassium iodide, 2 grams; iodine, sufficient quantity for saturation; water, 100 cc.

M.E. Blood cells very colored and strongly contracted.

Liquid of Vibert: *Composition.* Mercury bichloride, 0.5; 394/ Sodium chloride, 2.0; water, 100.

M.E. Many blood cells of normal form and dimensions; average, after sixty measurements, after two and four days, $\frac{1}{126}$ millimeter; some blood cells crumpled.

Liquid of Paccini: *Composition*. Water 300, glycerine 100, Sodium chloride 2, Mercury bichloride 1.

M.E. Results comparable to preceding, but not as good.

Solution of Sodium Sulfate at 5 or 6%.

M.E. From the beginning, mixture of blood cells dilated to $\frac{1}{100}$ millimeter and contracted in the form of blackberries; then some regular, spherical globules appear, contracted to about $\frac{1}{165}$; these latter become more and more numerous and all the blood cells look this way after forty-eight hours.

Artificial Serum of Malassez and Potain: *Composition.* Solution of gum at 1.020, 1 volume; solution of sodium sulfate and sodium chloride, equal parts, at 1.020, two volumes.

M.E. Same action as the solution of sodium sulfate.

Of all of them the liquid of Vibert alters the blood cells of fresh blood the least, the greater part of them conserving their characteristic form, with a normal diameter. The expert must use this in preference to all the others when he has to examine blood not yet dried, liquid, clotted or saturating a fabric.

But, whenever practical, the procedure of Welcker, which seemed to us to have been quite successfully used in this circumstance, must be preferred to the liquid of Vibert, despite this liquid's qualities. This procedure, to be treated in the second part of this work, surpasses all the others: simple and rapid, it delivers to the expert all the diagnostic elements, definitively reunited and fixed, and at the same time material evidence of considerable importance.

If the blood is liquid, the operation is one of the simplest; if it is clotted, it suffices to take a fragment from the most fluid part, bring it to the bottom slide, and push it before the needle held horizontally, This leaves a smear of blood cells which are immediately fixed by dessication, and can later be measured.

/395 With this procedure we have obtained excellent results with clots of beef, mutton and chicken blood.

B. Action of Liquids on Dried Human Blood on Varied, More or Less Permeable Substances

Liquid of Virchow: *O.P.*† With a scraper, small scales are separated and macerated in liquid, protected from all evaporation, for a time varying from 1½ to 4 hours. A portion is then placed on the bottom slide with a very small drop of liquid; the slide is covered, and subjected to light sliding movements. If the maceration is sufficient, the dissociation is easy. If too prolonged, the small crust can take on an elastic consistency, the dissociation becomes difficult, and the blood cells undergo a light contraction.

M.E. Numerous characteristic blood cells, well isolated, more or less regularly circular, flat, biconcave. Average of 100 measurements, $\frac{1}{127}$ of a millimeter.

†For each liquid, we indicate the operating procedure (*O.P.*) which gives the most favorable result.

Liquid of Bourgogne: *O.P.* Place a drop of liquid on the stain, leave for five minutes, then brush with a fine paintbrush and transport the drop to the microscope.

M.E. Characteristic blood cells, more or less regular; the discoid form is slightly accentuated and the thickened blood cell has a diameter reduced to about $\frac{1}{134}$.

Liquid of Roussin: *O.P.* More or less prolonged maceration. Dissociation difficult.

M.E. Irregular plaques, formed by a mass of discolored blood cells, in the middle of which very clear, very colored white blood cells are distinguished; a few rare, isolated, irregular, transparent, dilated blood cells; abundant granular debris.

Liquid of Ranvier: *O.P.* More or less prolonged maceration. Dissociation difficult, hard, breaking masses.

M.E. Blood cells with very clear contour, very colored and very contracted.

Solution of Sodium Sulfate: *O.P.* Maceration; hemoglobin dissolves, the liquid colors.

M.E. Irregular plaques, uniformly pale yellow, on which 396/ very apparent white blood cells stand out; attempts to dissociate the blood cells result only in irregular debris, not very colored and granular.

Artificial Serum: Result similar to the preceding.

Liquid of Vibert: *O.P.* Maceration for about an hour.

M.E. Characteristic globules, but with no flexibility and not very clear contours; the preparation is congested with debris.

Liquid of Paccini: Results about the same as the preceding.

From these experiments it is evident that the liquid of Virchow certainly gives the best preparations under these conditions, most favorable to the diagnosis of the source of blood. Blood cells, which resist its action so poorly while in fresh blood, seem to have acquired sufficient resistance from dessication to undergo its action without alteration during all the time necessary for their dissociation and after. With a thin blood stain, dried on a knife blade two and a half months before, we obtained a splendid preparation, which we edged in paraffin, after three hours of maceration in liquid of Virchow. Fifty measurements done immediately gave us an average of $\frac{1}{128}$ of a millimeter; after forty-eight hours $\frac{1}{142}$, and after eight days $\frac{1}{153}$.

This liquid liberates blood cells by dissolving the matrix uniting them. The preparation is splendid, with no debris which can alter the clarity: all is dissolved but the blood cell! In the middle of the blood cells, isolated in infinite number, characteristic, but most often more or less deformed by reciprocal compression, are always found those that escaped any deformation, and can serve as a basis for serious measurement.

After the liquid of Virchow comes the liquid of Bourgogne, which, by the procedure recommended to us by the inventor, a procedure which marvelously suits the properties of this liquid, also gives very good preparations, but in general inferior to the preceding.

Besides, the liquid of Bourgogne, in the presence of dried

/397 blood cells, has, although to a lesser degree, this property of dilating fresh blood cells, in exaggerating the discoid form, and diminishing, however, the diameter. This action appeared appreciable to us in blood from beef and pig, whose globules seemed contracted to $\frac{1}{192}$ and $\frac{1}{180}$ millimeters. Let us now add that, in contrast to the liquid of Virchow, the liquid of Bourgogne gives poor preparations with dried blood from animals with elliptic blood cells.

To sum up, when called upon to examine dried blood stains forming a crust, thin though it might be, on wood, iron or any not very permeable substance on a weapon, flooring, woodwork, paving-stone, on straw and on certain papers, as well as on pieces of bone or fiber of clothing, the expert must not hesitate to use the liquid of Virchow, following the procedure which has worked so well for us. It is under these circumstances that he will obtain, with dried blood, the most convincing results and the most affirmative conclusions.

It is not necessary that the stain be thick or extensive. Would that every examining magistrate were well aware of this principle. It is true, if not probable, that a very small stain, as big as the eye of a needle, under the conditions we have just studied, is more amenable to yielding useful information to the expert than a shirt of cloth or calico stained all over with blood.

C. The Action of Liquids on Human Blood Dried on Fabrics

Whether the fabric is wool, fur, flax, cotton or hemp, the precise distinction as to its influence on the medico-legal investigation of blood has not been established until now. This is a gap which can explain, in a certain measure, the contradictions as well as the skepticism of certain experimenters.

/398 The following experiments permit a grouping of the different fabrics into two essentially different classes: 1) material whose natural element is not dampened by blood; 2) material whose element is dampened by this liquid.

The laws of capillarity justify this differentiation. When a drop of blood falls on a fabric, the capillary phenomena vary with the nature of the material. If this fabric is formed by elements which dampen with blood with difficulty, like wool and fur of certain animals, the liquid is almost as if pressed down at the points of contact; the blood dries in a mass more or less independent of its support. If a drop of blood is projected onto cloth or felt, the mutual attraction of the liquid molecules prevailing over that exerted between these molecules and the material, the drop tends to conserve its spherical form and to dry on the surface of the material without penetrating it.

If, in contrast, the material is susceptible to dampening by blood, the capillary phenomena are altogether different: they draw and indefinitely disperse the blood cells, which penetrate far into the central part of the material and deposit around the filament, (a barrier they cannot overcome, a veritable filter) a more or less regular sheath, constituted by blood cells strongly united, applied and drawn in every direction by the force of capillarity, without intervention from plasma, which goes beyond, and penetrates the filament itself. Does not the drop of blood which falls on linen instantaneously make an oil stain?

1) **Fabrics not dampened by blood.** With the exception of liquid of Bourgogne, with which it is suitable to follow the procedure already indicated, the procedure which gives the best results consists in scraping the surface of the stain, when it forms a crust or light coating on closely-woven linen or felt; or, if the fabric is loose and aerated like certain linens, like the fabric of Arab burnoose or of a knit, in cutting out the stain, and unravelling the subjacent material with needles on a watch glass. In both cases, a more or less coarse 399/ powder is obtained, which is subjected to the action of the liquids.

In proceeding as we have done previously, the results are perhaps less favorable, but substantially the same as those obtained from blood forming a crust on impermeable bodies: the liquid of Virchow again gives the most splendid preparations and the most favorable to a good measurement.

We will not summarize the results of our experiments in order not to be redundant. These results show nothing which should be surprising! Are not the blood cells here in conditions analogous to those in which they find themselves in dried blood on an impermeable body? Are they not independent of support and united by plasma which forms, on drying, a matrix which dissolves so easily in the liquid of Virchow, which thus liberates them without altering them?

2) **Fabric dampened by blood—Liquid of Virchow:** *O.P.* — The imperceptible powder obtained in unraveling the dry material again gives better results than direct maceration of the stain.

M.E. Very irregular blood cells, contracted from $\frac{1}{160}$ to $\frac{1}{240}$ of a millimeter, rarely isolated; more or less complete envelopes, fragmented, formed from distorted blood cells, contracted and intimately united.

Liquid of Bourgogne: *O.P.* More or less prolonged maceration. *M.E.* Results similar to preceding.

Liquid of Roussin: *O.P.* More or less prolonged maceration or absorption.

M.E. Identical results, but clearer. The limits of each blood cell are more easily appreciable. Not many blood cells isolated, and all are contracted and irregular.

Solution of Sodium Sulfate: same results, less clear.

Artificial Serum: similar to preceding.

Liquid of Ranvier: does not seem to contract the blood cells more than they already are.

Liquid of Vibert: *O.P.* More or less prolonged maceration. 400/ *M.E.* contracted, granulous blood cells, quite clear mosaic casings, but formed by granulous elements.

Liquid of Paccini: analogous to the preceding.

What is amazing here is the consistent uniformity of results and their imperfection. Whether the stain is few days or months old, whether the powder obtained in unraveling the dry material stained with blood is used, or whether the material is moistened by absorption or more or less pro-

longed maceration, the liquids, no matter whether they are dilators or not, are impotent in regenerating distorted and contracted blood cells. In every case, microscopical examination reveals the same phenomena of which these are the essential ones: filaments of cotton, flax or hemp appear covered in a yellowish varnish, forming a casing more or less complete, which the maneuvers were able to remove and sometimes to separate completely and fragment into irregular plaques. This casing, like its debris, under careful examination with the liquid of Roussin rather than any other, appears very clearly constituted by the immediate juxtaposition of irregular blood cells, contracted and intimately cemented together: a veritable mosaic at the surface of which a few independent blood cells, more or less regular, but contracted, are sometimes distinguished on a higher plane. Between the filaments, in the spaces which they circumscribe, debris of variable form is seen and in the middle of it some rare, isolated blood cells, but always distorted and contracted. An isolated blood cell is exceptionally found which has escaped the common fate by who knows what luck, and has conserved its form and perhaps its normal dimensions. But, not counting that this good fortune is very rare, what expert would dare establish a diagnosis on such a narrow basis?

Furthermore, the dissociation of blood cells in the present case is not an easy thing to do. The blood cells being intimately cemented together, the mosaic quite often fragments outside the lines separating the various elements, due to the sliding movements, and if the observer is not careful,
/401 he runs a strong risk of mistaking fragments, constituted by debris from neighboring blood cells, for isolated blood cells.

In the realm of the ideas we are pursuing, the most important phenomenon, along with deformation, is the contraction of the blood cells, an enormous contraction which resists the action of all liquids.

Why are the blood cells contracted whose dessication was very rapid, more rapid than in any other circumstance, since capillarity has dispersed them on a greater surface? Does the filament, swollen by plasma and returning to a lesser volume by evaporation of water, form its casing, drawing together the elements composing it, in this reaction? This is an hypothesis to which the following experiment seems to lend some weight. If blood drops are deposited on a strip of cloth or calico which is immediately placed under a bell-jar, in an atmosphere saturated with humidity, the blood does not dry, and, if dissociated in the liquid of Vibert, after one, two, three and even six hours, blood cells more or less distorted, but not contracted, can be noted.

In summary, as for blood stains on fabric, the expert, called upon to give an opinion on their origin, cannot ignore the fact that the results which he reports will be most conclusive if the fabric is wool or fur, but very limited, by contrast, if it is flax, cotton, or hemp. In the last case, he would search in vain for blood cells of characteristic form and dimension: he might be able to confirm that it is blood and distinguish mammal from oviparious blood, but that is all! And again, it is the presence of nuclei, more than the dimensions and the form of the blood cells, which permits him to express an opinion. However, a restriction must be made in favor of certain new, very compact material, whose finish might inhibit the destructive action of capillary phenomena; it must be also made for the case where the blood is in such abun- 402/
dance that it dries in clots.

SECOND PART
Causes of the Alteration of Blood Cells

As causes of the alteration of blood cells, without deter- 530/
mining the role of each one of them, it is generally admitted: the nature of the substratum of the stain, the thickness of the stain and its age, water, dessication, temperature and the humidity of the environment.

From the experiments summarized in the first part of this work, the influence of the supporting substratum can be deduced.

The age of the stain, within limits of one day to ten months, did not appear to us to have appreciable influence. Once fixed by dessication, the blood cell no longer tends to change, if no cause of alteration intervenes.

The experiments which follow will permit judgment of the
importance of the thickness of the stain, the rapidity of des- 531/
sication being inversely proportional to this thickness.

Under the influence of water, the blood cells swell, become spherical, and diminish in diameter. Hemoglobin is dissolved, but the cellular stroma is not completely dissolved. This last phenomenon explains how, in certain cases, blood cells could be found without having obtained the reaction of Teichmann. When water acts on fresh blood, not yet dried, only contracted, distorted blood cells, unfit for the diagnosis of the origin of blood, are obtained after dessication. If, by contrast, water acts on dried blood, on a stain forming a more or less thick crust, the deepest parts can escape all alteration.

As for dessication, its influence is poorly interpreted, in attributing to it a destructive action not belonging to it. When dessication is rapid, it suddenly fixes the blood cells in their primeval form; comparative examination has demonstrated that, under these conditions, their dimensions have not been modified. Supporting this principle, Velcker has done measurements, whose evaluations have been recognized as perfectly exact by all hematologists. Renaut[17] recommends for the measure of blood cells the procedure of Velcker, which consists in depositing a droplet of blood on the bottom slide, heated to 60°, and spreading it out immediately in a thin layer with a needle held horizontally.

If the dessication does not occur rapidly, by contrast, within limits which the experiments which follow will permit one to appreciate, the blood cell remains a very fragile element, and soon alters in form and dimension.

It is evidently not the dessication, but rather the causes tending to delay it, which wreak destruction on the blood
cells: the most powerful, which seems to sum up all of them, 532/

is the degree of humidity of the environment. Here, the element of temperature intervenes, for with a low temperature, there can be a lot of humidity with little water vapor; with a high temperature, little humidity with a lot of water vapor. No matter what other variable conditions combine to prevent the dessication (such as an article of clothing, folded up, imprisoning still humid stains and let this article of clothing be concealed, hidden in a basement, or left to the night air for nights during which the air chills below the saturation point), it is always the humidity of the environment, confined or not, which opposes the evaporation of the water of plasma during a time more or less prolonged, in a continual or intermittent fashion, and thus favors alteration of the blood cell.

To study this influence, and to define its consequences as much as possible, we deposited drops of blood on different objects (a plate of glass, a knife blade, wooden planks, pebbles, and cloth), which we immediately placed under a glass bell-jar, over a saucer filled with water. After a specific time, this blood was examined, on its removal from the humid chamber before dessication, and later, after dessication. When the blood drop was still fluid, sometimes separated as a small clot bathed in serum, we made a preparation according to the procedure of Velcker, with a droplet of this serum in which we had diluted a piece of clot. If the blood were thick, we would dissociate blood cells from it in liquid of Vibert; after dessication, in liquid of Virchow.

In order not to repeat ourselves, let us now say that, with a similar stain, after a stay of the same duration, we observed essentially the same phenomena on removal from the bell-jar, or after dessication. This is a consequence of and a new proof of the influence attributed to dessication, which suspends all alteration, and fixes the blood cells in the form they have at the moment it begins to affect them.

/533 **Summary of Results Obtained**

1) Influence of humidity on fresh human blood:

After one hour in the humid chamber: no appreciable alteration.

After two hours: blood cells generally healthy; some rare blood cells with wavy contours.

After three hours: Twenty measurements done on a preparation (Velcker procedure) give an average of $\frac{1}{130}$ millimeters; the blood cells are, in general, regular.

After four hours: regular blood cells; one in ten show buddings. Some rare contracted blood cells have become spherical.

After eight hours: healthy blood cells mixed with wavy, angular, jagged, more or less contracted blood cells and spherical blood cells.

After twelve hours: still some regular blood cells; altered blood cells predominate.

After twenty-four hours: rare regular blood cells, the others more or less altered.

After forty-eight hours: in certain cases, as a consequence of more or less great changes in humidity, due to variations in temperature, there are no more blood cells, but granulous debris; in others, morphologic alteration continues, thorny, berry-like blood cells, more or less contracted, and spherical blood cells.

After four days: all the blood cells are regular, spherical, contracted to $\frac{1}{200}$ millimeters, not changing form in passing under the microscope and more colored.

After eight days: same state; the blood of this preparation, left to dessicate, gives with the liquid of Virchow,after a month, blood cells absolutely simulating those of sheep blood, less the discoid form.

2) Influence of humidity on fresh rabbit blood:

After an hour and a half: numerous regular blood cells (average of thirty measurements, $\frac{1}{145}$ millimeters); some wavy, thorny blood cells, more or less contracted and sometimes spherical.

After three hours: three in four blood cells were altered.

After six hours: nine in ten were altered.

After twenty-four hours: all the blood cells are contracted, more or less spherical, thorny or regular.

After four days: all are spherical, contracted to about $\frac{1}{230}$. 534/

After eight days: same.

3) Influence of humidity on fresh quail blood:

After one hour: healthy blood cells, a few circular globules, tending toward the spherical form.

After six hours: some circular blood cells cracked around their edges.

After twelve hours: healthy blood cells in a ratio of 1 to 3, the others circular, more or less spherical.

After twenty-four hours: sometimes granulous debris or alteration, continuing to take on the spherical form, with an avarage diameter of $\frac{1}{160}$ millimeters. Elliptic blood cells do not take the berry-like, notched appearance of discoid blood cells; they pass directly to the circular form, then become spherical[18].

The different phases of alteration of blood cells in fresh blood, sheltered from dessication, can easily be followed step 535/ by step, by making a preparation of fresh blood which is immediately bordered with paraffin.

4) Influence of humidity on human blood dried beforehand:

Blood stains twenty days old are placed in a humic chamber for twenty-four hours, then left to the atmosphere for forty-eight hours. Under the microscope, after dissociation in the liquid of Virchow, the blood cells appear paler, smoother, their contours less well defined, but do not seem contracted; there is a lot of blood cell debris.

The same stains are placed once again into the humid chamber and left for four days. The blood cells seem almost dissolved, effaced in part. They are more transparent, the contours more blurred; but a certain number still have their normal form and dimensions.

Though it varies slightly, according to the source of the blood and the diverse conditions of temperature and humidity, successive alterations of mammal blood cells can be summarized thus: as early as the first, second or third hour, slight alteration or contours, which, wavy in the beginning,

soon become angular, sometimes giving a very clearly hexagonal or octagonal form to the blood cell; later, the sides of these geometric figures depress, the angles become more protruding, and their area is sprinkled with small projections, a kind of budding; finally, the blood cells, contracting more and more, become berry-like, thorny and reach their ultimate state from the first hour to the fourth day, the spherical form, which they can conserve a long time, and which dessication does not modify.

No liquid can regenerate altered blood cells. All that can
/536 be expected from the best of liquids is that it isolate the blood cells as they are, altered or not, without acting on the blood cells themselves. Causes of error are thus diminished, in that those which might result from alterations produced by the liquid itself are not added. Besides, would it not be mere fancy to expect a dilator liquid to exert this action with a variable intensity, proportional to the degree of alteration of the blood cell?

The contraction of the blood cell is the essential characteristic of its alteration under the influence of humidity: a contraction accompanied by diverse distortions before getting to the regularly spherical form. This ultimate state, to which hardly any attention has been drawn, is, however, the most durable. Always consistent in the same animal, it is represented by a spherical blood cell, having $\frac{1}{200}$ millimeter in man, $\frac{1}{230}$ in the rabbit, $\frac{1}{160}$ in the quail. These figures adequately indicate that the diameters of blood cells, having become spherical, are appreciably proportional to the diameters of healthy blood cells.

The essential data resulting from the preceding experiments can be summarized thusly:

1) If the support substratum of the stain is not one which would alter them, most of the blood cells conserve their characteristic form and their normal diameter if dessication of the blood occurs within the first two hours.

2) If any cause whatever delays dessication beyond this period, the blood cells are altered; the alteration becomes more profound as the dessication is delayed more, and as the blood, as a function of its source, is more rapidly alterable. Human blood seems to be the one whose blood cells present the greatest resistance to various destructive influences.

3) Dessication holds spontaneous alteration of blood cells in abeyance, and fixes them in the form they have at the moment they start to dry.

/537 4) In dried blood, the blood cell resists the causes of alteration for a much longer time.

5) No liquid is capable of rendering the primeval form and dimensions to an altered blood cell.

6) No element permits evaluation of the coefficient of dessication of Virchow, which should be more correctly called the coefficient of non-dessication; once distorted and contracted, all mammalian blood cells can resemble each other at a certain moment in their destructive evolution. In the last phase, however, the diameter of the spherical blood cells seems proportional to the diameter of normal blood cells.

7) The contraction of blood cells accompanies characteristic distortions, which are very easy to distinguish from those resulting from reciprocal compression; from the moment a blood cell conserves its flat, biconcave form with clear contours, it can be considered as healthy, and serve as a basis of serious measurement. In the opposite case, the expert must abstain: where the blood cell is altered, all diagnosis, already very uncertain, soon becomes impossible with the beginning of the alteration.[19]

THIRD PART
Measurement of Blood Cells—Source of Blood

The diameter of blood cells constituting the essential distinctive characteristic, its exact evaluation would necessarily 538/
lead to the diagnosis of the source of mammalian blood. Unfortunately, the measurement of a blood cell is a delicate operation in itself, which, furthermore, occurs here under conditions and on a basis which render the data that can result from it imprecise.

Vibert, in his work[20], studied the diverse causes which oppose an exact measurement and limit the scope of our evaluations. Let us sum them up in a few lines:

> The diameter of blood cells, even in the absence of every pathological state, varies in relatively considerable limits, not only for a given animal species, but even for a given individual
>
> The most competent authors are far from alloting the same average diameter, or the same extreme limits, to blood cells of an animal.
>
> Using this table (Table I), to what species would the 539/
> expert attribute the blood cells measuring between 0.006 and 0.008?
>
> In admitting that blood cells have absolutely fixed dimensions, it is not possible to distinguish with certitude a blood cell of 0.0075 (man) from another having 0.0073 (dog) or even 0.0069 (rabbit).
>
> Even on perfectly immobile blood cells, a measurement cannot be made within $\frac{1}{10}$ μ.

With the ocular micrometer, likewise even with the camera lucida, it is impossible, as says Vibert, to arrive at a rigorously exact evaluation of blood cells: according to our experience an approximation of 1 to $\frac{3}{10}$ of a thousandth of a millimeter can be attained.

But, if one considers that these $\frac{3}{10}$ μ intervals sometimes diminish, and sometimes increase the actual diameter, it seems to us it can be admitted that the average of the measurements will not be appreciably far from reality, in any case, within $\frac{3}{10}$ μ.

As for attributing to such and such an animal a blood cell of a determined diameter, no prudent expert shold consider it. It's not the diameter of a blood cell, but the average diameter of 50, or of 100 blood cells, which must serve as the basis of a serious diagnosis. Do not the large blood cells of pig and even of beef have a diameter near the average diameter of blood cells of man?

TABLE I

DESIGNATION	FREY	WELCKER	GUIDELINES OF THE SOCIETY OF LEGAL MEDICINE	TOURDES	DRAGENDORFF
Man	0.0046 to 0.0069	0.0045 to 0.0097	0.0075	0.0074 to 0.0080	0.0077
Dog	"	0.0073	0.0073	0.0066 to 0.0074	0.0070
Rabbit	0.00713	0.0069	0.0069	0.0060 to 0.0070	0.0064
Cat	"	0.0065	0.0065	0.0053 to 0.0060	0.0056
Horse	0.00575	"	0.0056	0.0055	0.0057
Ox	"	"	0.0056	0.0056 to 0.006	0.0058
Sheep	"	0.005	0.005	0.0047 to 0.0050	0.0045
Pig	"	"	0.006	0.0060 to 0.0065	0.0062
Goat	"	0.0041	0.0046	0.0040 to 0.0046	0.0062

The most obvious cause of error results from a variable diameter of blood cells in the same individual. Indeed, from this variability stems a very great difficulty, that of obtaining averages representing different blood cells in a normal proportion. Hayem admits that, of 100 blood cells of human blood, 75 are of average size, 12 are big and 12 are small. As a consequence, if the average obtained does not represent them exactly in these proportions, it will be too high or too low, according to whether the large or the small blood cells will have been measured in greater number. Such is the /540 cause of divergence which is inevitably produced between averages of measurement done on the same preparation under identical conditions. The expert would not know how to correct for this effect; he can only attenuate the effect by multiplying the measurements.

To this cause of permanent error is added another, exceptional it is true, but which must be taken into consideration, however, to inspire in the expert the sentiments of reserve which must always preside over the establishment of his conclusions. It results from the possible alteration of the diameter of blood cells under various pathological influences. Kelsch determined an increase in the volume of red blood cells under the influence of malaria, and Malassez demonstrated that healthy blood cells of a man which are 7.6, are 8.29 in chlorosis and 6.64 in cancer. In the presence of such considerable differences, is it not permissible to concede that, in animals, certain afflictions can likewise modify the diameter of blood cells in such a way as to impose them on the expert?

Such are the principal causes of error which oppose an exact evaluation of the diameter of blood cells, and remove from the results obtained all characteristics of absolute verity. Let us now look at the data of the experiments.

We performed our experiments with an ocular micrometer, adapted for a Nachet microscope, giving a magnification of 800 diameters.

The blood cells were immobilized by bordering the preparation with paraffin; currents resulting from evaporation of the liquid at the limit of the top slide are avoided.

1) **Measurement of fresh blood cells by the procedure of Welcker.** This operation permitted us to appreciate the degree of precision that can be attained under these eminently favorable conditions, quite exceptional in legal medicine, it is true! It gave us at the same time, an evaluation of 541/ differences resulting, all other things being equal, from the variability of the diameter of globules, a basis for comparison, permitting us to better judge the results obtained with dried blood by the same observer and with the same apparatus.

The following table (Table II) represents the averages of measurements carried out on preparations of known and unknown origin:

Table II

Origin of Blood	Average of 25 measurements Preparations of *known* origin					Average of 125 measurements		Average of 100 measurements Preparations of *unknown* origin
Man	1/129	1/125	1/130	1/127	1/127	1/127.8	0.0078	1/127
Guinea pig	1/129	1/130	1/129	1/126	1/128	1/128.4	0.0077	1/126
Dog	1/138	1/140	1/135	1/141	1/141	1/139	0.0071	1/140
Rabbit	1/145	1/139	1/140	1/142	1/144	1/142	0.0070	1/144
Pig	1/159	1/160	1/164	1/161	1/165	1/161	0.0062	1/144
Beef	1/162	1/167	1/162	1/163	1/166	1/164	0.0060	1/144
Cat	1/174	1/176	1/172	1/173	1/175	1/174	0.0057	1/144
Chicken	1/86	1/176	1/140	1/173	1/175	1/174	0.0057	1/144
Carp	1/79	1/176	1/106	1/173	1/175	1/174	0.0057	1/144

From the analysis of figures, it results that:

1) To the blood cells of dog and rabbit, which were considered by different authors as approaching closest to those of man, must be added those of the guinea pig, whose aver-

age diameter, more considerable, is so close to those of the
/542 blood cells of man, it cannot be determined if they are larger or smaller.

2) Divergences existing between the different averages of 25 measurements done on the same preparation of known origin, divergences which could likely be attributed to the variability of the diameter of blood cells in the same individual, are 3 to $\frac{4}{10}$ μ. Then, even under conditions as favorable as this, if the difference of the average diameter of the blood cells of two animals is not over 3 or $\frac{4}{10}$ μ, it will be impossible to distinguish them with certainty. It will be impossible to distinguish the blood of man from that of guinea pig, the blood of dog from that of rabbit, the blood of pig from that of ox; but one can distinguish with certainty blood of man and guinea pig from that of dog and rabbit and blood of the latter from that of pig, ox and cat.

If the figures given in the Guidelines of the Society of Legal Medicine are taken, if human blood cells are admitted to be 0.0075 and those of dog 0.0073, it will be deemed that distinction of the blood of these two animals is impossible; if, on the contrary, figures are accepted from Roussin, Tourdes and Dragendorff, 0.0077 and 0.0070, figures which closely approximate our own, it is permissible to consider that a distinction can be established. In fact, working with preparations of unknown origin, we have always obtained figures such that we could give an opinion with certainty.

3) The difference existing between the large diameters of the elliptic blood cells of chicken and carp is such that the distinction of blood from these two ovipari is an easy thing.

2) **Measurement of dried blood cells, isolated with liquid of Virchow.** To measure blood cells of known origin is an operation of little value, from which serious information
/543 cannot be drawn. Indeed, no matter what one does to maintain objectivity, one is involuntarily dominated and led too easily to preconceived results. So, neglecting the results of numerous measurements we had done under these conditions, we entrusted to our colleague and friend, regimental adjutant pharmacist Péré, fifty varied objects stained with blood asking him to remove small crusts, and to return them to us furnished with a serial number that we might determine the unknown origin.

These stains, from different sources, were one to six months old. Their support was of variable nature: wood of ash, walnut and oak (used in the manufacture of axe handles, rifle butts and floor boards), paper, knife-blade, plates of glass, straw, material of wool or fur.

We had absolutely excluded from this study material of flax, cotton or hemp, after having acquired the conviction that blood cells of different animals undergo the same destructive influences on contact with them as blood cells of man; and that the principles propounded on this subject in the first part of this work are fully confirmed by these new experiments. These principles seemed to invalidate the conclusions of the memoir of Professor Ch. Robin[21], who was able not only to find numerous intact blood cells on a smock of blue cotton, but could affirm their origin. This contradiction is only apparent, for it must be considered, as Robin wrote himself in his memoir, that he was able to remove small superficial crusts from each stain. Contrary to the opinion of Briand and Chaudé, one is led to believe that the eminent histologist found himself confronted with one of the exceptional cases noted above. In ordinary conditions, when blood drops fall on material of cotton, they do not form a crust at the surface, but are absorbed by the material so as 544
to give a stain the same appearance and equal diameter on both sides.

To finish with these materials, it remains for us to sum up their influence on nucleated elliptic blood cells. Under the conditions recommended in the first part of this work, examination of oviparious blood, dried and absorbed onto material of cotton, flax or hemp, treated with liquid of Virchow or Roussin, shows the following:

The filaments are covered in a yellowish coat seeded with more or less brilliant nuclei, of a pale rose, that much more apparent when the thickness of the coat, separated from the filament, is less. The edges of different blood cells are discerned only with difficulty: it is a smooth varnish. The elliptical form is gone. The isolated blood cells are contracted and their very irregular form recalls very little of their primeval form.

The nuclei are then the essential, the only durable characteristic, that which permits easy, certain distinction, even in these unfavorable circumstances, of oviparous from mammal blood.

Let us return to blood stains forming crust, to the measurement of their blood cells and the diagnosis of their origin. In the following table (Table III) the results of twenty assessments done on blood of absolutely unknown origin are summarized:

Table III

SERIAL NUMBER	STATE OF BLOOD CELLS	NUMBER OF MEASUREMENTS	AVERAGE DIAMETER	ATTRIBUTED TO	ACTUAL ORIGIN	NATURE OF SUBSTRATUM	AGE OF STAIN (MONTHS)
1	rather well conserved	120	1/142	dog or rabbit	dog	wood	1
2	well conserved	120	1/128	man or guinea pig	man	glass	1
3	altered in general	75	1/172	ox or cat	ox	glass	1
4	"	75	1/162	pig, ox or cat	pig	cloth	1
5	"	75	1/166	"	cat	wood	1
6	well conserved	150	1/129	man or guinea pig	man	straw	5
7	altered in general	75	1/172	ox or cat	ox	knife-blade	2

Table III—Continued

SERIAL NUMBER	STATE OF BLOOD CELLS	NUMBER OF MEASUREMENTS	AVERAGE DIAMETER	ATTRIBUTED TO	ACTUAL ORIGIN	NATURE OF SUBSTRATUM	AGE OF STAIN (MONTHS)
8	distorted at nucleus	75	1/172	oviparious	carp	cloth	4
9	rather well conserved	120	1/143	dog or rabbit	dog	wood	2
10	"	100	1/130	man or guinea pig	man	"	5
11	"	150	1/136	dog or rabbit	rabbit	"	5
12	passable	100	1/157	pig or ox	pig	cloth	2
13	"	75	1/169	pig, ox, cat	cat	wood	2
14	rather well conserved	120	1/129	guinea pig or man	guinea pig	cloth	2
15	"	150	1/138	rabbit or dog	rabbit	wood	5
16	"	25	1/233	goat	goat	"	3
17	elliptic, clear	25	1/135	(large diameter) chicken	chicken	"	4
18	passable	200	1/137	rabbit, dog	rabbit	paper	4
19	"	75	1/165	pig, ox, cat	cat	knife-blade	2
20	well conserved	75	1/129	man, guinea pig	man	wood	6

/544 From these figures, and from the numerous observations we have performed, the following information can be drawn:

Blood cells of man preserve the best of all. After them, come, in order of resistance, those of dog, goat, rabbit, cat, guinea pig, ox and pig; the blood cells of these last are always more or less profoundly altered, even in the most favorable circumstances.

The blood cells of man and dog are most colored; the others, those of the rabbit in particular, are paler, more transparent and more difficult to measure. Here is an element which, in certain cases, can be taken into account; but it must not be forgotten that man's blood cells discolor and become paler under certain influences.

/546 The blood cells of chicken and carp give excellent preparations with liquid of Virchow. The blood cells, a bit dilated it is true, conserve their elliptic form with clear contours and a very apparent nucleus. Under these conditions, it is easy to distinguish not only oviparous blood from that of mammals, but again, in measuring the large diameter of the blood cells, the blood of chicken from that of carp.

As for the diagnosis of mammalian blood, the preceding table (Table III) shows that, in short, the results we have obtained obviously approximate, in appearance at least, those given us by the procedure of Velcker. We have been able to distinguish the blood of man and guinea pig from that of dog and rabbit, and blood of the latter from that of pig, ox and cat. But, it must be admitted, this diagnosis, easy and sure in certain cases, was quite difficult and unsure in others. If, after fifty measurments, we could give an opinion with certainty when it was a question of blood from pig, ox or cat; if the diagnosis of blood of dog appeared to us, though very delicate, to have, however, a fair degree of certainty, it was only in performing one hundred fifty to two hundred measurements that we could arrive at a simple probability in favor of blood of rabbit.

The different averages of thirty measurements, done on rabbit blood from the same source, presented considerable differences so as to mislead the expert: $\frac{1}{132}$ *and* $\frac{1}{145}$ *mm.* for example. Moreover, these averages have always been greater than those we obtained by the procedure of Velcker, whereas, 547/ for other animals, the cat excepted, they have been, in general, essentially equal or less. To what can these differences be attributed? The measurement of blood cells of rabbit, paler and more transparent, is certainly more difficult; but that does not explain a constant increase in the average diameter of these blood cells.

If the dimensions of the largest blood cells of rabbit blood prepared by the procedure of Velcker are compared to that of these same blood cells isolated by liquid of Virchow, it is noted that these have a diameter appreciably greater than that of the former. These blood cells, whose diameter is exaggerated, have a peculiar aspect: they seem spread out, larger and flatter. This is not a dilation; the liquid of Virchow, after twenty-four hours, contracts them from $\frac{1}{137}$ to $\frac{1}{168}$ mm! This is a collapse of the less resistant globular stroma, which has the consequence of flattening the blood cell and slightly increasing its diameter, to the detriment of its biconcave form.

This phenomenon, especially evident in large blood cells, proves that the best of liquids is not perfect! None of them can adapt equally to the variable qualities of blood cells coming from different species and even to those of different blood cells from the same animal. However that might be, experience shows us that, relative to human blood, the diagnosis of blood of pig, ox and cat are easy, that of blood of dog, delicate, that of blood of rabbit, uncertain, that of blood of guinea pig, impossible.

In light of these facts, instilling in us controlling sentiments of reserve and the gravity of the subject and the varied causes of uncertainty and error which we have pinted out, as well as the responsibilities of the expert who owes to justice as much enlightenment as possible; for the future, when called upon to give our opinion on the origin of blood stains, we will proceed and form conclusions in the following manner.

The expert is only very exceptionally called upon to give 548/

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could likely be attributed to the
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rabbit, the blood of pig from that
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120	1/128	man or guinea pig	man	glass	1
75	1/172	ox or cat	ox	glass	1
75	1/162	pig, ox or cat	pig	cloth	1
75	1/166	"	cat	wood	1
150	1/129	man or guinea pig	man	straw	5
75	1/172	ox or cat	ox	knife-blade	2

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On the Possibility of Distinguishing Human Blood from that of Mammals. (Medico-legal Study)*

Ch. Vibert

I

/48 When medical experts have determined that stains found on clothing, weapons or other objects are composed of blood, it is not uncommon that they are asked if the blood comes from man or a domestic animal. When the species of the animal has been specified in the question and the animal does not belong to the mammalian class, the problem is relatively simple. The form and dimensions of the red blood cells, the presence or absence of internal nuclei, form very distinct characteristics, clearly differential, and which generally permit a certain reply after a well-conducted examination by microscope. But when it is a matter of differentiating the blood of man from that of another mammal, the problem becomes more difficult, for the only distinctive discernible characteristic consists of the differences in dimensions which are most often minimal. Moreover, the difficulty of an assessment such as this has long since been noted. Already in 1857, Virchow remarked: ". . . I do not believe a micrographer should ever be allowed to let the life of a man depend on the yet so uncertain evaluation of the coefficient of dessication of blood cells. Blood undoubtedly dries sometimes in a way so as to clearly recognize individual blood cells . . . but dessication occurs under so many variable conditions, and blood, once dried, can be exposed to such unfavorable circumstances, that a judgment on the size /49 of its constituent parts cannot be exercised with certainty."[1]

Most histologists share this viewpoint. However, treatises of legal medicine admit or seem to admit the possibility of recognizing from which mammal blood stains derive, and they limit themselves to recommending reserve, without explaining the numerous motives of such wise counsel. It is regrettable that the guidelines for blood stains drawn up by the Society of Legal Medicine, guidelines which merit being the official guide of experts in every other regard, limits itself to simply giving the dimensions of red blood cells of various domestic animals and then to comment laconically in its conclusions: "He (the expert) will measure the blood cells and can thus affirm if it is a matter of human blood or not."[2]

As a result of the incomplete manner of presenting the question in classical works, experts have, several times, not hesitated to affirm in court that stains submitted for their examination were produced by human blood. Now, in general, and without taking into account the circumstances specific to each assessment, such an absolute affirmation would never appear to us to be permissible. This is what we are going to try to demonstrate at the same time as we make an effort to specify the difficulties of the question.

II

At our present level of knowledge, the only characteristic which can be invoked for the differentiation of blood of various mammals consists in the difference of the diameter of the blood cells. An important remark must now be made; it is that this diameter, even outside of any pathological state, varies in relatively considerable limits, not only for the same animal species, but also for the same individual. It is thus that in blood preparations, Malassez observed about 150 blood cells in the same microscopic field with the following dimensions:[3]

	Max.	*Minim.*	*Average*
Man	0.009	0.007	0.0074
Dog	0.0087	0.0062	0.0074
Dog	0.0095	0.0065	0.0074
Rabbit	0.0085	0.006	0.0072

For man, Welcker assigns as limits 0.0045 to 0.0097, and Frey, 0.0046 to 0.0069.

It might be claimed that these extreme figures represent exceptions which should not be taken into consideration. It is possible, though not likely, that an expert, who often experiences difficulties in isolating one or two blood cells, might come across precisely these dwarf or giant blood cells. But, in any case, the intermediate blood cells vary enough between themselves so that it is impossible to attribute a blood cell with a diameter of 0.007, for example, to man rather than to dog or rabbit.

These differences in dimensions are so accentuated that very competent authors are nowhere near assigning the same average diameter or the same limits to blood cells of the same animal. Consultation of the table below is convincing:

*Translation of: "De la Possibilité de Distinguer le Sang de l'Homme de Celui des Mammifères (Étude Médico-légale)."

in *Archives de Physiologie Normale et Pathologique* **14** (2nd series **9**): 48-58 (1882).

Designation	Frey[4]	Welcker[5]	Guidelines of the Society of Legal Medicine	Tourdes[6]	Dragendorff[7]
Man	0.0046 to 0.0069	0.0045 to 0.0097	0.0075	0.0074 to 0.0080	0.5077
Dog	"	0.0073	0.0073	0.0066 to 0.0074	0.8070
Rabbit	0.00713	0.0069	0.0069	0.0060 to 0.0070	0.0064
Cat	"	0.0065	0.0065	0.0053 to 0.0060	0.0056
Horse	0.00575	"	0.0056	0.0055	0.0057
Ox	"	"	0.0056	0.0056 to 0.006	0.0058
Sheep	"	0.005	0.005	0.0047 to 0.005	0.0045
Pig	"	"	0.006	0.0060 to 0.0065	0.0062
Goat	"	0.0041	0.0046	0.0040 to 0.0046	"

/51 With this table at hand, what will the expert choose as the standard figure serving as the reference point for his research; to return to the preceding example, to what species would be attributed blood cells included between 0.006 and 0.008? Such blood cells might belong to a dog or rabbit, as much as to man, and it appears evident to us that a differential diagnosis of this kind is absolutely impossible, especially with the absolute certainty required in legal medicine.

But let us disregard this difficulty, however considerable it might be. Let us allow that for each animal the red blood cells have an absolutely fixed diameter, and let us take the figures given by the Guideline of the Society of Legal Medicine. Even working with fresh blood just drawn from a vessel, is it possible to differentiate with certainty a blood cell of 0.0075 (man) from another of 0.0073 (dog), or even 0.0069 (rabbit)? All who have performed measurements on blood elements know that such precision is almost impossible. Other than the fact that the evaluation of such minimal differences is always extremely delicate, the difficulty is singularly increased in the particular case where the blood cells are always subject to variations on whatever vehicle they might find themselves. In taking care to let the preparation lie still, to avoid even the slightest movement on the table on which the work is being performed, and to refrain from breathing near the slide or bringing your hand near it, it is sometimes, though quite rarely, possible to make out a blood cell immobile enough so that its contour can be exactly delineated with a camera lucida. But even with a perfectly immobile blood cell, we wonder how a measurement within $\frac{1}{10}$ of a μ can be done, if an ocular micrometer is used, as recommended by the Guidelines of the Society of Legal Medicine.

The preceding considerations appear to us to justify considerably our earlier assertion: that it is unthinkable for an expert to assert that these stains originate from human blood. If a very capable histologist is not in a position to /52 determine whether fresh blood, just drawn from a vessel, and prepared with all suitable precautions, belongs to man rather than to a dog or a rabbit, then all the more reason will the question be unanswerable for an expert almost always investigating dried blood. Indeed, here appear difficulties of another order which most often render the problem impossible to resolve, even if the blood to be examined comes from an animal whose blood cells are appreciably smaller than those of man.

III

It is known that in drying, blood cells lose their characteristic form to become characteristically spherical or polyhedric, to form spikes, etc. At the same time, their diameter appreciably diminishes. The conditions of heat and humidity in which the dessication took place, the nature of the substance on which the blood had been deposited, the size and thickness of the stain, and the time which has passed, play a considerable role in the degree of these deformations. But it would be nothing but day-dreaming to hope that, by taking all these factors into consideration, an estimation can be made of what Virchow calls the "coefficient of dessication" or that calculation can be made of the primeval dimensions of blood cells isolated from the preparation. On the other hand, these changes are definitive, and no reagent can restore the primeval form or dimensions to dried blood cells. All that can be asked of the various liquids used for the examination of blood stains is that they promote the dissociation and isolation of blood cells. The imperfection of this dissociation constitutes a very frequent cause of error against which it is important to be on guard. Indeed, the blood cells most often break at the same time as they separate. Either a blood cell missing a part or, on the contrary, an entire blood cell to which a fragment of a neighboring blood cell remains fixed, without any clear limit of separation, are seen under the microscope. The naturally irregular form and jagged contour then render difficult the 53/ determination of whether it is actually a matter of an intact, perfectly isolated, globule. Finally, the different diameters of the same blood cells are most often unequal, leaving it doubtful as to which it is convenient to adopt.

The reality of all these difficulties is put into perspective by a perusal of the figures below.† They are reproduced from an observation by camera lucida, at a magnification of 1000 diameters, of blood cells from blood stains of varied dates and origins. These stains were made by us under well-defined conditions or were of absolutely certain origin. The examination concerned either the small bloody crusts often found on the surface of these stains or solely on the impregnated material. In this latter case, the stained linen or fabric was divided into small pieces; each piece was wetted with a few drops of one of the following solutions:

† [The figures have not been reproduced in the translation.]

A. Mercury bichloride 0.50
Sodium chloride 2
Water 100

B. Solution of sodium sulfate of a density of 1.020

C. Solution of sodium sulfate of 1.020 ..... 100 g
Mercury bichloride ..... 0.50 g

After a prolonged maceration for ½ hour or an hour, the material was unravelled by fine glass needles, then the red or reddish liquid thus obtained was covered with a slide and brought into the field of the microscope. The blood cells appearing the least isolated and the most clearly delimited were then outlined. The figures do not represent a single unique field but a collection of blood cells chosen from within the preparation. Besides, it is less difficult to immobilize blood cells under these conditions than in a preparation of fresh blood, for here, they are often stopped and maintained by undissociated threads or fragments of the stain which are found in the preparation. For the design, we placed the paper
/54 on the plate invented by Malassez,[8] a plate which can be inclined exactly along the same angle as that of the prism of the camera lucida, so as to eliminate all deformation of the image. Use of this procedure facilitates the assurance these deformations actually do not exist. It suffices to delineate the divisions of an objective micrometer, being sure they are of rigorously equal distance from each other.

Figure 1 represents a preparation obtained from small crusts of dried blood found on the shirt of a murdered infant. Examination was performed one month after the murder. Liquid A was used.

Figure 2 was obtained from a non-scaly stain found on the same garment and examined with liquid B after 45 days.

Figures 3 and 4 represent preparations made from the blood of a rabbit, deposited on linen, and placed under conditions as identical as possible with those to which the preceding shirt had been subjected. In figure 3 the examined blood was in small crusts; it was treated with liquid A after a month. In figure 4, the blood impregnated the linen without formation of crusts. After 43 days the stain was treated with liquid B.

/55 Comparison of these four figures clearly demonstrates that it is impossible to differentiate blood cells coming from man from those coming from rabbit. It is evident how difficult it is, with such irregular forms and such unequal diameters for the same blood cell, to compare these elements either among themselves, or with typical blood cells whose exact dimensions are known.

Figure 5 is more instructive in that it demonstrates that even blood coming from an animal whose blood cells are relatively very small, such as the sheep, cannot easily be distinguished from human blood when dessicated. This figure is a reproduction of a preparation obtained from sheep blood, deposited on linen 10 days before, and treated with liquid C. It can be seen that many of the blood cells have dimensions that are equal to and even greater than those of figures 1 and 2. In *a*, ‡ a blood cell is seen to which a portion of another blood cell is adhering; there was not found, however, the line of demarcation or traces of fusion proving it is actually so. It might be objected that the stain is recent, that the liquid employed was not the same as that used for the child's blood, etc., but these objections support precisely the hypothesis we are putting forth.

We don't maintain, however, that searching for blood cells 56/ in blood stains always gives results as incomplete and dangerous with regard to interpretation. Recently, we had the opportunity of examining blood deposited two months before on a woolen garment, and we could find blood cells, the greater part of which were perfectly isolated and had preserved their normal form almost intact. This observation depends on a particular combination of numerous factors enumerated above and whose mode of action, we repeat, is yet unknown. It can be said only that, when blood is protected from evaporation, the blood cells preserve their morphological characteristics very clearly for a long time. This circumstance is not as rare in legal medicine as might be believed. It is enough that a linen or fabric be folded several times immediately after being stained for the blood to remain liquid between the folds for several days. We dipped linen into blood of a kid. After having let the linen drip a bit, it was folded in half several times, then wrapped in paper and carried to the laboratory. Only after five days was the package opened. The blood was still in the liquid state in the center of the piece of linen. A small piece was removed with 57/ a scalpel and placed on a slide without the addition of any reagent. Figure 6 represents blood cells, delineated rather haphazardly, without choosing those which were clearest as in the preceding investigation. It is evident that, in this case, it could be concluded that the blood did not come from a human being and it is the same in every case where the assessment can be done under favorable conditions, and that the blood belongs to a species whose blood cells are relatively very small. We can easily believe that Richardson[9] was able to differentiate successively the blood of calf and of sheep, which he had someone else deposit on white paper from human blood. But, even while operating under such exceptionally good conditions, the diagnosis would not have been made if the sheep or the calf were animals whose blood cells normally offered dimensions more closely approximating those of human blood cells.

It is here that it cannot be repeated enough to warn experts against rash assertions, profoundly regrettable from every point of view. The limits within which an affirmation is permitted can be indicated in the following conclusions:

1) It is always impossible to assert that a stain is formed 58/ by human blood. One can only say, in certain cases, that it *could* have come from human blood.

‡ refers to a label on one of the cells in Figure 5, which is not reproduced in the translation.

2) It can sometimes be asserted that a stain comes from a mammal other than man. But for this, it is necessary that the animal whose blood produced the stain belong to a species whose blood cells are much smaller than those of man, and that the investigation be able to be executed under very favorable conditions.

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6. In *Diction. encyclop. des Sciences médic.,* 3rd series, v. VI, p. 644.
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A Simple Method for the Forensic Differentiation of Human and Mammal Blood*

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First Communication

/293 In the biological process of Wassermann-Schütze and Uhlenhuth, we possess such an excellent method for the forensic identification of human blood that it could appear almost superfluous to publicize a new method in addition to it. If we dare to do this, however, we do it because our method is easy and because it can be a useful preliminary or auxiliary test along with the other process.

The preparatory studies were begun by one of us (Marx) in February 1903; they will be published in the April issue of this year's volume of the *Vierteljahrschrift für gerichtliche Medizin.* The principle of the method relies on distinguishing, with the aid of a microscope, the difference between the effects of homologous and heterologous sera on fresh human blood. The human blood corpuscles are quickly agglutinated by a foreign serum in such a way that, under the right conditions, the erythrocytes flatten out immediately after the addition of serum and stick together in small piles. If the foreign serum is less concentrated and older, the agglutination takes place less dramatically; in all cases, though, the differences, when compared to the effects of a homologous serum on fresh blood, are unusually clear. Figures 1 and 2 [not reproduced in the translation] show a reaction of medium strength. In Figure 1, human serum has acted upon human serum; in Figure 1, pig serum has acted on human blood. The human blood serum came from twelve-month old dried human blood, the pig blood serum from a ten-month old dried sample. The photomicrographs were taken with a Leitz objective no. 5 and ocular no. 3, which corresponds to a magnification of 1:250.

In Figure 1 (human blood with human serum), the blood corpuscles lie next to each other, though clearly separate and not gathered together into piles; in Figure 2 (human blood with pig serum), there is a most complete agglutination, in some places agglutination of the erythrocytes so that individual blood cells become unrecognizable. The difference, therefore, is that the erythrocytes of fresh human blood are influenced by homologous serum only in that they reach a state where they lie close to one another, arranging themselves next to each other, an effect which one can scarcely call agglutination compared to the reaction of heterologous serum. Here, the individual erythrocytes remain recognizable, each one clearly isolated from the other. On the other hand, the human blood corpuscles are quickly agglutinated by heterologous serum, are tightly bound together in little piles, and are finally no longer recognizable as individual cells (hemolysis).

It interested us greatly to find out that ape blood serum produced an effect similar to homologous serum; we were able to recognize a difference, in that the human erythrocytes usually took on the shape of thorn apples when acted on by human blood; when ape blood was added, on the other hand, they shrank, became polygonal, and did not show the thorn apple shape. We had at our disposal ten-month old, dried blood from an Indian ape.

The technique used in our process is the following. From dried blood in some substratum, or on linen, wood, sand, blotting paper, or similar objects, a brown to black-brown-red solution, as concentrated as possible, is produced on a slide by adding one or more drops of 0.6% saline solution. One then extracts a small drop of blood from one's fingertip with a glowing hot needle and mixes it for five to six seconds into the blood solution on the slide with a glass rod. This is covered with a cover slip and observed under higher and lower magnification for the next fifteen minutes. The fresher the heterologous blood and the stronger the concentration, the quicker the reaction is finished. With blood only a few months old, it takes place for the most part in a few seconds but becomes still more pronounced from minute to minute; with blood only a few weeks old it takes place quite drastically, almost immediately after the initial mixing. Instead of covering the preparation immediately with the cover slip, one can smooth it out on the slide and let it dry for two or three minutes. One thus obtains neat, long-lasting demonstrative preparations.

We have examined the following types of blood with definite success:

*Translation of: "Eine einfache Methode zur forensischen Unterscheidung von Menschen- und Säugetierblut." I and II Mitteilung.

in *Muenchener Medizinische Wochenschrift* **51** (7): 293 and **51** (16): 696-697 (1904).

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fluid blood		*dried blood*
human	horse	3 years old, dried on a piece of linen
dog	dog	
horse	calf	
cattle	human	dried on linen, blotting paper, wood, and in substrata two weeks to two years old
pig		
mutton	rabbit	dried for a period of two weeks to one year on linen, sand, blotting paper, wood, and on substrata
rabbit	pig	
white mouse	cattle	
	sheep	

If one wishes to proceed to a follow-up test of these experiments, we recommend that one begin by studying the action of fresh, defibrinated animal blood, diluted to half strength with saline solution, on human blood, while observing our technical prescriptions. In order to determine with certainty the variations, a certain amount of practice is necessary, practice which can be quickly acquired. Our colleagues in the institute were very soon in a position to recognize from among the preparations which we put before them whether we had allowed homologous or heterologous blood to act upon the human blood. For practical reasons, one might want to allow the term "serum" for blood solution, though it is not completely correct.

We hope that we will soon be able to report on further successful experiments with our method. We have expressly refrained from reporting whether it was possible to distinguish animal tissue sections from those of humans by our process.

In our second communication we wish to examine more closely the works of Lansteiner, Ascoli, von Decastello and Sturli, and Landsteiner and Richter concerning isoagglutinins. We wish to point out once again with special emphasis that the agglutination, caused by heterologous serum, is always accompanied by hemolysis (by a progressive decay and dissolution of the cells)†, while in the case of occasional clumping formation caused by homologous serum the erythrocytes remain visible to the last as individual, well preserved and colored cells.

We need scarcely affirm that this communication does not concern the publication of new facts but rather represents an attempt to render well-known facts useful for forensic purposes.

Second Communication[1]

/696 Since Landois[2], we have known that blood serum has the characteristic that it agglutinates and dissolves the blood corpuscles of other animal types. Our procedure is based on this phenomenon. We would have to spell out here an outline of all of our knowledge about transfusion if we wanted to indicate the foundation of our work in all its aspects. It is self-evident that we must be satisfied here with this reference to the fundamental experiments of Landois.

† Modification in stroma fibrin (Landois)

We are obligated, on the other hand, to deal with a series of more recent works since they are very closely related to our theme. Indeed, Landsteiner[3] was the first to point out that under certain circumstances the blood corpuscles of one species are agglutinated by the serum of another individual of the same species. An attempt was then made to determine the causal connection between the appearance of these so-called isoagglutinins and the pathological conditions of the individuals from whom the agglutinating serum originated.[4] Further experiments by Landsteiner[5] himself, by Ascoli[6], by von Decastello and Sturli,[7] by Langer[8], and others, however, have shown that any normal human serum can possess the characteristic that it agglutinates the blood corpuscles of another human being. In any case this characteristic is not constant; it varies in each individual. Landsteiner and Richter[9] attempted to devise a method based on such individual blood differences that would enable them to assert with certainty that a given blood stain did or did not come from a specific person. Both authors, however, came to the conclusion that, when agglutination fails to take place, one 69 cannot exclude the possibility that the blood stain under examination could have come just as well from some other individual as from the one who provided the blood corpuscles for the test, precisely because the isoagglutinins are not present in every serum. On the other hand, there are human blood corpuscles which apparently are influenced by no other human serum (compare the tables of Landsteiner and Richter, 1. c.). In any case the existence of isoagglutinins is of decisive importance for our process. We will have to come to terms with them in what follows.

First, we were in the fortunate situation for our purposes that the blood corpuscles of one of us (Marx) belonged to the insensitive group which were influenced by none of the many fresh and old human blood types which we tested,[10] while Ehrnrooth's blood corpuscles belonged to the group whose blood corpuscles were easily influenced, i.e., easily agglutinated. Our blood corpuscles thus represented two opposite types of erythrocytes with regard to isoagglutination. Besides our own blood corpuscles, we tested, in defibrinated blood, the relative susceptibility of the blood corpuscles from other persons by means of homologous and heterologous sera. Having established this in advance, we achieved the following results.

Agglutination by means of homologous[11] serum never appears so markedly as does that produced by a heterologous serum of the same age. In the case of isoagglutination the erythrocytes arrange themselves next to each other or in rouleaux forms (pseudo-agglutination: compare the article, cited above, by Decastello and Sturli); the individual blood corpuscles as such remain clearly recognizable; their pigment does not dissipate or disintegrate; there is no formation of "stroma fibrin" (Landois). Dried, homologous blood looses its isoagglutinins relatively quickly so that, after a few weeks (two to four), there are left only traces of recognizable isoagglutinating action. Dried blood from mammals shows the liveliest agglutination and hemocytolytic action to all

human blood corpuscles even years later (more than three years later according to our experience to date). If, accordingly, the isoagglutinins are only to be seriously considered in the case of relatively fresh blood (up to one month old), then in every case a reaction with a trace of animal blood, known to be of the same age, must be carried out by way of comparison in order to clear up for the practical observer whether he is observing the forces just mentioned. To aid us in cases which still remain doubtful, we use in every case the following reactions which we recommend be carried out in a specific order.

The experiments of Malkoff[12] have demonstrated that a serum of type A, which will agglutinate the blood corpuscles of type B, looses this characteristic when treated with serum B. On the other hand, we have noticed in repeated experiments that human serum C, which will agglutinate human blood corpuscles D, undergoes a strengthening of its agglutinating action against blood corpuscles D, when it is treated with any other non-agglutinating human serum or with serum D itself. Accordingly, we observe the following. In a two cc. test tube, we produce a 20–25% blood solution from our own blood, taken from the fingertip, in 0.6% saline solution; after 24 hours a layer of clear, diluted serum had formed at room temperature. We introduce a drop of our serum into the blood solution to be tested, a solution which is produced according to the technique presented in our first communication.[13] The drop of our serum is at least the same size as that of the solution to be tested; the sera are thoroughly mixed. If the agglutinating serum is heterologous, then the agglutination effect is weakened, or it is completely halted. If the agglutinating serum is homologous, then the agglutination effect is considerably strengthened. One can also carry out the experiment in the following simple fashion. One sets up our experiment according to the method indicated in *Communication I,* once with animal blood and once with human blood. If one now adds to each preparation just one drop of the particular serum, the erythrocyte clumps, formed by the heterologous blood, break up again into individual blood corpuscles, which then only gradually arrange themselves anew into loose associations. In a preparation, made with homologous blood, on the other hand, clear piles or rouleaux formations appear, or the piles and rouleaux formations grow stronger, if they were already present. We are fully aware that the introduction of this experiment in doubtful cases, where the blood traces are very fresh, means a complication of the process; we can, however, assert that we have put our method on a surer foundation by it.

The indications for using our process follow. In a case where there is a relatively large quantity of dried blood available, one will, of course, set up first the Wassermann-Uhlenhuth reaction, as one does in all other cases. From the material which is left over, one produces with a very small amount of physiological saline solution a highly concentrated blood solution of a somewhat reddish, brown-black hue; then he sets up our reaction in the manner described. In the case of blood traces which are over a month old, one can make a diagnosis without any further hesitation. If agglutination does not occur throughout the concentrated solution, then one is surely dealing with human blood (or ape blood?[14]). If very strong agglutination appears immediately and is followed by cytolysis and finally by the formation of stroma-fibrin, the blood definitely comes from an animal. If, in the case of a blood trace less than a month old, no agglutination follows, then we clearly have before us human or ape blood. If, a short time after the beginning of the reaction, agglutination appears without clear cytolysis, it could be human blood; in this case, our auxiliary reaction, mentioned above, will soon clarify the situation for us. It goes without saying that one sets up, in every case, comparative reactions with dried human and animal blood of known provenance and age, if possible of the same age as that of the blood trace under scrutiny. Last but not least, the *alpha* and *omega* of our reaction will always be the comparison with the result of the Wassermann-Uhlenhuth reaction. We believe, however, that under certain conditions the conclusion of our test can be a valuable support to the results of the biological process.

To close, a few technical observations. Our reaction takes place most clearly at room temperature. Its results must be evaluated fifteen minutes after the blood has been introduced. One tests the effect of fresh sera best in a dilution with physiological saline solution in the ratio of one part serum to two of NaCl. Serum, preserved over chloroform, soon looses its potency. Moreover, the action of sera, in a forensic context, is naturally not of the same significance which attaches to the action of old, dried blood. It can be observed, furthermore, that fresh, homologous sera can call forth an intensive rouleaux formation which has nothing to do with agglutination.

Footnote made during the correction: In cases of older and less concentrated blood solutions, the following modifications of technique are recommended. A drop of blood solution is placed on the microscope slide; a small drop of blood from the finger is placed on the cover-slide. The two slides are put together. The changes, then, make their appearance most clearly on the edges of the preparation. At the same time, such preparations make it very easy to recognize hemolysis caused by the heterologous sera by means of numerous blood-corpuscle shadows.

The blood of a different species of monkey (Meerkatze), which we were able to test in the meantime, behaved as a homologous blood. On the other hand, we did not see the polygonal form of erythrocytes which we noticed in our first communication.

Notes and References

1. I Mitteilung in No. 7, this journal, 1904.
2. Landois: *Die Transfusion des Blutes.* Leipzig 1875. *Beiträge zur Transfusion des Blutes.* Leipzig 1878. Article "Transfusion" in *Enlenburgs Realenzyklopädie* 1890.
3. *Zentralbl. f. Bakt.* 1900 **XXVII.** page 357.
4. Compare the works of Lo Monaco and Panichi. *Rif. Med.*, 1902; referred to in this journal, 1902, No. 25.
5. This journal 1903, and *Wiener Klin. Wochenschr.* 1901

6. This journal. 1901. 31
7. This journal. 1902. 26
8. *Zeitschr. f. Heilk.* **XXIV.** 1903
9. *Zeitschr. f. Med.-Beamte* 1903, No. 3
10. Compare the blood of Hübler, Mealy, Mechauk, Eiff. in the Tables of Landsteiner and Richter in *Zeitschr. für Med.-Beamte,* l.c.
11. Since we allow the different sera to work only on corpuscles of human blood, then homologous serum is always to be understood to mean human blood serum.
12. *Deutsche Med. Wochenschr.* 1900. No. 14
13. One must extract older blood spots by processing them for about two to three hours in order to obtain very concentrated solutions. In the case of fiber materials, we recommend that they be moistened in some saline solution and then be pressed vigorously between the pincers of a tweezers.
14. We hope soon to be able to continue our experiments concerning ape blood.

A New Contribution to the Specific Identification of Egg Protein Using the Biological Method*

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734 Our experiments concerning immunity have placed before our eyes in a striking fashion how the serum of animals which are pretreated with increasing doses with various poisonous substrates, whether of an organic or inorganic nature, is able to react in a very specific way. Thus the animal body reacts to the injection of toxins of diphtheria, tetanus, or of snake or eel poison, etc., by forming antitoxins which neutralize the poison. When the animal body is inoculated with cholera, typhus, or plague bacteria, it answers by producing substances which agglutinate these bacteria in the test-tube, and which break up the bacteria in the stomach cavity of the guinea pig.

These facts, established in the field of immunity against bacteria and their metabolites, have their analogues in a similar area, as the latest research has demonstrated. Thus Bordet could detect in the serum of animals pretreated with repeated injections of blood corpuscles, agglutinating and hemolytic qualities developed against these blood corpuscles.

Ehrlich and Morgenroth then provided an explanation for these specific, hemolytic qualities of sera when, by following exactly the explanation of R. Pfeiffer for the serum which destroys typhus and cholera bacteria, they attributed the effect to two substances which form in the body of the immune animal, the so-called immune body and the activating enzymes, the so-called addiment. Through the agency of the immune body, the addiment is bound to the substance of the red-blood corpuscles, through which process their dissolution is achieved.

The immune body is very stable and can bear a one-hour heating to 60°, whereas the addiment, which is also present in normal serum, is extremely unstable. Similar specific substances as these also appear in the serum of animals pretreated with injections of other animal cells.

Von Dungern was able to produce an antibody, by repeated injections with ciliated epithelium, which destroyed these cells in the stomach cavity of the guinea pig. Metschnikoff experimented with rat spleen and lymph glands from rabbits and produced, with repeated injections of these substances, a substance which agglutinated and destroyed leukocytes. In an analogous manner were found immune sera against spermatozoa (Metschnikoff, Moxter, Landsteiner), liver epithelia, etc. (Lindemann). The next step was to examine the products of animal cells with reference to their capacity to produce antibodies. Such experiments were then carried out with rennin (Briot) and trypsin (v. Dungern). Further, Bordet confirmed that substances formed in the serum of animals pretreated with repeated injections of cow's milk, substances which precipitated protein bodies when added to milk. According to Wassermann's experiments these substances of the lactosera are specific, in that the serum of animals pretreated with cow's milk precipitated only the protein bodies of cow's milk, and that of animals pretreated with goat or human milk similarly reacted only to the protein substances of these types of milk.

I was interested now in determining whether specific antibodies developed in the serum of animals pretreated with egg protein and whether the protein substances of various birds' eggs could be distinguished from one another in this fashion. For my experiment I chose first hen's egg protein.[1] I let this protein flow out of a cleaned and carefully cracked egg into a sterile beaker of sterile physiological saline solution. By beating this solution with a sterile glass rod I made it thin enough that it was suitable for injection. In this way I injected each time the whites of two to three hens, eggs into the stomach cavity of a rabbit at intervals of several days. Despite the rather high quantity of liquid, which at times reached 100 cc, the animals withstood the injections very well and appeared to be in good health with this animal nutriment. When one has administered a certain amount of albumin—the albumin from five or six eggs is enough—a few drops of the serum from these animals demonstrates definite turbidity when added to a solution of 5–10% hen egg albumin made up with physiological NaCl solution. This occurs at the bottom of the test tube because the serum, which has a greater specific gravity, sinks downward and then spreads gradually throughout the rest of the liquid. If one observes these tubes further, one can observe how the turbidity settles and a flocculated sediment forms.

This reaction becomes all the more striking the more egg albumin the animal receives intraperitoneally.

One can thus confirm that no chemical reaction can com-

*Translation of: "Neuer Beitrag zum spezifischen Nachweis von Eiereiweiss auf biologischem Wege."

in *Deutsche Medizinische Wochenschrift* **26** (46): 734–735 (1900).

pete with the exactness of these biological reactions.

In a comparable way I tested the most common protein reagents regarding their effectiveness vis-a-vis the biological reaction. I was able to obtain a clear reaction with a few drops of my serum even in a protein solution diluted to 1:100,000, while the chemical agents, concentrated potassium nitrate, acetic acid, potassium ferrocyanide, a mixture of magnesium sulfate and potassium nitrate, are no longer capable of calling forth a reaction in a dilution of over 1:1000. I am confident that the titer of my serum can be raised even higher.

Obviously, a great number of control experiments were also set up. Normal rabbit serum of a great number of rabbits never produced this reaction. At the beginning of each treatment of the rabbits their serum was also checked. It never showed the reaction described.

At the next step this serum was added to a great many different solutions of protein preparations. I selected nutrose, somatose, Deyche's alkalialbuminate, Heyden nutrients, peptone, Riedel, casein, and horse, cattle, mutton, and donkey serum. Not once was the reaction positive. Moreover, no reaction took place with a serum albumin preparations obtained from various sources.

Now, to test the reaction with egg albumin from other birds' eggs, I set up the same experiment using pigeon egg albumin. Here, too, the results were clearly positive, although they were decidedly weaker than in the hen's egg albumin solution. From this it follows that this reaction is not specific for hen egg albumin.

Moreover, the serum of a rabbit repeatedly injected intraperitoneally with pigeon egg albumin produces, when added to a solution of hen egg albumin, a definite turbidity, which is, however, not as strong as in the solution of pigeon egg albumin. From this observation it seems safe to conclude that the same albumin substances are contained in hens' and pigeons' eggs.

Unfortunately, it has not yet been possible for me to expand my experiments to other birds' eggs, since in this season those are impossible to obtain. I remain determined to continue as soon as possible these very interesting experiments with other eggs, and I will report on them later. Likewise, I will busy myself further with the very difficult chemical aspect of these reactions in order to clarify the
/735 process of this reaction. The important question arises here as to whether the precipitation takes place in the protein solution or in the serum added. So far I am able to report only that the serum still causes as clear a reaction as before after being heated to 60°C for an hour.

It was of further interest to discover whether, after repeated intrastomach doses of hen egg protein, these bodies formed in the serum of such rabbits. In order to determine this, I administered with a probang for several weeks a daily dose of hen egg protein, beaten, and diluted with a physiological saline solution. The serum of these animals was tested every eight days; the reaction remained at first negative. After 24 days a positive reaction took place. In order to multiply the antibodies as fast as possible in the serum of the animal. I gave it a hen's egg white both morning and evening for twenty-four days. It turned out that the reaction produced by the serum was essentially not more definite even though the animal had up to that day received forty-three egg whites *per os* (orally).

The observation seems to me to be of special significance because it proves that, despite the effects of stomach acids in the case of intrastomach application over a long period of time, specific antibodies can form in the animal's body, a fact that is also to be considered in immunization experiments *per os*.

As we see, the results, briefly sketched here, encouraged us to approach the question of the biological differentiation of protein substances; all the more so since we have not come very far with the purely chemical approach to this problem in the last few years. The biological method is so much more full of promise because the reaction far exceeds all the chemical methods in exactness.

Following this line of thought, I set up, among other things, an important problem for myself: to prove whether it would be possible to distinguish by means of the biological method a great variety of blood types. One observation, which I had made with the serum of a rabbit pretreated with hen's blood, especially inspired me to go on. Such serum produces a definite, rapidly developing turbidity when added to a laked hen's blood solution, which was extremely diluted (a weak red color). The turbidity gradually settled as a flocculated sediment. This same serum produces no turbidity in similarly prepared solutions of horse, donkey, cattle, mutton, and pigeon blood. I wish also to mention that this serum has not as yet called forth any turbidity in a solution of hen's egg albumin. Rabbit serum also does not produce turbidity in any of these blood solutions.

If we summarize briefly the chief results of our experiments, we obtain the following:

1. When rabbits are repeatedly injected intraperitoneally, as well as in the stomach, with a solution of hen's egg albumin, substances form in the serum of these animals which produce a turbidity, i.e. a precipitation, when added to a solution of hen's egg albumin. This reaction occurred also in a solution of pigeon's egg albumin.

2. The serum of a rabbit pretreated intraperitoneally with pigeon's egg albumin contains substances which produce turbidity, i.e. precipitation, both in a solution of hen's egg albumin and in one of pigeon's egg albumin.

3. The reaction, caused by the serum of rabbits pretreated in this way, occurs only in egg albumin, not in the many other sorts of protein which I tested.

4. This biological method of protein identification surpasses in accuracy the chemical reactions and is suited, most likely to a high degree, for differentiating the different varieties of protein substances.

5. The serum can stand a one-hour heating to 60° without losing its reactive capacity.

To close, allow me to express my most humble thanks to

Professor Dr. Loeffler for the kind interest he took in my experiments.

References

1. After I determined the facts mentioned here a preliminary communication of Myers concerning immunity against proteins appeared in the *Centralblatt für Bacteriologie* (vol. 28, no. 819). He experimented with crystalline egg albumin, serum globulin, and Witte's peptone, and he came to similar results as mine.

A Method for the Differentiation of Various Specific Blood Types, In Particular for the Differential Diagnosis of Human Blood*

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/82 In my study entitled "A New Contribution to the Specific Identification of Egg Protein Using the Biological Method" (this Journal, 1900, No. 46), I reported an observation in the serum of a rabbit which had been intraperitoneally pretreated whith chicken blood. When a chicken blood solution, laked by adding water, was mixed with the serum of this animal, definite clouding developed rapidly. The clouding gradually formed a flaky precipitate at the bottom of the container. On the other hand, horse, cattle, ram and pigeon blood serum solutions showed no clouding when treated in the identical manner. The above finding induced me to undertake further investigations, so as to determine whether it would be possible to discriminate between the blood of various animal species with the aid of this biological method. I considered these studies important, the more so since the problem could not be solved so far with any procedure. My principal aim was to answer the forensically significant question: how to discriminate between human blood and other specific blood types.

Before dealing with this interesting problem, however, I conducted a few orientation experiments with cattle blood.

At intervals of 6 to 8 days, I injected 10 cc defibrinated cattle blood into the abdominal cavity of rabbits.

After five of these injections the animals already yielded an active serum, as shown by the experiment described below.

I first prepared solutions of the various specific blood types
/83 with ordinary tap water; I added enough water to these solutions to obtain a pale red color (dilution 1:100). I eliminated interfering stroma residue either by letting them deposit in the test tube, or by means of filtration. I removed approximately 2 cc from the resulting clear solution and placed it into a small test tube with a diameter of 6 mm, mixing an identical volume of a double concentrated physiological salt solution (1.6%) with it. It is extremely important to put the blood solution in a physiological salt solution for these experiments, since normal rabbit serum will cloud when water is added, and could interfere with the determination of a specific clouding. No clouding occurs in the normal rabbit serum when physiological salt solution is used.

The absolutely clear, reddish blood solutions, prepared as indicated, originated from the following animals: cattle, horse, donkey, hog, ram, dog, cat, stag, fallow deer, hare, guinea pig, rat, mouse, rabbit, chicken, goose, turkey, pigeon. Human blood was included in the experiment as well.

When I then added to each of my small test tubes 6 to 8 drops of the rabbit serum, pretreated with cattle blood, using a capillary tube with elongated point, clouding developed quite soon in the cattle blood solution only; it was especially conspicuous in penetrating sunlight. The rest of the test tubes showed completely clear contents. Prolonged observation subsequently revealed that the clouding intensifies and finally drops to the bottom as a definite, flaky precipitate. Normal rabbit serum causes no clouding in cattle blood solutions.

Privy Councillor Loeffler asked me to select the test tube containing the cattle blood among the above-mentioned 19 test tubes containing blood, which were unmarked and arbitrarily aligned.

After adding a few drops of my serum, I was immediately able to determine which test tube contained cattle blood.

Encouraged by the specificity of the above reaction, I used the identical method when injecting human blood intraperitoneally into rabbits. When added to each of the aforementioned series of 19 blood solutions, the serum of these animals developed clouding and precipitated only in the human blood solution. All other solutions remained absolutely clear. I wish to stress once more that normal rabbit serum causes no clouding in human blood solutions. Accordingly, I was able with this reaction, to differentiate reliably between human blood and the rest of the specific blood types listed.

It seems reasonable to assume that the specificity of said reaction applies, appropriate changes having been made, to other specific blood types as well. I am at this time engaged in studies concerning this problem; I wish to determine in particular whether the specificity exists also in closely related animal species such as the horse and donkey, for example, or whether the relationship between thse animals becomes evident in the reaction as well. It should be investigated in this

*Translation of: "Eine Methode zur Unterscheidung der verschiedenen Blutarten, im besonderen zum differential-diagnostischen Nachweise des Menschenblutes."

in *Deutsche Medizinische Wochenschrift* **27** (6): 82-83 (1901).

context, for example, whether the serum of rabbits pretreated with human blood causes clouding in monkey blood, which I was regrettably unable to obtain so far.

The reaction is extremely sensitive and, therefore, traces of blood are sufficient to determine from which species the blood originates. Accordingly, the verification of each specific blood type requires pretreatment of the animals with the various blood samples, so as to obtain a serum usable for diagnostic purposes in suitable cases. The pretreatment of the animals should be continued until the serum shows rapid clouding and produces a precipitate.

It is of particular interest that I was also able to determine with the aid of my serum the human blood among samples of human, horse and cattle blood; these blood samples had dried for four weeks on a plank and were then dissolved in a physiological NaCl solution. This is certainly a fact of special significance.

As for the nature of the reaction in question: the process presumably involves the formation of "coagulinen" in the animal organism, as defined by Ehrlich, similar to those resulting from the injection of various milk caseins, as performed by Bordet and Wassermann, and as observed by me with egg albumin; Myers observed the same phenomenon simultaneously and independently from my own findings.

In conclusion, I wish to thank Privy Councillor Prof. Dr. Loeffler for his interest in my investigations.

Additional Reports on my Method for the Identification of Human Blood*

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/260 My investigations on the differentiation of human blood from other specific blood types with the aid of a certain serum[1] were fully confirmed by the detailed work of Wassermann and Schütze[2] published shortly after my own report, as well as by the reports of Stern[3] and Mertens.[4] My method is based on the fact that the blood serum of rabbits, and of some other animal species, which were pretreated intraperitoneally with defibrinated human blood or with the defibrinated blood of certain other animal species, produces a precipitate in the laked solution of the blood in question.

I determined the above fact in the course of my work on the biological differentiation of various egg albumins,[5] while investigating whether the albumins of chicken eggs and those of chicken blood are identical. For this purpose, I had injected larger quantities of defibrinated chicken blood into a rabbit. I found that the serum of the pretreated animal, as indicated by the standard serum titer at that time, formed a precipitate in a chicken egg albumin solution, while no such precipitate resulted in a laked chicken blood solution.

I wish to refer in particular to the course of my investigations here because—as I subsequently determined—Bordet and Tschistovitsch[6] described, with reference to another matter, a similar precipitating effect in the serum of a rabbit pretreated with blood; the serum reacted with the corresponding blood despite the fact that this blood was not laked.

I continued to elaborate the aforementioned experiments, related to my studies on egg albumin; the experiment is fundamental for my subsequent investigations aimed at the discrimination between various specific blood types, as will be shown below. It became evident that the serum of a rabbit pretreated with a chicken egg albumin solution can be induced to show definite clouding and to form a precipitate subsequently, in a considerable diluted laked chicken blood solution. A less intensive clouding occurs in a goose blood solution. The serum was so effective that it produced a precipitate within seconds when a 2.5% chicken egg albumin solution was added. The effect was almost as powerful when the serum was mixed with goose, duck or guinea fowl egg albumin solutions; the reaction was weaker with pigeon egg albumin. Moreover, a rabbit pretreated with goose egg albumin solution yielded a serum which caused significant clouding in a laked goose blood solution, while the clouding was less pronounced in a chicken blood solution. When the same serum was added to goose or duck egg albumin solutions, a substantial flaky precipitate formed within seconds, while only clouding was observable in guinea fowl, chicken and pigeon egg albumin solutions. I will not elaborate on these interesting studies here; instead, I intend to report on them later in connection with my planned studies which should include as many various bird eggs as possible.

The investigations performed so far nevertheless indicate that chicken, goose, duck, guinea fowl and pigeon eggs contain albumins, some of which are found in the blood of the above-mentioned birds as well. However, the albumins of the various bird eggs cannot be as reliably differentiated with the reaction as the albumins in blood.

The fact that the serum of rabbits pretreated with human blood has a precipitating effect on laked blood solutions indicates the forensic usefulness of the phenomenon. However, the following finding was decisive for application in practice: even old blood, desiccated for a prolonged period, retains its reactivity, since material of this type is presumably under examination in most cases by forensic experts. My blood samples, dried for three months, still react efficiently. I therefore feel entitled to assume that this blood could tolerate even much longer periods of desiccation. But the human blood to be examined by the expert is not always 261 desiccated; for example, blood which putrefied some time ago could be involved.

Accordingly, the important question must be asked as to whether such material is still suitable for the reaction. To solve the problem, I let the various blood samples decompose at room temperature in the laboratory; some of the blood samples were obtained from cadavers in an advanced stage of decomposition and from anatomical preparations.

The following putrefied blood samples were used for the experiment performed on March 19:

1. Blood from a cadaver dissected on January 22 of the current year (phthisis pulmonum).
2. Blood from a cadaver dissected on January 22 (uremia).
3. Blood from an infant stillborn on January 20.

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*Translation of: "Weitere Mittheilungen über meine Methode zum Nachweise von Menschenblut."

in *Deutsche Medizinische Wochenschrift* **27** (17): 260-261 (1901).

4. Blood from the anatomy mortuary; the blood was already significantly putrefied when the sample was collected on March 1.

5. Blood from a subject whose death was caused by military tuberculosis.

6. Blood samples from healthy persons, which were subjected to putrefaction a) since February 20, b) since March 4, and c) since March 10.

The following served as controls: blood samples from the ram, hog, horse, donkey, cattle, cat, dog, goose, chicken, hare, rabbit and stag, which underwent putrefaction for the same length of time.

All these samples were discolored, reddish to blackish-brown, with a penetrating odor, partially indicating the presence of H_2S. When a glass rod immersed into HCl was held over the samples, ample volumes of ammonium chloride vapors were released by the putrefied fluid. The reaction was weakly alkaline.

These putrefied blood fluids were then diluted considerably with a physiological salt solution, according to the method described by me. Filtration through a sterilized Berkefeld's filter, which retains all bacteria and other corpuscles, followed. A filtration of this type can be rapidly performed with the aid of a water jet suction device fitted to any water system. The filtration is definitely necessary because an absolutely clear blood solution for the reaction is obtainable only with this method. The filtration also sterilizes the blood solution, which can then be stored for a prolonged period of time. Subsequently, approximately 4 cc of the resulting, partially yellowish-brown and partially reddish fluid is mixed with 12 drops of my serum. All test tubes containing human blood[7] showed clouding; the fluid in all other test tubes remained clear.

The above experiment shows that the reactivity of human blood was not eliminated by up to three months of intensive, odorous putrefaction. This fact is presumably of general biologic interest. Further tests should be carried out to determine whether a still longer period of putrefaction alters the blood, thus preventing a specific reaction. Such an effect, however, seems highly unlikely because putrefaction, like fermentation, stops after a certain period of time, before all substances subject to putrefaction or fermentation, respectively, are completely converted.

I included still other questions, important in practice, in my studies. Since the blood to be evaluated can be suspended in a wide variety of liquids, the forensically significant question arises as to whether blood in such liquids is eventually still reliably determinable. Among various blood wash waters prepared with weakly alkaline soap, the water containing human blood could be readily verified. Human blood in menstrual urine could likewise be successfully determined; all other urine samples mixed with cattle, hog, ram, chicken, horse and cat blood failed to react.

Moreover, I was able to diagnose traces of human blood immediately among various blood traces frozen in snow at $-10°C$ for 14 days.

As expected, the reaction was likewise definitely positive in human blood solutions in which hemoglobin had been converted into carbon monoxide-hemoglobin.

Stern[3] and Mertens[4] recently pointed out that rabbit serum, formed after the injection of human blood, also causes a precipitate in human urine containing albumin. I can fully confirm their finding: several urine samples with high albumin content showed a characteristic reaction. The reaction was especially intensive in a urine sample containing fetid pus, originating from a cystitis and pyelonephritis case. The serums from rabbits pretreated with chicken and goose egg albumin caused no clouding in such urine samples containing albumin.

As for the serum used for the reaction: it will tolerate heating to 60° for 1 hour without loss of its precipitating property. The specifically coagulating substances seem also quite resistant to preservatives, such as carbolic acid (Carbol), for example. Admittedly, my findings in this respect are not yet conclusive. It is certain as of now, however, that serum mixed with 0.5% Carbol remained reactive for three months. But, whenever feasible, I always prefer to use quite fresh serum for the reaction. It is suggested that five to six large and vigorous rabbits be subjected to pretreatment; the serum volume needed for the examination can then be obtained at any time by taking blood samples from the ear vein, without killing the animals as a result of exsanguination. When the blood has clotted, the serum is removed and centrifugation is performed, so as to obtain a clear serum. I collect the blood needed for the pretreatment with a sterilized Heurteloup cupping device, like that used for therapeutic blood elimination in ophthalmology. With this method, 10 to 20 cc fluid blood is readily obtainable; defibrination and injection into the rabbits can follow immediately. Accordingly, blood is obtainable without difficulties; the required volumes are readily available at any time from healthy persons as well. As for the chemical nature of the reaction, I am engaged in the study of the same at this time. I wish to state now merely that the precipitate originating from the serum is soluble in excess NH_3 as well as in H_3PO_4.

In conclusion, I wish to thank Privy Councillor Professor Dr. Loeffler for his interest in my investigations.

Notes

1. *Dtsch. Med. Wochenschr.*, 1901, No. 6
2. *Berl. Klin. Wochenschr.*, 1901, No. 7
3. *Dtsch. Med. Wochenschr.*, 1901, No. 9
4. *Dtsch. Med. Wochenschr.*, 1901, No. 11
5. *Dtsch. Med. Wochenschr.*, 1900, No. 46
6. *Ann. Inst. Pasteur*, Paris, 1899
7. I used small test tubes with a diameter of approx. 8 mm. I added the serum drop by drop, from a capillary tube, the point of which had been elongated over a flame. When the reaction is to be accelerated, the test tube is placed near a hot oven or into the incubator at 37°.

Additional Reports on the Practical Application of My Forensic Method for the Identification of Human and Animal Blood*

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/499 I stated in earlier publications that the identification of human blood—under a wide variety of practically important conditions, such as blood dried for a prolonged period, putrefied for several months, or frozen—will definitely succeed with the method initially indicated by me.[1] The observations made somewhat later by Wassermann and Schütze[2] have by now been fully confirmed by Stern,[3] Mertens,[4] Dieudonné,[5] and quite recently by medical examiners, including the reports of Ogier[6] from the Toxicology Laboratory in Paris, and on the quite extensive material of the State Medical Institute at Berlin through the investigations carried out by Ziemke.[7]

All these studies prove the forensic usefulness of my method brilliantly. Its value will become most evident when investigating as large a number as possible bloodstained *corpora delicti,* such as those submitted in forensic practice to judges and experts. I had several recent opportunities to examine such objects, kindly made available by the State Prosecutors, in particular by First State Prosecutor Mr. Hübschmann at Greifswald, as well as by the Director of the Local Institute for Legal Medicine, Dr. Beumer. Sentence had already been passed on some of the cases at issue here; the origin of the blood adhering to the submitted *corpora delicta* was not in doubt; however, at my request the information was initially withheld by the aforementioned gentlemen, so as to control the accuracy of my diagnosis. Some of the cases were new, and the specific blood type was subject to doubt, either from the start, or during the legal proceedings. Since the last-mentioned cases are not yet *res judicatae,* I am unable to report on them at this time, but will do so later.

As for the old (*res judicatae*) cases: I will briefly summarize the results of my investigations.

1. 1 meter long, ridged club, with a few faded brownish, stains, from the year 1900.

Some of the suspicious material was scraped off and diluted in a physiological salt solution. The resulting liquid shows no definite color; it is clear and foams slightly when shaken. Five drops of serum from a rabbit pretreated with human blood (Serum E) are added to 4 cc of the above liquid. Clouding results almost immediately; it soon deposits in the form of a precipitate.

Diagnosis: human blood.

Subsequent information: a case of serious bodily injury; blow on the head. Bleeding lesion.

2. Reddish sand, from the year 1896.

The sand is placed into a physiological salt solution. A pale, yellowish, clear liquid results. Serum is added as in Case 1. Precipitation occurs almost immediately.

Diagnosis: human blood.

Subsequent information: track of blood, originating from a murder committed in the vicinity of Greifswald.

3. Cotton cloth with a few reddish stains, from the year 1897.

The suspicious stains were rinsed out with a physiological salt solution. Admixture to the yellowish liquid as above. Almost immediate clouding, which soon drops to the bottom as a precipitate.

Diagnosis: human blood.

Subsequent information: the cloth was found near a strangled person.

4. Trousers with reddish, faded, small stains on the trouser fly and on the lining in the area of the genitals.

Procedure as above.

Diagnosis: human blood.

Subsequent information: suspected rape; in fact, intercourse with a menstruating person.

5. Hatchet with a few blood traces on the handle. From the year 1900.

Procedure as above.

Diagnosis: human blood.

Subsequent information: case of serious bodily injury.

Accordingly, the accuracy of my diagnosis was confirmed in all cases. The procedure used by me seems indeed to be the simplest and fastest way to demonstrate my method's forensic usefulness. Privy Councillor Dr. Loeffler therefore kindly proposed to his Excellency, the Minister of Justice, that

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*Translation of: "Weitere Mittheilungen über die praktische Anwendung meiner forensischen Methode zum Nachweis von Menschen- und Thierblut".

in *Deutsche Medizinische Wochenschrift* **27** (30): 499–501 (1901).

bloodstained objects submitted to the Courts as evidence be forwarded to the Hygienics Institute at Greifswald, where the *corpora delicti* in question could be examined by me without additional information, so as to compare my diagnosis with the pertinent documentation.

The Minister of Justice then ruled that all *corpora delicti* of the above type, from the sphere of jurisdiction of the Breslau State and Supreme Courts be handed over to me; accordingly, I expect that a larger amount of material will soon be put at my disposal.

Thanks to the good offices of Prof. Beumer, I also had the opportunity to investigate the following bloodstained objects and blood samples, respectively, without receiving preliminary data on their origin:

1. Blood-soaked linen cloth. Procedure as above. Serum E added. Negative reaction.

Admixture of serum from a rabbit pretreated with ram blood in the same test tube: negative reaction.

Admixture of serum from a rabbit pretreated with horse blood in the same test tube: negative reaction.

Admixture of serum from a rabbit pretreated with hog blood; strongly positive reaction.

Diagnosis: hog blood.

When I notified Prof. Beumer of the diagnosis, he stated that the cloth had been soaked with hog blood several years ago, for use in a demonstration.

2. Dried blood from the year 1897. Procedure as in Case 1.

Diagnosis: hog blood. Confirmed by Prof. Beumer.

3. Dried blood from the year 1900.

Diagnosis: human blood. Confirmed by Prof. Beumer.

4. Dried mixture of blood from various mammals, from the year 1889.

Diagnosis: hog and ram blood. According to information received from Prof. Beumer: hog and ram blood.

Moreover, I wish to state that I was able to identify hog blood on a bloodstained and singed music sheet found in a large puddle of blood on the Gützkower highway; this excluded any suspected crime from the start. I was also able to determine hog blood in an extract from hog organs dried for 1-½ years. I likewise identified human blood—a fact which could be added to my earlier statements—in rinsing water containing considerable volumes of carbolic acid (Carbol), sublimate and soap; the color of the water was a murky, brownish-red. The method proved successful in a 3% dissolved mixture of borate and human blood, as well as in blood-soaked garden soil, after desiccation for three months.

As these reports show, I was able in each case to diagnose human blood as well as hog and ram blood accurately.

/500 At the start of my investigations it already seemed to me of considerable forensic interest to answer the question: from which animal species a blood sample originates in cases when no human blood is at issue. To determine whether or not the blood originates from man in any given case will be of decisive significance; it is nevertheless obvious that, whenever the human blood reaction is negative, the reliable identification of the animal species from which the blood stems could, under certain circumstances, provide important cues for the further progress of the criminal investigation. I refer in this context to poaching, for example. The diagnosis is also occasionally important when the statements of a defendant on the origin of blood stains found associated with him/her are to be investigated from the viewpoint of truthfulness. Not infrequently, murderers pour animal blood over traces of human blood, so as to conceal the same. In some of these cases, besides identification of the human blood, the determination of the animal blood species could be significant.

I am therefore making every effort to prepare such specific sera, useful for solving the above problem. These studies are extensive. So far, I can report the following results:

1. The serum of a rabbit, pretreated with hog blood, yields a precipitate in hog blood solution only; the precipitation is somewhat weaker in a wild boar blood solution, while all other specific blood types used as controls remain clear. Blood solutions from the following animals served as controls for all additional experiments:

Cattle, horse, donkey, ram, goat, hog, chicken, bat, pigeon, duck, goose, owl, crow, sparrow, rabbit, guinea pig, rat, mouse, hedgehog, dog, fox, cat, stag—Man.

2. The serum of a rabbit pretreated with horse blood yields a precipitate in a horse blood solution, and a slightly weaker precipitate forms in donkey blood solution. The other blood species remain clear. The serum of a rabbit pretreated with donkey blood shows reversed behavior.

3. The serum of a fox blood-rabbit yields a precipitate in the fox blood solution, and a weaker precipitate forms in dog blood; all other solutions remain clear. (Blood solutions from the wolf and jackal were not available; their behavior is presumably similar to the reaction in the dog blood solution).

4. The serum of a hedgehog-rabbit forms a precipitate in the hedgehog blood solution only. The controls are clear. (Animal species closely related to the hedgehog could not be investigated so far).

5. The serum of a cat blood-rabbit yields a precipitate in cat blood solution only. The controls remain clear. (Blood from other predators related to the cat was not available).

6. The serum of a ram blood-rabbit forms a precipitate in a ram blood solution; its precipitate is near-identical in the goat blood solution, and weaker in the cattle blood solution.

7. The serum of a cattle blood-rabbit forms a strong precipitate in the cattle blood solution; the precipitate is weaker in goat and ram blood.

The aforementioned facts reveal that it is possible to demonstrate the relationship between various animal species *ad oculos* in the test tube, a fact that was determined earlier concerning Man and monkey as well. This biologically significant finding should be taken into consideration in the forensic diagnosis of a specific animal blood type. However, definite results are obtainable with my reaction on the various aspects of inter-species relationships only when the serum is of the highest possible quality. For example: while

determining the relationship between the ram, goat, and cattle, the serum from a rabbit pretreated with ram blood immediately forms a strong precipitate in the ram blood solution; the precipitate is slightly less strong in goat blood and still weaker in the cattle blood solution. It becomes clearly evident, that cattle are less closely related to sheep than to the goat. When the strength of the serum is not high, no clouding whatsoever is obtainable in the cattle blood solution.

Excellent, high quality serum is the precondition required for any forensic application of my method. When the effectiveness of the serum is reduced, fateful errors could occur in the course of blood evaluation. I therefore require a serum for forensic use which, when added to a pale yellowish blood solution to the ratio of 1:40, will almost immediately, or at least within 1 minute, cause definite clouding. The clouding should not be delayed for one, much less for several, hours. Using such high-quality serum, I was able to produce a precipitate almost immediately even in very old blood, desiccated for twelve years. In my lecture delivered before the Scientific Association at Greifswald on June 5th of the current year, I demonstrated my reaction in human blood which had been dry for six years. While I was still adding serum to the 12 control test tubes, definite clouding was already evident in the first test tube containing human blood. The clouding was so obvious that it was visible from the highest seat rows of the large auditorium. The result of the reaction, of course, also depends on the concentration of the blood solution.

When only small blood samples are available, the fluid frequently shows hardly any color at all. In such cases the formation of foam during the shaking of a small test tube indicates that sufficient blood albumin has been dissolved.

It is occasionally difficult to obtain such high quality serum. The results depend not merely on the volume of blood used for pretreatment; I found that the condition of each rabbit is very important as well. Some rabbits yield an excellent serum after a few injections; others yield a completely useless serum after a much longer treatment period. I even found that despite continued treatment, the serum failed to improve; instead, it showed pronounced deterioration.

Ziemke's report indicates how much depends on the quality of the serum. He investigated the same blood solution with two different serum types. The reaction was positive in one case, and negative in the other. As a matter of course, such failures must be entirely excluded when using my method for forensic purposes. In my opinion, it is therefore imperative to assign the manufacture and control of the serum to an institute. In that case, experts would be able to obtain a tested, high quality serum at any time. It is certainly undesirable to let each medical examiner himself prepare the serum to be used for the diagnosis of human blood needed for a given case. The preparation requires prolonged practice and experience.

Larger volumes of the serum should be stored at a central location; it is necessary, therefore, to manufacture it in larger quantities. The use of larger animals than rabbits for obtaining serum would be desirable. My tests in this respect with a small lab failed completely. The animal yielded no trace of any precipitate, despite the fact that it had received, within five weeks, injections of approximately two liters of human blood and exudate fluid. Therefore, rabbits will have to be used for the time being; when they are large and vigorous, the serum yield will be approximately 50 cc.

I proceed as follows to obtain larger serum volumes: the serum of the rabbit is tested serveral times in the course of the treatment by taking blood samples of approximately 8 cc from the ear vein. When the serum proves to be usable, i.e. when it yields an almost immediate precipitate in the tested blood solution, the rib cage of the rabbit is opened in deep chloroform narcosis and a heart section is performed. The blood flowing into the sterile chest cavity is collected with a sterile pipette, collected into cylindric test tubes several cm wide and left to coagulate, while the test tubes remain in an oblique position. After separation of the serum, centrifugation is performed to obtain a completely clear serum. Since this does not always succeed with small, manually operated centrifuges I pass the serum through a Berkfeld filter. No obstacles whatsoever were encountered with this procedure. The serum obtained was absolutely clear and entirely sterile as well. For preservation I used either 0.5% carbolic acid (Carbol) or, more recently, chloroform as well. This mixture proved to be highly efficient so far.

With the above procedure, a larger quantity of serum can always be kept ready for mailing.

Other researchers determined that the serum from rabbits pretreated with human blood forms a precipitate in urine containing albumin. I used a similar method, therefore, to investigate other human albumins. I found that the serum from a rabbit pretreated with human blood also causes clouding in human semen and in the purulent sputum (of tuberculosis patients). These are facts which deserve to be taken into consideration in the practice of medical examiners. Therefore my reaction is specific for human albumin.

Notes

1. *Dtsch. Med. Wochenschr.*, 1900, No. 46; Greifswalder medizinischer Verein am 1 December 1900, Referat *Muench. Med. Wochenschr.*, 1901, No. 8; *Dtsch. Med. Wochenschr.*, 1901, No. 6 (February 7); *Dtsch. Med. Wochenschr.*, 1901, No. 17; *Arch. Kriminalanthropol. Kriminalistik*, 1901, May; Verhandl. Naturwissenschftl. Vereins at Greifswald, Sitzung on June 5, 1901
2. *Berl. Klin. Wochenschr.*, 1901, No. 7 (February 18)
3. *Dtsch. Med. Wochenschr.*, 1901, No. 9
4. *Dtsch. Med. Wochenschr.*, 1901, No. 11
5. *Muench. Med. Wochenschr.*, 1901, No. 14
6. Societé de Médecine Légale, Referat *Dtsch. Med. Wochenschr.*, 1901, No. 26
7. *Dtsch. Med. Wochenschr.*, 1901, No. 26

Concerning My New Forensic Method to Identify Human Blood*

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/317 Judges and experts have for a long time been most deeply concerned with the all important problem of distinguishing human blood from other blood types. Until now, though, a sure answer to this question has been impossible. One was able to diagnose fairly accurately human blood in the case of relatively fresh blood with the aid of blood-corpuscle measurement. In the case of dried blood, on the other hand, where the formed elements have been destroyed, even with the blood crystal test, the diagnosis was so unreliable that one could say it was impossible. Since in forensic practice one is almost exclusively concerned with such dried blood, one must be equipped with a practical, forensic method to determine also the origins of blood in this condition.

I was recently successful in discovering such a reliable method, which I will briefly describe in the following article. Concerning the details I refer the reader to my thorough studies which appeared in the *Deutsche Medizinsche Wochenschrift*.[1]

Busying myself with the biological differentiation of protein bodies of different birds' eggs, I established that the blood serum of rabbits, which were injected in the hollow of the stomach continuously with the whites of hens' eggs for a rather long time, produced a precipitation when added to a dilute solution of hen's egg protein. The same serum failed to produce any precipitation in other protein solutions which are not derived from eggs. In the course of my studies it was of great scientific interest to establish whether the protein substances of hen's eggs and hen's blood could be distinguished from one another with the help of this reaction. Following this, I injected rabbits in the stomach cavity with increasing doses of defibrinated hen's blood and discovered that the serum of animals pretreated in this way, produced no precipitation in a solution of hen's egg protein—at least at the serum's present titer. In the hen's blood solution, on the other hand, in which the blood corpuscles were dissolved by water, that is laked, precipitation appeared. The same serum produced no precipitation in the blood solutions of other animal species so that I had to assume the specificity of this reaction.

I now repeated this experiment *mutatis mutandis* with cattle blood. The serum of these animals pretreated with cattle blood produced precipitation only when added to a solution of cattle blood, never in the blood solutions of other animal species, brought in to act as a control. I prepared now to pretreat rabbits with human blood. At six-day intervals I injected into the stomach cavities of these animals approximately 10 cc of defibrinated human blood. After five of these injections, the animals produced an effective serum, which the following experiment demonstrates.

First I prepared solutions of a large number of blood types with ordinary tap water. To do this, I added water until the solutions were uniformly colored a weak red (dilution 1:100). In order to eliminate the remains of dissolved red-blood corpuscles which disturb the reaction, I either let the solution sit in a test tube or I filtered out the particles. I took approximately two cc from the clear solution I had extracted, placed it in small test tubes of approximately six mm in thickness and mixed it with the same amount of saline solution, double physiological strength (1.6%). It is very important to use in the experiments a blood solution in physiological saline solution, since normal serum, when mixed with tap water, frequently gives rise to turbid disturbances which can impair recognition of the specific turbid reactions. In physiological saline solution such disturbances do not occur when serum is added. These blood solutions, absolutely clear and colored reddish, were produced in this way from the following animals: cattle, horses, donkeys, pigs, mutton, deer, goats, dogs, foxes, cats, stags, female red deer, hares, guinea pigs, rats, mice, rabbits, chickens, geese, turkeys, pigeons—humans.

With a capillary tube removed from an injection needle, I now put 10–12 drops of the serum from the rabbits pretreated with human blood into each of my glass tubes. Relatively quickly a clear, especially striking turbidity made an 319/ appearance in indirect sunlight only in the solution of human blood. All the other test tubes remained clear.

After observing longer, one noticed how the turbidity became increasingly intense and how finally a strong flocculated sediment formed.

I need scarcely mention that normal rabbit serum produces no turbid reaction in all these blood solutions.

I am now in a position (with the help of this reaction) to distinguish with certainty human blood from all the other blood types.

*Translation of: "Ueber meine neue forensische Methode zum Nachweis von Menschenblut."

in *Archiv für Kriminal-Anthropologie und Kriminalistik* **6**: 317–320 (1901).

The reaction is very fine so that extremely small amounts of blood are enough to determine from which species the blood comes. In order to be certain in every case concerning the type of blood it is necessary that one pretreat rabbits with a great variety of blood types so that their serum can be utilized for diagnosis in suitable cases, provided that the specificity of this reaction occurs also *mutatis mutandis* with other blood types, which according to my experiments is most probable. I am presently occupied with clarifying this point.

That my reaction enables one to identify washed (laked) human blood points the way to the forensic use of the reaction. The deciding stroke was my observation that blood which had dried for a long time and then been dissolved in a physiological saline solution also produced a fine reaction.[2]

I have further busied myself with some important practical questions. I was able to establish that the reaction could discriminate human blood in foul-smelling blood samples which had been left three months in the laboratory to de-
/320 compose. When it is a matter of decayed blood, one must naturally make the solution, diluted with saline solution, absolutely clear. To do this I use the Berkefeld Kieselguhr filter which I combine with a suction device which is easily attached to any water pipe. Since such a filter holds back all bacteria and sundry other corpuscular elements, one obtains a beautifully clear and sterile liquid with which one can then set up the reaction. I was also able to diagnose human blood stains without difficulty when the stains had been frozen in snow at $-10°$ for more than fourteen days.

It was equally possible from an assortment of soapy-water samples containing different types of blood to determine immediately the sample which contained human blood.

These experiments which I have briefly outlined here have already been confirmed in many other quarters, chiefly through the study of Wassermann and Schütze,[3] which appeared shortly after my publication.

These experiments also answered the question which I had raised, whether the reactions went so far as to differentiate very closely-related individual subjects such as man and ape. They showed that the serum of a rabbit pretreated with human blood produced a cloudy disturbance in a solution of ape's blood, though the disturbance was faint. This fact, though of great interest to natural science, should be of no importance whatsoever for our forensic practice.

Thus I am convinced that my method has been shown to be most useful for judges and experts.

I would be most thankful to these men if they would send me blood-stained *corpora delicti* to test in doubtful cases.

Notes and References

1. *D. Med. Wochenschr.*, 1900, No. 46; 1901, No. 6 and No. 17
2. *Note made during correction:* A short while ago Professor Beumer of the local institute for forensic medicine and the local First States Attorney, Mr. Hübschmann, handed over to me for testing several samples of blood dried on various objects, blood both from humans and from other mammals without any indication of origin. In every case I was able to diagnose with absolute certainty the blood type. One case involved dried pigs blood from the year 1889, another human blood dried in sand, which came from a murder committed in 1896. The other blood samples (human, pig, etc.) were from the years 1897, 1898, and 1900.
3. *Berl. Klin. Wochenschr.*, 1901, No. 7

Concerning the Development of the Biological Method of Protein Differentiation in the Service of Legal Medicine with Special Consideration of Our Own Research Results, (Personal Recollections)*

Paul Uhlenhuth

Freiburg i. Br.

/309 Professor Ponsold has requested that I report in a coherent fashion on the results of my efforts in the area of *biological protein differentiation* and especially mark out the method as well as point out the considerations and lines of thought, which led me to the discovery of forensic blood differentiation. I followed the request at first only hesitantly, because I believed that I had already presented this in its essentials in my first works. But, as I must assert after a review, there exists much between the lines, which one cannot express in an objective presentation of the research results, such as my many reports, but which might be of historical interest in understanding the development of the biological method of protein differentiation in the service of forensic medicine.

What made my decision especially easy, however, is the happy memory of those young years which like friendly stars are intelligible in the darkness of this time. During those years, I, as a young researcher at the beginning of my scientific career with light enthusiasm but also through hard work and difficult struggles, was able to join in the conquest of new territory, labor which was above all of critical importance for the law and for legal medicine in investigating the truth.

Even if in what follows, I try to represent this part of my life's work together with its practical results almost *in statu nascendi* in the spirit of Ponsold's historical viewpoint, permit me to express my personal experience. I would also heartily desire such an opportunity for our younger generation of researchers.

It was at the turn of the century when, as a young military doctor and assistant of our great master Robert Koch, I had /310 the good fortune to be able to work and educate myself in his laboratory at the Institute for Infectious Diseases in Berlin, the so-called "triangle" on the Charité. At that time Koch stood at the height of his fame. His classical works on anthrax, wound infections, and on tuberculosis and cholera had made his name famous throughout the world, while his research on tropical diseases enticed him to undertake enthusiastically expeditions to distant corners of the world. At that time I came to know Friedrich Löffler at the Institute: he was the oldest student of Robert Koch. As his first and foremost assistant in the Imperial Ministry of Health, he had discovered in mucus the microorganisms of erysipelas, diphtheria, and trichinosis and had thereby already gained world renown. He was Full Professor of Hygiene in Greifswald. As the leader of the *Commission to Study Hoof-and-Mouth Disease*, which was set up by the Prussian Ministry of Culture and which carried out its work at the Institute for Infectious Diseases, he had made, together with Frosch, the significant discovery that the causative agent of this devastating animal disease was a microscopic filterable virus. When Frosch dropped out of the Comisssion in 1898, Löffler chose me as his successor. The facilities available in the Institute were not sufficient, particularly the stalls for the large animal subjects to be used in experiments. These animals were being housed in a make-shift manner at the city railway barn. As a result of these conditions the Commission was transferred to the Hygienic Institute in Greifswald, and I resettled there in 1899 as Löffler's co-worker. Although it was not easy for me to leave Berlin and the famous research laboratory with its disease section, nevertheless I surely viewed it as a lucky turn of fortune that I had the opportunity to continue this useful and important work in the more favorable country surroundings of Greifswald, a lovely little university town. It was also fortunate that I could continue this work, which had already led in Berlin to important results, with a man like Löffler to whom I was bound by a friendly devotion. Our further experiments concerning the nature and the control of this disease (hoof-and-mouth disease), especially concerning the active and passive immunization, were carried out with great difficulty in the Hygienic Institute and in a rented farm house close to the city. There in the country I took part in them until I was called to be director of the bacteriological section of the Imperial Office of Health in 1906. These experiments led, above all, to the discovery of a highly effective remedial and prophylactic serum which achieved great importance in the fight

*Translation of: "Über die Entwicklung des biologischen Eiweissdifferenzierungsverfahrens im Dienste der gerichtlichen Medizin unter besonderer Berücksichtigung eigener Forschungsergebnisse (Persönliche Erinnerungen)".

in *Deutsche Zeitschrift für die gesamte Gerichtliche Medizin* **39**: 309–348 (1949).

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/311 against this devastating disease.

The serum research, the youngest child of our bacteriological science, still stood at that time at the beginning of its development, but it already had celebrated illustrious triumphs. In 1890, Von Behring had made the important discovery that in the blood serum of animals, pretreated with diphtheria-toxin, specific substances appeared which were capable of neutralizing the toxin used in the injection while it was in the test tube and also when it was in the animal's body. This discovery has clearly shown itself to be extremely beneficial in the fight against this murderous disease of our children. It was the take-off point for all of immunology, a study to which I dedicated at that time a great part of my life's work. In 1894, Richard Pfeiffer was able to prove that in the blood serum of animals which were inoculated with cholera and typhus bacilli, specific immune bodies (antibodies) then appeared, which influenced the bacteria in a certain way, in that they broke up the bacteria when they were injected into the abdominal cavity of a guinea-pig (Pfeiffer's phenomenon). Two years later (1896) Gruber and Durham were able to detect more specific substances in the above-mentioned serum, namely substances which agglutinated the cholera and typhus bacteria in their culture suspensions (agglutinins). This reaction, well known as the Gruber-Widal reaction, has achieved great practical importance diagnostically. As a young assistant doctor in Oldenberg, I was one of the first who was able to affirm in practice the worth of this method in 1897. It was my very first experiment which I published.

These findings suggested that similar reactions would also appear in extracts produced from bacterial bodies as they had in the cultures themselves. It was already established that one could immunize with sterile filtrates from typhus and cholera bacilli cultures and thus obtain a serum with the same agglutinating properties as that which had been produced by inoculating with the pure cultures themselves.

Following this path farther, Rudolf Kraus then produced in 1897 the evidence that immune serum produces in filtrates of the bacteria cultures in question specific precipitates, and indeed these precipitates only appeared when an immune serum was brought together with the filtrate of the matching bacterial culture. Because of the demonstrable specificity, one had to assume that an equally important diagnostic significance would of necessity result from this precipitation reaction, as in the case of agglutination and the Pfeiffer phenomenon. That was, moreover, repeatedly the case with
/312 glanders (Wladimiroff), anthrax (Ascoli), etc. Bordet then made in 1899 the important observation that, after inoculation with defibrinated blood, substances formed in the serum of animals which were pretreated in this fashion, substances which broke down the blood corpuscles (hemolysins) and coagulated them (hemagglutinins). Specific agglutinating and lytic antisera can also be produced from pretreatment with other animal cells i.e. ciliated epithelium, white blood cells, kidney cells, spermatozoa (Von Dungern, Metschnikoff, Moxter, Landsteiner, Lindemann, and others). Bordet found further that precipitins also formed in the blood serum of a rabbit after inoculation with cow's milk. Moreover, the reaction of milk serum was specific, so that one could differentiate the protein bodies of cow, goat, and human milk from one another (Fish, Ehrlich, Wassermann).

All these experiments concerning specific antibodies which gave us important suggestions in our research into immunity and serum, treatment of hoof-and-mouth disease, also powerfully inspired me to use every free moment to undertake my own experiments in this area. I can say with confidence that nothing in my career as a researcher has made a greater impression on me, and nothing so captivated me, as the *law of specificity* which governs the whole study of immunity. Indeed here, nature, our great teacher, reveals for us the splendid capacity of her smallest living organisms, the cells. Here she shows us that these cells are our greatest chemists and physiologists. We only give them the raw materials, and as playful as goblins, they produce from them the finest reactions, so sharp and certain in their reactions that the investigative soul stands still in pious reverence, as it would before a miracle.

With this impression I began my work in 1900 and I started *ab ovo* in the truest sense of the word, in that I set out in my work to determine whether specific precipitates developed in the serum of animals pretreated with egg albumin. I wanted to determine whether protein substances of different birds' eggs could be differentiated in this way. Thus, I inoculated rabbits in the stomach cavity with large doses of hen's egg-white solution and extracted in this fashion a serum that still produced turbidity, i.e. a precipitation, when added to a solution of hen's egg white diluted to 1:100,000, while the chemical reactions in protein ceased at a dilution of 1:1000. The serum produced no reaction when added to solutions of various other kinds of protein (nutrose, somatose, Heyden nutrients such as peptone, casein, or horse, deer, mutton, or donkey serum). Only compounds of 313
egg albumin obtained from various sources reacted positively, a reaction that was also of practical interest (see below). The reaction was thus specific for egg albumin, as was demonstrated at almost the same time by Myers, working completely independently of us. Then, I tried to establish further whether it was possible with the help of this so unusually fine reaction to distinguish the albumin substances of various birds' eggs. The experiments, which I expanded to include eggs of chickens, doves, geese, ducks, turkeys, pheasants, sea gulls, and lapwings, led to positive results insofar as it was possible in this way to differentiate to a certain extent the albumin substances of the eggs, excepting the closely-related bird species. Pursuing further these biological attempts at differentiating albumin, I set up the task of proving whether it was possible to detect the differences between albumin bodies from a chicken's egg and those from chicken's blood, in other words, between two protein bodies from one and the same organism. Therein lay the key to the method which could distinguish different blood types, since this experiment demonstrated that *egg protein* could be dis-

tinguished without a doubt from *plasma protein* by means of the specific egg antiserum in that this antiserum produced precipitates only in the egg albumin, but *not* in the solution of hen's blood. At the same time, rabbits were inoculated with defibrinated hen's blood. The serum of animals, pretreated in this way, showed in a solution of hen's egg albumin no cloudiness after a rather long time or only a very weak effect, while in an equally diluted solution of laked hen's blood, the serum produced a strong precipitate.

Through this test it was proven that one was in fact able to differentiate egg albumin from the plasma protein of the hen. At the same time and through this test a fundamental fact was established; for the above-mentioned serum produced a precipitate only in a solution of hen's blood, while all the other blood solutions, from horse, ass, deer, ram, and pigeon blood, which had been introduced for comparison, remained completely clear. Moreover, normal rabbit serum produced no cloudiness in these blood solutions. This *interesting observation was the starting point for perfecting the biological method of differentiating the various types of blood.*

After I had reported on the results of these tests in my work, "A New Contribution to the Specific Test for Egg Albumin by Biological Means", which appeared in the November 14, 1900, edition of *Deutsche Medizinische Wochenschrift,* I presented these biological reactions of albumin and blood at the Greifswald Medical Association on December 1, 1900, at which time I was also able to demonstrate a corresponding specific reaction in donkey's blood. I took this
/314 opportunity to make known that "I would be busy trying in an analogous way to decide the important forensic question concerning the distinguishing of human blood from that of animals". I will never forget that memorable session in which so many excellent men of the medical faculty, such as Löffler, Bier, Grawitz, Bonnet, Hugo Schulz, Beumer, Peiper, Schirmer, Moritz, Martin, and Krehl followed my presentations in suspense, and participated in the discussion in a lively fashion. I can still see in my mind how the famous physiologist, "the old man" Landois, a meritorious blood researcher, pushed back his glasses and, fascinated at seeing the reaction, shouted, "Blood is a very extraordinary juice".

I then proceeded to produce precipitating sera to test a great variety of blood types, a task I completed with considerable difficulty. Thus, I first achieved a high-grade serum to test cow's blood. My privy councillor, Löffler, set up a problem for me. From eighteen unlabeled blood solutions, which had been made from laked blood, and which Löffler had arbitrarily arranged in order, I had to select the tube containing cow's blood. The blood solutions were from the following animals: a cow, a horse, a donkey, a pig, a dog, a cat, a stag, a female deer, a hare, a guinea pig, a rat, a mouse, a rabbit, a hen, a goose, a turkey, and a pigeon. Human blood was also introduced into the test. After a few minutes I had solved the problem. The solution of cow's blood was the only one to display a typical cloudiness and a precipitin reaction, while all the other test tubes remained clear. My patience was put to a hard test, however, since I was at first unsuccessful in obtaining a serum which would precipitate human blood — a step which was surely the most important for forensic purposes. This was in itself not particularly noteworthy, since it turned out after further experiments that the individual characteristics of rabbits played a crucial role in the production of precipitating serums, so that approximately six pretreated rabbits sometimes yielded only one or two usable sera. Finally I had achieved a usable serum, which, when added to the same blood solutions mentioned above, only produced a precipitate in the solution of human blood, so that I was able to select the human blood without further ado. Of decisive importance, however, from a forensic standpoint was the fact that blood from humans, horses, cows, etc., which had dried for weeks on a great variety of materials, and had then been dissolved in a physiological NaCl-solution, could be differentiated immediately, even when it was a matter of very small blood stains.

Even when there could be no doubt after that, that the problem of forensic blood differentiation was solved in principle, still I could not at first decide whether to publish, since 315.
I was most aware of the huge responsibility which was bound up with the publishing of a method, often critical in the administration of justice. I repeatedly checked my results using all imaginable controls. I let dry on a board (see below, p. 335) a number of smaller and larger stains of human blood and of blood from a great variety of animals and had Löffler and my trusted assistant, Schirmacher, who participated in these experiments with bright enthusiasm, hand over to me concealed samples, which they continually scraped off the board in order for me to determine their origin. Without exception I delivered the correct diagnosis, even though the extremely critical and careful Löffler in a joking manner repeatedly set traps for me. For a long time the manuscript lay completed. And at that point it was my young wife, who had followed my work with growing excitement and joy and who, full of apprehension as only women are, advised me not to wait any longer, especially since I had already published the fundamental tests on November 15, 1900 and had demonstrated them on December 1 at the Greifswald Medical Association.

When one day, I had identified by means of my own tests all the blood samples before me with mathematical certainty, in the evening I again sought out my privy councillor, Löffler, in his apartment. Loffler had assumed until then a cautious wait-and-see attitude. I read him my work. He was in agreement with it on every point. That same evening I brought my work to the post office. In about a week I received the proofs and shortly thereafter, on July 2, 1901, the article appeared with the title, "A Method To Distinguish the Various Blood Types, Especially For the Differential-diagnostic Test of Human Blood."[1] Fourteen days later Wassermann and Schütze reported in the *Berliner Klinische Wochenschrift* on similar results. Nevertheless, I was undoubtedly the first, and I had to thank my anxious wife for this honor.

If I have presented all this in such detail, I have done so because I wanted to show the younger generation how monstrously difficult it was for me to seize upon the right moment to make public a process involving such responsibility, a process on which, under certain circumstances, the fate of a man might rest. That means that together with the happy joy of the discoverer, the joy the young researcher knows, the joy with which one cannot get to the laboratory fast enough each morning, with that exultation, a calm and critical self-control must be maintained in order that one not be carried along to a hasty publication. This needs to be learned first.
/316 When reminiscing, I reckon such times of tension and expectation, as I have often experienced in my later days, among the most beautiful moments of a life, filled with successes and also disappointments. On the other hand, the self-criticism and the period of testing and consideration ought never to last too long, lest the power to decide should suffer; otherwise, one is too late. For me that would have perhaps been fatal, since this discovery was the determining factor in the rest of my scientific career.

In this connection I must point out that, a short time after publication of my article, I received information of an observation which Tchistovitch had made in 1899 while studying immunization against the serum of eels, well-known as extremely poisonous. When he mixed this eel serum with an antitoxic serum, a cloudiness appeared after a few moments; if he used horse serum instead of the poisonous eel serum to pretreat his animals, he was able in the same way to establish analogous conditions immediately. Thereupon, Bordet confirmed this observation with the serum of a rabbit pretreated with defibrinated hen's blood. Accordingly, there can be no doubt that these creditable authors have established specific precipitins of plasma protein. What is of special interest, however, is that they have done this in a completely different context, through their experiments which were at that time totally unknown to us.

On the other hand, there should be no doubt that we first indicated the method to recognize and differentiate the different blood types in the course of our experiments concerning the biological differentiation of various birds' eggs, and especially through the difference which we established between the protein bodies of a chicken's egg and of chicken's blood, and, what is of chief importance, that we first worked out and recommended this method for forensic use. We were also the first to succeed in recognizing human blood, as such, in an old and dried state and to distinguish it from the blood of various animals, something previously impossible. These facts, which were not expressed clearly in the literature, or in my earlier works, deserve to be emphasized in the historical presentation of the development of biological blood differentiation.

II. In the greatest number of legal cases which were determined by the recognition and differentiation of human and animal blood, one had up to that point relied almost completely on the microscopical measurement of red blood corpuscles. However, in the case of dried blood, with which indeed forensic practice deals almost exclusively, these measurements established in no way a certainty, but a best only a *probable diagnosis* because of the contraction of blood cells caused by the drying process. A judge, however, could not begin anything with that. The medical examiners, 317/ who carried out such experiments throughout the years, know best how depressing this deficiency was for the legal experts. Now, as if by magic, a change entered on the scene.

Even though it appeared that the method which we worked out for blood differentiation was finally perfected from the legal standpoint, not only after my own conclusive experiments with old blood dried on various substances, but also after decayed and frozen blood as well as blood mixed with different chemicals, such as soap, revealed its origins with certainty, still I went on using every opportunity to form a judgment myself concerning the forensic meaning of the method in its practical application. Thus I tested a great number of blood-spotted articles which were placed at my disposal in Greifswald by the director of the Medico-legal Institute, Professor Beumer, and also by the first state prosecutor, Hübschmann. I especially concentrated on state's exhibits of expired criminal cases, which were handed over to me by the Justice Minister for testing. After testing the blood-stained *corpora delicti*, which had been given to me without any further information, my verdict was compared with the relevant official reports. In every case I was able to make the right diagnosis, whether it concerned human blood or that of any animal. After the efficiency and dependability of the method was proven in the laboratory in this way, it was a lucky coincidence that it was able to stand its crucial test in the sensational murder trial conducted by the prosecutor's office in Greifswald against the sex murderer Tessnow. *This process was the very first in which our method found practical application*. Besides the charge of a sex murder against him, Tessnow was suspected of having butchered sheep in a grizzly, sadistic manner. In fact, I was able to detect on the pieces of clothing which were handed over to me for examination both human and sheep blood, a result which was of crucial importance to illuminate the facts of the case and convict the murderer. On the strength of my conclusions, Tessnow submitted a sweeping confession and was condemned to death.[2] Since I was naturally the only one at first who was fully conversant with the method of forensic blood testing, I was brought in as an expert to 318/ delivery judgments, and I was thereby able to travel to every section of Germany at the request of the courts so that I could personally present the results of my tests at the various proceedings. This activity brought me uncommonly great satisfaction and compensation since I could grasp first-hand what importance this method of protein differentiation, which was at first a purely scientific method, had won in the search for the truth in legal proceedings, particularly since it contributed not only to condemnations but also to the freeing of defendants.

Even though the results of my labors were recognized and confirmed through many tests, still there were raised here

and there, as was to be expected in any delicate biological reaction, certain objections, which were, however, grounded simply in the faulty handling of the method, especially on the part of experts who were inexperienced and uneducated in serology. In view of the serious decisions which rested on the blood test in a legal process, I considered it necessary that the test be worked over according to certain uniform viewpoints which until then had held true. Thus, together, with the thoroughly critical, totally conscientious, and unforgettable forensic physician, Professor Beumer, the director of the Institute for Legal Medicine in Greifswald, I completed in 1903 an article entitled "Practical Primer For Forensic Medicine regarding the Blood Test Using the Biological Method".[3] This article underwent still further elaboration as a result of experiments carried out in the following years with my students, Weidanz, Steffenhagen, Seiffert and others.[4]

/319

III. Without going into all the details, I consider it necessary once more to point out in this context the most important of the precepts which we worked out.

Since the biological method is not a specific blood test, but rather a specific protein reaction so that suppurative sputum, seminal protein (gonorrhea secretive), albumin-containing urine, ascites, and other exudates, and possibly milk and colostrum, can react with an antiserum to human blood, so it is the first task of the expert to identify blood as such with the help of well-known chemical and physical methods. Only then does one move on to the biological determination of the origin of the blood. Thus in our "primer" we thoroughly treat of the production of a completely perfect antiserum, which must be absolutely clear, not opalescent, sterile, species-specific, and of a high-potency, not a very easy task granted the individuality of rabbits which are almost exclusively the animals in question.

The antiserum must above all display a prompt effect, i.e., it must be of a high potency because I require that the reaction or specific turbidity develop before our eyes within a few minutes, and that it appear so fast and clearly that even for a layman any doubt about the commencement of the reaction is out of the question. An antiserum which has this effect must be of the following strength: In a solution of 1 cc of serum diluted 1:1,000 with 0.85% saline solution, 0.1 cc must produce an immediate reaction within one to two minutes when carefully layered in. In a dilution of 1:10,000 or 1:20,000 serum in saline, the turbidity should set in within three to five minutes in the bottom of the test tube, a ring-like turbidity, gradually increasing in strength. One can see the reaction best by holding up a piece of black cardborad.[5] By observing these criteria, one has the inestimable advantage that he himself can successfully test tiny blood spots since we have seen the reaction take place within a short time even in dilutions of 1:20,000. Of especial importance, moreover, is the species specificity. Besides the relationship reaction, which we will pursue in more detail below, one sees from time to time "heterologous turbid reactions", even in unrelated protein solutions. Such reactions

/320

are especially common when one adds high-potency, specific sera to protein solutions which were insufficiently diluted, that is when one does not work quantitatively. In immunity reactions such quantitative work is absolutely necessary.

This "overlapping" can of course be so strong in individual sera that even in greater dilutions turbid reactions, i.e. precipitates, can form. Still the practiced investigator will not confuse these reactions with specific turbid reactions if he notices the swiftness and intensity with which the reaction takes place. Nevertheless, they can be the cause of errors for the inexperienced.

The species specificity test of antisera, known to be of high potency (titer 1:20,000), takes place with solutions of heterologous antigen solutions of 1:100, 1:200 and, if necessary, 1:1000. After very careful tests of a great number of antisera, carried out in the Imperial Office of Health by my students, Manteufel and Beger, eighty-seven percent were shown to be absolutely specific, i.e., they alone did not produce a trace of turbidity at concentrations of 1:100 and 1:200.[6] Sera which still cause heterologous reactions even at 1:200 are unusable for forensic practice and ought not to be distributed (For more details, see below, "State Testing of Precipitating Sera", p. 347).

Regarding the execution and the course of the biological reaction, moreover, we have given exact directions, chiefly concerning the handling of the material used in the experiment to produce the extract from blood-besmirched surfaces. As in all forensic blood tests, the most important fundamental rule is that all containers, test tubes, and instruments be meticulously clean and sterile, and that all liquids be absolutely clear. In our experience simple physiological (0.85%) saline solution is the best as a solvent, but it must be allowed to act upon the relevant, pulverized substratum for a sufficiently long period. In order to achieve clear solutions by this process, every agitation must be avoided as much as possible. The test fluid, which under certain conditions is still clearly filterable, must display a foam when shaken, as the sign that enough protein has gone into solution. Then, the fluid must be diluted to 1:1000, and, when tested with nitric acid, can be recognized [as suitable] by means of a light turbidity. Besides the test liquid, control solutions must be introduced, controls which are prepared in the same way as the test liquid, using a solution of the same blood-species whose identity is to be established by the reaction. Solutions prepared from heterologous blood species must also be prepared. the selection of heterologous blood-species is unimportant (one must select steer and pig blood controls to identify horse blood). Animal-blood solutions which display related reactions (see below) are naturally to be avoided. Then, the clear extracts must be tested with litmus paper for their reaction. At a dilution of 1:1000 they ought to show a neutral reaction. Strong alkaline and acidic solutions are to be discarded, but in practice they rarely come into play in view of the high dilution of the test liquid. If, in exceptional cases, they react acidically (leather, tree bark, etc.), they can be neutralized with 0.1% soda solution

321

or magnesium oxide.

The test solution is then put in test tubes I and II, one cc of the solution produced according to prescription from the blood stains in question being placed in each tube. The same quantity of homologous solution, i.e., a blood solution corresponding to the antiserum,[7] goes into tube III, while one cc of heterologous blood solution is put into tube IV and one into tube V (for example pig and cow blood). In tube VI is physiological saline such as that which was used to produce the test liquid. As a further control for certain cases we include still another tube, no. VII, filled with an extract from a blood-free piece of the substratum in question (see below).

0.1 cc of the antiserum which has been pretested (and with the prescribed titer 1:20,000) is then added with a graduated pipette (one cc with graduated marks) to each of the tubes filled with their respective solutions, with the exception of tube II. To this tube 0.1 cc normal rabbit serum is added. In adding the serum one must be careful that the serum runs down the wall of the test tube and is not dropped directly on the liquid. When the serum is added, it usually sinks to the bottom because of its greater specific gravity. The layering must be done very carefully or otherwise, the developing reaction will not appear clearly as a ring formation.

The following are valid criteria for judging the reaction. Tubes III, IV V and VI provide an indicator of the fitness of the serum used. In tube III obvious turbidity must appear at the bottom of the tube within a minute (the value of the antiserum). Tubes IV and V (specificity of the serum) and likewise tube VI (clarity of the serum) must not show a reaction, i.e. turbidity, within twenty munutes. Tube II must provide the evidence, i.e. the lack of any precipitation, that normal rabbit serum does not in itself bring about any turbidity. Only when the reaction in the six control tubes has run its course in the manner described above, and only when
/322 tube I, in a positive reaction, shows a turbidity, i.e. a precipitation, of the same kind as in tube III, can the test be considered certain. Turbidity which sometimes develops after the twenty-minute period cannot be considered a positive reaction. In order to execute the test in the manner presented here, the test tubes *must not be shaken*. The reaction must be done at room temperature and not in the incubator. It is useful to repeat the experiment several times and it should be carefully observed and followed *in statu nascendi*. If the experiment is undertaken according to this prescription, all so-called heterologous turbidities, i.e., unspecific reactions and other "interference factors", can be excluded.

Here I must draw attention to an "interference factor" which merits close observation. Early we determined that strong extracts of tree bark and leather gave a "pseudo-reaction" as a result of their acidic content (tannic acid), i.e., that by the addition of antiserum as well as any other serum an obvious, often cloud-like turbidity or precipitation can result (Uhlenhuth and Dürck, Graham-Smith). This was not the case with other substrata which we examined, such as wood, glass, fabric material, iron, paper, stone, coal, cork, straw, sand, earth, etc. With the prescribed dilution of the test fluid to 1:1000, however, the turbidity resulting from the acid no longer occurs. In any case such a false reaction would reveal itself straightaway in tube II. For certainty, we have also required tube VII as a control on the substratum so that every possibility of error is ruled out. Where the failure to observe our suggested rules and controls can lead is demonstrated by a report from the state chemical laboratory in Lagos, recently published by Heindl. Here the careless testing of a stain on a waterproof raincoat, a stain suspected of being human blood, simulated a positive reaction, in that the extract from the raincoat alone gave a positive reaction with any serum whatsoever, a reaction which was later determined to have resulted from the rain-proofing substance (Fritz). If the expert had adhered to our rules, such a dangerous error would have been impossible, since tubes II and VII would have immediately revealed the interfering factor, as was the case in our earlier test with tree bark, leather, and so on. I refer in addition to the research of Fritz, Bessemann, and Baert, which was stimulated by this case of Heindl's, and also to my own treatise on that problem.[8]

Schoenherr was the first to research thoroughly this ques- 323/
tion in my laboratory. In eighty-one extracts of various materials (tree bark, oak,[9] leather, rubber, plastic, roofing felt) he was able to establish twenty-eight pseudo-reactions (acidic reactions), and indeed these took place whenever he tested the extracts at the ratio of 1:10 (one part substratum and ten parts physiological saline solution). After diluting to 1:500 the pseudo-reactions disappeared so that, in fact, they do not come into play, if our rules are observed.

Concerning the details I refer to the work presently in print and appearing in the *Archiv für Kriminologie* as well as to the article of Fischer in the same journal.

Thus, the biological method, as we had worked it out, achieved such perfection that it satisfied all conceivable demands regarding its trustworthiness and dependability. It goes without saying that this is true only in the hands of an experienced expert. The best evidence of that is the fact that over the years our prescriptions were not altered in their essentials.

I would, however, not want to miss this opportunity to refer to the *capillary method* which was outlined by my friend Hauser and modified in my laboratory by Carnwath. Here I cannot go into the execution. For this method only the smallest quantities of the blood solution, i.e., the fragments of a droplet, are sufficient for the test. As a result, there would in practice rarely be a case in which the testing of a blood spot would meet with insurmountable difficulties, provided that the solubility of the blood was not diminished too much through aging or other causes. This method naturally required special practice and experience. Together with my students Weidanz and Angeloff, I have used the capillary method successfully to identify the provenance of blood in leeches and in blood-sucking insects (bed-bugs, fleas, lice, mosquitos, gnats, flies). By means of this test we were still

able to identify human blood in bed-bugs after fourteen days. The results were the same with human, cow, and goat blood in fleas, sheep ticks, and dog ticks.

In a selection of *Anopheles* mosquitoes, the transmitters of malaria, we were able to determine the presence of pig and cow blood, but not of human blood, which we had expected to find. The mosquitoes, we later discovered, had been caught in pig and cattle barns. So it was possible in a simple manner to determine the blood suppliers of individual carriers, a fact which can be of great importance in epidemiological research.

/324 The capillary method has come into vogue in many places for forensic practice, in place of the test-tube or Uhlenhuth-tube method (see also Merkel).[10] We ourselves have used it with advantage because the ring formation, which arises when layering the test liquid with the antiserum, appears in an especially striking fashion in the capillary tubes.

IV. To illustrate the forensic significance of the biological method, it is sufficient to refer to the opinions of court physicians which have played a determining role in illuminating the facts in countless cases of murder, bodily injury, moral transgression, theft of household animals, poaching, etc. I have brought together a collection of my own opinions, the especially important ones, and published them with Weidanz in my book, referred to above.

From the abundance of opinions which I have rendered during the years I want here to select only a few strking examples in order to illustrate the practical importance of the method.

1. A butcher, accused of a triple robbery-murder, alleged that the blood stains found on his shirt sleeves were due to his having butchered a cow. With the aid of the precipitin reaction I was able to establish with certainty that the stains were human blood stains. On the grounds of overwhelming circumstantial evidence, including this finding which was an important consideration, the accused was condemned to death. Shortly before his execution he made a comprehensive confession.

2. A man, on whose clothing were found blood stains, was arrested under heavy suspicion of murder. He asserted his innocence, however, stating that the blood came from a wound his horse had suffered. His story was not believed until I was able to prove the truth of his testimony by means of the precipitin reaction. The man was thereupon released from prison.

3. A man was accused of having stolen and butchered a pig, and of having concealed the body in a sack. He maintained that the blood stains on the sack came from a female dog which had given birth. I, however, was able to establish that it was a matter of pig's blood and thereby cleared up any doubt about guilt.

4. The following case is also interesting: a man who wanted to cheat on his pension was discovered one morning in his blood-stained bed. He maintained that he had suffered a violent hemorrhage. Since the medical examination gave
/325 no evidence of this, I brought into play the precipitin reaction in order to determine the origin of the blood. The test indicated the presence of cow's blood. When the man was directly confronted with the result, he admitted that he had poured out a bottle of cow's blood, which he had fetched for himself from the slaughter house, with deceitful intention.

In view of the great responsibility which such a forensic test brings with it, the conditions surrounding it are similar to the bacteriological determination of diseases which endanger the public, such as cholera. Considering the far-reaching consequences of such a diagnosis the imperial administration has issued exact instructions which are to be strictly followed, and, if these are not observed, the diagnosis of cholera is not recognized as valid. Further, experts are only admitted who have obtained proof of special training. If these demands are present in public health, they ought also to be necessary here, where the determining of human blood often decides a life-or-death issue in a murder trial. There even is a special training program for the carrying out of the Wassermann reaction for syphilis, a program which was worked out in the imperial health office and required by regulation for all official examinations.[11] Accordingly in 1903, I demanded that central offices be instituted where experts could be instructed in carrying out the forensic blood test. I felt that university institutes of legal medicine were the best suited for the job. From the central offices the experts can also acquire high-potency sera, which has been tested by the state.[12] It is a matter here of a serum reaction which brings about extremely fine biological processes; to observe and judge these processes requires a special course of studies. If these methods of testing are unfamiliar even to the court physicians, how much more are they strange to the court chemists, who are often called upon to carry out such experiments.

At this point it was a welcome turn of events that the official departments took a stand regarding this matter, so important for the administration of justice, on the recommendation of the Scientific Deputation for Medical Affairs. This recommendation ran as follows:

> The practical uses regarding the serum method of blood testing are already so widely disseminated in Germany as well as abroad, the results of the research so unanimous in their essentials, that no doubt can any longer be raised that this new biological method enables one in the majority of cases to determine with great certainty the origins of fresh and dry blood and to distinguish human blood from the blood of different animals. Though this excellent method naturally should 326/
> not drive out the old, tested methods of blood identification, but rather should supplement and complete them, we vigorously urge that it be used in judicial practice.

On the basis of this recommendation the Prussian Justice minister issued on September 8, 1903 a disposition dealing with this question wherein the biological method was introduced into legal practice.[13] The Hygienic Institute of the

University of Greifswald, the Institute of Infectious Diseases in Berlin, the Institute of State Pharmaceutics in Berlin, and the Institute for Experimental Therapy in Frankfurt am Main were all named as institutes which were straight-away invited to undertake forensic blood research. Similar dispositions were released in Austria, Bavaria, Württenberg, Baden and also abroad in almost every nation. In order to have ready at all times a satisfactory serum, the Hygienic Institute in Griefswald was intrusted in Spring 1904 by the Prussian Ministry of Education with the production of high-potency serum, where I controlled the process myself. Later the bacteriological department of the Ministry of the Interior was also named. I was transferred there as director in 1906. The production and regulation of sera was all the more necessary as I was able to establish that sera produced by court physicians themselves, or obtained from other sources, fell totally short in many ways of meeting the prescriptions we outlined above. Above all were found among these strongly opalescent sera, which were not of sufficient potency to eliminate errors, i.e. false results. Unfortunately, as the result of adverse circumstances, it has in recent years not been possible for these bureaus to carry out the production of the necessary antisera so that the court physicians were no longer able to obtain satisfactory, officially approved antisera. Now that the governmental testing of sera,[14] testing we had formerly worked out in its details, and had held to be strictly necessary, has been introduced at the Institute for Experimental Therapy in Frankfurt am Main, and now that private serum laboratories have undertaken the production of the kind of antisera practical experience requires, one hopes that the constant difficulties in this respect have been removed. I also consider it strictly necessary that more attention be given the instruction of court experts in this matter
/327 and that the blood tests be limited to state forensic medical institutes and possibly to hygienic-serological institutes which ought to be designated by name to the courts and police officials. In these institutes such experiments can continually be carried out by experienced experts. Since, in consideration of circumstances, the old official regulations are now forgotten and since considerable abuses now exist, abuses which my questionnaires to the forensic medical institutes brought to light, the question of forensic blood testing ought to be uniformly established and regulated. As the result of these regulations, chemists, criminal experts, official doctors, and health offices should not, in general, carry out these tests.

V. Of forensic importance, and also of interest to the natural sciences, are the results published in my first writings concerning the differentiation of albumin from various birds' eggs (see above). I established that the bonds of relationship among the animals achieve visible expression in the biological reaction. Thus, I hit upon the closely-allied idea of recommending the use of the precipitin reaction for the study of the relationships among the animals. By this means the blood relationships between horses and donkeys, among sheep, goats, and cattle, among dogs, foxes, wolves, and jackals, between pigs and boars, hares and rabbits, chickens and pigeons are demonstrated before us *ad oculos.* With certain gradual and temporal differences, the biological reaction runs approximately parallel to the degree of blood relationship and, in general, agrees with the animal taxonomy. In the case of reptiles and amphibians (v. Dungern) and of fish (Neresheimer, Dunbar, Kodama, and others) similar relationships exist. Nuttall confirmed these experiments and expanded them greatly, testing 900 different blood species with thirty different antisera and 16,000 reactions.[15] Of very special interest are the tests concerning the blood relationship between men and apes. The identification of these blood species was first brought forward by Wassermann and myself, and was confirmed and thoroughly studied by Nuttall. It was shown that a human antiserum produced almost as strong a precipitation in the plasma protein of the human-like apes (chimpanzees, gorillas, orangutans)—when analyzed quantitatively—as did in human blood. This serum reacted somewhat more weakly with the blood of baboons and long-tailed apes. Weaker still was the reaction in the case of new-world monkeys, the *Cebidae* and *Hapalidae.* The blood of the *lemurs* (Halbaffen) reacted either only very 328/
weakly to serum of very high potency, or it did not react at all (Nuttall). That the serum of a rabbit pretreated with human blood calls forth precipitation not only in human blood but also in ape blood, and in addition produces such precipitates in no other types of blood, is forceful evidence for the blood relationship between man and the ape family. Moreover, considering the differences in the precipitates from the biological reaction, one must accept that different grades of relationship, some closer, some more distant, exist between man and the various types of apes, especially that the anthropomorphic apes (chimpanzees) stand closest to man and that, in general, the monkeys of the old world are more closely related to man than are those of the new world. Clearly, this evidence for the blood relationship between man and the ape family is worthy of being placed along side of all the other evidence which follows from comparative anatomy and from the history of evolution. Indeed this might be the most striking and startling, since one can demonstrate it in a flash in the test tube *ad oculos.* Thus this biological reaction is a solid prop for the theory of evolution as it was founded and developed by Darwin, Lamarck, and Häckel.

These experiments have been confirmed by many researchers (Hansen, Bruck, W. A. Schmidt, Yamanuouchi, and so forth). The serological studies of Mollison and von Krogh are especially impressive and interesting from the standpoint of animal taxonomy. By carefully measuring the quantity of precipitation, and by using transverse reactions, these studies came to the conclusion "that the phylogenetic relationship among the forms of life can be grasped more clearly and surely through the tested precipitin reaction than it can with the aid of morphological similarities.[16]

"If protein substances of related species are common, and if these substances are found no where else in the animal or

plant world, this fact must lay claim to greater importance than any morphological feature. The protein which a creature has inherited from its ancestors is to a certain extent a passport wherein the infinitely complex marks of its forefathers have been entered." It proves definitely, "that both species have a common stretch in their phylogenetic development which they completed before their present
/329 differentiation. A distinction between protein relationship and phylogenetic relationship does not exist" (v. Krogh). If Ehrhardt is of a different view, his judgment at best must have reference to older experiments in which unsuitable methods were used.[17] Thus, our precipitin reaction as a method for biologically distinguishing protein must be considered at least equal to all the morphological research, and both of these methods will be most useful when they control each other's results.[18] Our precipitin reaction has thereby achieved great meaning for anthropology and zoology.

This rich biological method of observation has not yet been properly evaluated. For example, the apparent variation in susceptibility to infections and tumors (cancer) in supposedly closely related rodents led me to study thoroughly their blood relationships. To our surprise I was able to establish that, for example, between rats and mice there exists only a distant relationship so that it is easily possible to differentiate mouse and rat blood with an antiserum of not very great potency (see also Trommsdorf, Graetz, Steffenhagen, and Schönburg). This however, does not seem to take place with every antiserum in the same way (Otto and Cronheim). Rats and mice, of course, belong to two different families (*Epimys and Mus*). Recently we were also able to demonstrate that, by using an antiserum against field-mouse blood, one could distinguish field-mouse blood from that of a house mouse (white mouse), and that this was also possible in the reverse order, i.e. by using an antiserum against house-mouse blood.[19] Robert Koch already suspected such a difference between these blood types, when he conducted experiments concerning the varying susceptibility to the agents in mouse-septicemia and anthrax (1878). The field mouse displays a noticeable resistance against these agents compared to the house mouse. On the other hand, we observed that an antiserum against the blood of the house mouse reacted equally to the blood of a white mouse, a reaction which is perfectly understandable, because the white mouse is a pigmentless house mouse, and breeding is possible between them.

Although these relationship reactions are interesting, they are understandably the source of interference in forensic-medical practice. If, for example, the expert must confront the problem of distinguishing horse from donkey blood, or
/330 sheep from goat blood, he comes up against unconquerable difficulties, since the precipitin reaction breaks down in these cases. In the attempt to distinguish related blood types Weichardt employed the so-called "saturation method." I cannot go into this method more closely here, since it has achieved no practical forensic importance, even in the hands of an expert.

A forensic opinion gave me the occasion to work thoroughly with the distinguishing of closely related blood types. In this case it was possible to go a step further. The court sent me a blood-stained walking stick with the request to establish the origin of these stains. The man, in whose residence the walking stick happened to be found during a house search, was under suspicion of having killed a deer or a smaller wild animal (a hare, a fox, or some other similar creature) and of having taken it away on the stick. The man, however, claimed that the stains were caused by goose blood; his mother had supposedly slaughtered some geese and hung them up. The walking stick stood below these geese and the blood ran down onto it. First, it was possible to establish that the serum of a rabbit pretreated with goose blood did not call forth a reaction in the solution of the blood-stained material scraped from the stick. Thus, goose blood was ruled out. Similarly by using a deer-blood antiserum, deer blood could definitely be ruled out. Now in order to determine whether it was hare blood, I attempted to produce a hare antiserum. Toward this goal I pretreated rabbits with hare's blood, although in view of the supposed close relationship of the hare with the rabbit, theoretical doubts against this procedure were raised. In order to obtain an effective antiserum to hare's blood in any case, three chickens were pretreated with hare's blood along with three rabbits. Since at that time it was closed season and therefore impossible to obtain fresh hare's blood, I used four-year old, dried hare's blood, which I dissolved in a physiological saline solution. To my great surprise all three rabbits produced usable antisera which precipitated with hare's blood. The three chickens also produced effective sera after four or five intramuscular injections with hare's blood.

The antisera obtained from the rabbits as well as that from the chickens reacted to hare's blood, but they displayed the following differences. Though the serum from the rabbits pretreated with hare's blood was added to a great variety of blood solutions, it produced a reaction only in hare's blood. The blood solutions of tame and wild rabbits remained completely clear. On the other hand, with the hare antiserum obtained from the chicken, there was no positive difference between hare and rabbit blood, since it produced precipitates both in hare and rabbit blood.

With the aid of the hare antiserum obtained from the 331/
rabbits, I brought forth positive evidence that the blood found on the walking stick of the poacher was that of a hare. Through this "crosswise immunization," I was able in a similar way to differentiate positively chicken from pigeon blood. When I pretreated monkeys with human blood, I succeeded also in differentiating human from monkey blood by means of the human antiserum obtained from a monkey. The serum from this monkey, which has, since that time, accompanied me on life's journey as a stuffed specimen, called forth precipitation only in the human blood, not in the blood of apes. This interesting observation was confirmed by Landsteiner with human antiserum obtained from chimpanzees. Thus it is indeed possible in certain cases to produce

precipitins in the case of related animals such as chickens and pigeons, hares and rabbits, as well as humans and apes through reciprocal injections, and even to distinguish human from ape blood even when these antisera are, as a rule, not of high potency and are in general difficult to produce. On the other hand, it was not possible even with large doses of donkey's blood to produce from a horse specific precipitins against donkey blood. Even less was it possible to get such serum from sheep which had been pretreated with goat's blood because the plasma protein of these animals is too closely related. In this case, however, one must consider also that sheep are very poor producers of precipitins.

It is necessary that the expert take careful notice of the relationship reaction and, since he scarcely has at his disposal human antiserum obtained from apes, he should do as I have always done. He should add the note, "if ape blood has been ruled out by the judicial examination." Luckily this has practically no importance in our region. If need be, however, the decision can be reached with serum obtained from apes, as we have said. The same is true for the hare and the rabbit, the chicken and the pigeon, and so forth.

In practice, however, such an explanatory note is especially important when, for example, the case involves sheep blood (see above the Tessnow case), which by our method cannot at all be distinguished in practice from the blood of goats, deer, or cattle. The same is true of distinguishing horse from donkey blood. In these cases the diagnosis must be presented in conjunction with the judicial inquiry *per exclusionem.* In any case this must always be expressed in the opinion, since it has, in fact, happened that the accused has exposed the expert before the court.

We see, on the one hand, that the "crosswise immunization" means a certain progress in the differentiating of various blood types, but, on the other hand, the unsuccessful attempts at distinguishing horse from donkey blood, mutton from goat blood, show us that the precipitin reaction, otherwise so capable, finds its limit here. Where an equivalency of plasma protein can be biologically established, there is the possibility of crossing the animals, as is the case with the horse and the donkey. Mutton and goats are also indistinguishable biologically. Whether cross-breeding is possible here is still a disputed question. On the other hand, where a distinction of plasma protein is biologically demonstrable, cross-breeding seems to be ruled out. This fact is suited to quash any fantastic notions of breeding. Thus, these experiments can also be of practical use to the animal breeder.

It would be of particular interst from an anthropological standpoint if it were possible to distinguish the blood of different human races from one another. As is well known, C. Bruck, with the help of an antiserum against members of the white race, is said to have succeeded in distinguishing whites from members of the Mongolian and Malaysian races by means of weakly precipitating sera with the use of complement binding (see below). From the gradations in titer he derived the relationship of the different races to each other. According to our opinion, however, the differences in the titer were too small to permit sure conclusions. Moreover, Bruck's experiments could not be confirmed (Linossier and Lemoine, Marshall, Teague, Fitzgerald, and others). The experiments of Sutherland and Suk as well as those of Fischer and Raquet have not shown progress. Whether a "crosswise immunization" with the blood of whites and blacks would lead anywhere is unlikely, according to what we have said, since certainly among the individual human races mixing occurs to a wide extent (Europeans with Blacks, Indians with Eskimos). Nevertheless, such experiments should be attempted. Using antiserum to human blood obtained from the Robert-Koch Institute, we ourselves had the opportunity at our leisure to analyze quantitaively fresh blood serum from different races, from Englishmen, Armenians, Russians, Indians, Negroes, Arabs, and Mongols. In these experiments one must, of course, notice that the protein content of the blood sera can be subject to certain variations.

It was not possible to observe a distinction among blood samples of different races. The reaction proceeded very uniformly in a dilution of 1:1000 to 1:20,000. The reaction proceeded in the same manner in the blood of an ape which had been added to serve as a control.

We atempted also to distinguish the different canine breeds [20]/ which leave nothing to be desired regarding the variety of their outward appearance. Our experiment failed to distinguish with an antiserum to the blood of a pedigree German shepherd, quantitative differences in the blood of nineteen other supposedly pedigree dogs, a result which agreed with the well-known cross-breeding possibilities among the various canine breeds. [21] Although it would be very interesting from an anthropological standpoint we came no further with the present biological methods. The experiments, which we began with the Abderhalden protective-enzyme (Abwehrferment) reaction, could possibly reach the goal, but their execution is extremely difficult. Unfortunately, they had to be discontinued for other reasons. 333

VI. The idea came to me to use my method of distinguishing the different blood types to test whether it was possible to use this method in distinguishing the meat of different animals. From the start the prospects were good, because in a good cut of meat there is a large quantity of blood still present.

By numerous experiments I established that in a great variety of pig organs (spleen, liver, heart, muscle) dried for a year, a positive reaction occurred and, thereby, that the origin of these organs could still be ascertained exactly. This was my starting point in working out a method of distinguishing the different kinds of meat, which was of fundamental importance for meat inspection.

Through countless experiments we were able to demonstrate that the serum of a rabbit, pretreated with pig blood, produced precipitation only in an extract of pork, that from a rabbit pretreated with cat's blood only in an extract of cat's meat. Further, specific sera were produced for the identification of mutton and horse flesh, but at the same time the

possible relationship reactions between horse and donkey meat as well as that among sheep, goat, and cattle meat had to be pointed out. The importance of this method in testing chopped meat for the admixture of horse, dog and cat meat was accordingly self-evident. Moreover, I was able to establish the important fact for meat inspection that the specific identity is also successful in smoked products (pickled meat, pie). Thus it was possible to ascertain with certainty the origin of year-old smoked horse meat and ham. Similarly, we
/334 succeeded in determining the origin of horse sausage and sundry other German sausages, if the reacting protein bodies were not destroyed by cooking, as is the case with liverwurst.

The method of meat testing which I, together with Weidanz, Wedemann, and Borghmann, worked out in its smallest details for practical application, was confirmed and fully recognized by the work of Jess, Piorkowski, Nötel, Miessner and Herbst, v. Riegler, Groening, Ruppin, W. A. Schmidt, Schütze, Fiehe, and others. [22] Since it was impossible by using the current chemical and physical methods to identify with certainty horse meat, not to speak of the meat of any other animal, especially in sausage or other meat mixtures, the biological method for the practical inspection of meat was undertandably of extraordinary importance. For the inspection of foreign meat, the precipitin test to identify horse meat, carried out according to our instruction, has been required by law. Our instructions are found in the appendix "a" to the explicative guidelines "D" which went into effect 1 April 1908, regarding meat inspection[23] and the method is recommended officially for the inspection of domestic meat (see the relevant dispositions of Prussia, Würtemburg, Bavaria, and so forth as well as relevant opinions.[24] In the framework of meat-inspection laws, fresh, frozen, dried, smoked, pickled, cooked, and decaying meat can be subjected to testing by using the biological method. In all these cases the biological reaction reveals the origins of the meat, provided the protein bodies are not completely destroyed by cooking. Despite many efforts, the production of usable antisera for cooked-meat protein has not been successful. Regarding all the details of the technique and methodology I refer the reader to the works cited.

It should be mentioned that under the ban against prepared horse meat is included the introduction of horse intestines and dried horse blood. In these cases, too, the biological method has been used to advantage, as it has in the case of
/335 fish meat (Uhlenhuth, Weidanz, Borchmann). Moreover, if it is possible to extract from either animal or human bones enough soluble, reactive protein, one can determine their origins, something of importance for forensic medicine (Beumer, Schütze, Steffenhagen, and Clough).[25] In forensic cases, however, involving bones which have been burned, bleached, or carbonized, or which have been in water for a long time, this is no longer possible (perhaps, however, by means of the anaphylactic reaction, p. 343).

VII. Though as a rule forensic practice deals with fresh material with which it is easy to produce the specific identification by precipitation, the investigation of relatively old blood stains may still, under certain circumstances, contribute to solving a crime and thus be of importance. In my first efforts I was already able to demonstrate that a positive biological reaction occurred even with blood stains that had dried for weeks or months, indeed for three, five, and eleven years. It was successful, too, with dried organs 1.5 years old. These results were confirmed by others (Biondi, Ziemke, Graham-Smith). Even in the case of mummified organs thirty to forty years old, and of those sixty to seventy years old, I was still able to determine their origin with certainty, while in the cases of Egyptian mummies, a thousand years old, and of a horse muscle a hundred years old as well as with mummies from the lead-lined cellar of the Bremen cathedral (100 to 450 years old) and with the head skin of an Inca skull, the precipitin reaction no longer provided a positive result. The supposed positive results of von Hansemann in the case of 3000–5000 year-old mummies were due to mistakes in the experiments (pseudo-reactions, see above), as I was able to establish. Nevertheless, I would like to take this opportunity to mention that I was indeed able to establish the derivation of individual Egyptian and Peruvian mummies as well as of the one-hundred year old horse muscle, mentioned above, through the anaphylactic reaction (see below p. 343).[26-27]

As I mentioned above, in the Winter of 1900–1901, I had smeared a board (see above p. 315) with different blood types (human, horse, cattle, pig, etc.) on which I carried out my first experiments with dried blood. On the occasion of the celebration of the thirtieth anniversary of forensic blood testing, arranged in my honor by the Greifswald Medical Association, my student, Zimmermann, confirmed that these blood stains produced a prompt, specific reaction.[28] Zimmermann was also able to provide a positive reaction in 336/
most cases with the old judicial exhibits which I had tested successfully at that time. Some of these exhibits had been stored for twenty-five to thirty years in laboratory test tubes. Only in a few cases had the protein lost its solubility and, therefore, its reactive capacity, despite leaching for several days. This is especially true where the blood samples are very small and are solidly embedded through absorption in the tissue fibers. Also, blood stains on a smooth piece of note paper from the year 1900, which I had diagnosed as pig blood, had become insoluble.

From these experiments it also emerges that the testing of relatively old blood stains can still be successful in the subsequent solving of crime. It is not possible to give a time period after which one ought not to expect the biological reaction to be successful. The failure of the precipitin reaction seems to be due primarily to a loss in solubility rather than to a loss in specificity. Such a loss in solubility is dependent on the workings of many outside influences. It is important that the blood dry as quickly as possible, since the protein is only damaged a little by the drying and in such a state can remain intact for many years, since, most importantly, it has been removed from the spoiling process. I therefore recommended at the time that fresh blood, which is found at the scene of a crime, and which is to be handed

over to the experts for examination, be soaked up with a pure piece of blotting paper and allowed to dry. In a petri dish Zimmermann was able to test blood of humans and of a great variety of animal bloods, which I had dried and stored in its substratum in test tubes—thirteen to thirty years old. In almost every instance the result was positive, even when occasionally several days were necessary to produce the solutions. As I mentioned above, I myself was able to produce from a rabbit a practical, usable antiserum to hare's blood even with dried hare's blood, four years old, by dissolving it in a saline solution.[29] Indeed I succeeded in obtaining especially high-potency, specific antisera (titer 1:80,000) with similarly preserved human and canine blood and with egg albumin, all thirty years old. All of these facts might also be of forensic importance for the distinguishing of blood types which are difficult to procure (for example, wild animals in the off-season, etc.).

VIII. In addition, the biological process of protein differentiation according to our experiments has also achieved considerable forensic importance in the control of food products and in the identification of adulterations. Thus, with regard to establishing the presence of egg yolk in dough
/337 products and egg-yolk margarine we can succeed in distinguishing egg yolk from egg white with a specific antiserum to yolk.[30] (See also Otto-Lenghi, Emmerich and others). Adulterations of caviar with less-valuable fish roes could be demonstrated in my laboratory with certainty through the biological method (Kodama, Händel, Schern) since the sturgeon caviar can surely be distinguished from other fish roes by means of a specific serum. Combinations, i.e. adulterations, of nutrient preparations of protein for commercial purposes can be discovered through the biological method as we mentioned earlier. So we were able to prove that hematogin (Hommel) and marketable hemoglobin contained cattle meat. I call to mind the sensational legal process concerning a raw meat liquid extract "Puro" which was supposed to consist of fluid pressed from fresh ox meat, but which contained only dog protein. We were able to establish this by means of the precipitin reaction as did von Gruber and Horiuchi. Also, to identify bee honey (bee protein), i.e. to distinguish it from artificial honey, we advantageously called into service the biological process (see also Langer, Riegler, Galli-Valerio, Thöni and others). The same was true in identifying the provenance of milk products and cheese (Sion and Laptes). The biological method has also been called successfully into service to determine experimentally the origin of fat tissues (butter, bone marrow, margarine) insofar as soluble protein can still be extracted. The same is true for testing plant proteins (wheat, corn, rice, legumes, hemp, poppies, squash, almonds, mushrooms—champignons and others, yeast, etc.) as well as for inspection of animal-feed adulteration, for example, with Ricinussamen (Miessner) as the actual cases have demonstrated.

IX. In other areas such as physiology and clinical medicine, the precipitin reaction has also been shown to, be extremely valuable for the study of nutrition from a physiological standpoint (reabsorption relationships of foreign protein, the mechanics of albuminuria). Regarding this see Uhlenhuth, Citron, Ascoli, Hamburger, Moro, etc. One has also employed the precipitin reaction with more or less success to identify *Echinococcus* and *Taenia* tapeworm infections and for diagnosing cancer. In this respect, I refer the reader to our handbook article (see above). Attempts to feign sickness have been uncovered at the bedside, as was the case with a patient who simulated albuminuria. After chicken protein was identified in the urine instead of human protein, the patient confessed that he had put his breakfast eggs in 338
his urine sample (Wegner). A man who wanted to obtain an annuity by trickery was discovered one morning in his blood-stained bed. He pretended he had suffered a violent hemorrhage. Because the medical examination produced no clue concerning this affair, the blood stains were handed over to me for examination. I determined that it was cattle blood, When he was confronted with this statement, he confessed to having emptied with deceitful intent a flask of cattle blood which he had gotten from the slaughter house.

Concerning a similar case, Merkel reports that he was able to show that a woman, who was under a doctor's care for a year because of an alleged gastric ulcer and had been collecting an annuity, had secretly sprinkled cattle blood into her spittoon.

I would like to allude briefly to my research concerning organ specificity, which took its start from my first experiments, mentioned earlier, concerning the distinguishing of the protein substances in a hen's egg and in hen's blood and which consequently bear a close relationship to the biological differentiation of plasma protein.

I determined that the crystalline lens of the eye is the only animal protein body now known that does not produce a precipitin reaction with a blood antiserum.[31] On the other hand, an antiserum produced by injecting a rabbit with lens protein gave a reaction only in lens protein, but not in the proper blood solutions, or in solutions of other organs. Thus, plasma protein and lens protein—two protein bodies of the same organism—could be distinguished with certainty. These experiments led further to the scientifically interesting conclusion that the crystalline lenses of mammals, birds, amphibians, and, in some lesser respects, of fishes, possess a biologically identical protein. For example, rabbits, which were pretreated with cattle-lens protein, produce a serum that causes an identical precipitation in lens protein of a human, a pig, a dog, a frog, etc., so that here, the law of species specificity of the biological method appears to have broken down. The lens, therefore, must be viewed as though it were a foreign protein body in the animal organism. Perhaps the explanation for this lies in the fact that the lens is purely an epithelial organ which is completely without plasma protein. The organ specificity of the lens has become the subject of far-reaching studies on the ophthalmologens (Römer, Crusius, v. Szily, Doerr, Kraus, Okamoto, Shibata, Uhlenhuth, Händel). Sachs has termed this reaction a lipoid-antibody reaction, and has demonstrated a similar

organ specificity with the brain.

/339 Without going further into the other studies on organ specificity, which have achieved practical importance in differentiating the protein bodies of milk and birds' eggs (white and yolk), I would like to refer briefly to the special case of hemoglobin protein which is of forensic interest. A. Klein and H. Pfeiffer were able to prove that the precipitins which form after injection with erythrocyte extracts (hemoglobin) of various animals are specific, i.e. they produce precipitation only in erythrocyte extracts of the same animal family as the animal which was used for their production. In the corresponding blood sera, on the other hand, precipitation does not occur with such an antiserum. Klein, therefore, believed that one could dispense with making a chemical identification by using such an antiserum. Moreover, the hemoglobin antisera cross react with related animals (horse-donkey, human-ape) just as the antisera against plasma protein. We ourselves were able to confirm that the serum- and erythro-precipitins, if not completely specific, were rather strongly specific. At least, we could not produce a clear reaction in dissolved blood with serum precipitins, when the serum used for immunizing contained no hemoglobin. Despite many attempts, we have not been sucessful in producing high-potency hemoglobin antisera (Uhlenhuth and Weidanz), a fact observed in other quarters. Moreover, there is no need of such sera, which has scarcely been tried in practice. For the rest I refer you to the relevant works (Leers, Hektoen and Schulhoff, Heidelberger and Landsteiner, Hijaschi and others).

Regarding the specificity of serum precipitins and erythro-(hemoglobin) precipitins, it is important to observe the forensically significant point of Mezger, Jesser and Volkmann.[32] The extract of blood encrustations, dried on wood, produced no or only weak precipitin reactions with the usual specific antiserum to plasma protein. On the other hand, an extract from a piece of wood under the blood crust, where the serum, having been pressed out by coagulation, soaked in, produced a clear reaction. It seems important to me in this connection to point out this observation.

Here I think it necessary to make some observations regarding the biological differentiation of sexual protein which Dunbar and I carried out. Dunbar was able to establish in the case of plants, and of animals as well, that the male and female sexual cells react against one another serobiologically and react to other tissue components of the same
/340 organism as if they were foreign (see also Graetz). He was able to demonstrate this especially with the sperm and roes of fishes. I myself with my coworkers Händel, Kodama, and Schern was able to produce proof that fish-roe protein can be sharply distinguished from fish meat of the same animal. It was also possible to show that the eggs of sturgeons can be distinguished from other fish roes (carp, roach, fresh-water carp, tench, salmon, herring, trout). The identification of caviar adulteration, mentioned previously, rests on this observation.

In a similar way we were able to sharply distinguish biologically frog-egg protein from frog-meat protein, while frog-spawn antiserum precipitated, if only weakly, extracts of tadpole protein of the same frog, but not the meat extract of the sexually adult frog (Uhlenhuth, Wurm, Hsia.[33] In these experiments one was able to make the significant assertion that the viscous egg envelope of batrachians, which, according to our experiments consists of mucin, and is most likely made up of admixtures of true protein, possesses the qualities of antigens, so that it is also possible to produce precipitins of an apparently specific character to mucins. Here lie conditions similar to those in the building of antibodies which I first demonstrated, antibodies against almost pure carbohydrate gum arabic.[34] Numerous experiments concerning carbohydrate antibodies in bacteria conform to this conclusion (Avery, Heidelberger, etc.). Recently I made an interesting observation while busying myself with the biology of the potato beetle and the methods of fighting this insect. In the case of frogs, different stages in development with respect to their protein bodies can be differentiated by the precipitin reaction. My work showed that similar conditions pertain in the different stages of development in the potato beetle. Above all, I succeeded with a precipitating antiserum against the eggs of the potato beetle in identifying egg albumin in the sexually mature, egg-carrying beetles by means of the precipitin reaction, while this reaction failed to take place with male beetles.[35] One ought to expand such tests to include the developing phases of other insects (for example, butterflies, caterpillars).

I would also like to remark that I attempted earlier to establish differerences in the blood of sexually mature men and women by using high-potency antisera against human sperm protein. These attempts turned up completely nega- 341/
tive results, while by chance I was able to observe that high-potency antisera to hen's egg white produced a strong precipitation in the blood protein of sexually mature hens as well as in the blood of a rooster.

X. My exposition concerning the biological differentiation of protein would be incomplete if I did not at least briefly refer to the two methods which, from a purely scientific standpoint, are of great interest since one is in a position to detect the least traces of protein by using them. These are the complement binding reaction and the anaphylactic process.

Complement binding (Bordet, Gengou), which has achieved great practical importance in diagnosing infectious diseases such as syphilis (Wassermann), glanders, and others, was recommended by Neisser and Sachs as a control and supplement to the precipitin method, which it parallels, as a rule, in distinguishing human from animal blood. In cases where the precipitin reaction is only indicated in very great dilutions, the positive result of this reaction can be documented in a certain fashion by the absence of hemolysis, while the appearance of hemolysis indicates a negative result. Complement binding has rendered us exceptional service in scientific laboratory experiments where we have been dealing with pure protein solutions, and I myself have used

it with success in such experiments.

With regard to the utility of the method in forensic practice, it is extremely complicated and difficult to carry out, compared to the simple precipitin process. It is also extremely sensitive. This extreme sensitivity is its principal disadvantage in its practical application, since it can still be positive when $\frac{1}{100,000}$ or $\frac{1}{1,000,000}$ cc of blood protein are present. Even human sweat gives a positive reaction under certain conditions, a reaction that can be most portentous in testing a sweat-soaked, blood-stained shirt. With the precipitin method such is not the case, even with high-potency antiserum. We were also able to determine that extracts from different substrata (sacks, foot wrappings, wool stockings) can contain misleading material, which could give rise to error. In the face of its great complexity, its exceeding sensitivity, and the many sources of mistakes inherent in the method, which cannot be overlooked, it cannot be recommended in any case for forensic practice even in the hands of an experienced expert. In practice we do not need a more exact reaction than the precipitin method, conducted ac-
/342 cording to our directions. In every forensic case, I demand that the usual precipitin reaction be carried out according to the well-known prescriptions. If the reaction is positive, then a proper control is superfluous since no doubts can arise. If the precipitin reaction is negative, but the complement binding positive, then in practice, where indeed it is frequently a question of the life or of the death of a man, a judgement ought not to be given regarding the provenance of the blood, if that judgement is based solely on the positive outcome of the complement binding (Uhlenhuth and Löffler).[36]

The same point of view is valid also for using the method in meat inspection, where it can be called upon as a confirmation reaction to a positive result of the precipitin reaction.

Our judgement regarding the significance of the anaphylactic reaction is similar, a reaction well-known in living animal bodies. Its specificity, and the fact that here the smallest traces of protein are enough to induce typical anaphylactic effects in a guinea pig, suggest the thought that it has practical value in differentiating various sorts of protein. The comprehensive experiments, which I conducted with my friend and coworker Händel, led us to the conclusion that in all cases where the precipitin reaction can be used, the anaphylactic reaction can also be employed and that the result of the precipitin reaction alone can be viewed as decisive. The anaphylactic reaction is so sensitive that animals sensitized with urine—as with sweat—react positively with human serum so that the urines of different animals can be distinguished from one another.[37] This sensitivity, however, is a warning that one must be extremely cautious. Because of the circumstantiality, the considerable technical difficulties, as well as the difficulty in giving a judgement based on the hypersensitive reactions which appear differently in individual animals, this process is not sufficiently reliable for forensic practice and is, therefore, unsuited for it. Moreover, it is completely unnecessary. Nevertheless, the worth of the method should not be ignored fo pure, scientific experiments where it can be used to expand and confirm the results produced with the precipitin method. This was true, for example, in my attempts at differentiating frog protein from fish meat, as well as my work in distinguishing frog eggs, tadpoles, and frog-meat protein (p. 340). Here the results agreed with the results of the precipitin method mentioned earlier. Where the precipitin reaction fails or for technical reasons cannot be conducted, one can 343
with advantage bring in the anaphylactic reaction for scientific problems. I have thoroughly studied these conditions together with Händel. It was evident that the anaphylactic method also failed in distinguishing related blood types. Indeed, because of its sensitivity, cross reactions occur more frequently than with the precipitin reaction so that one cannot distinguish rats from mice, as one can with the precipitin reaction (p. 329). On the other hand, we were successful with guinea pigs in establishing definite anaphylactic symptoms with extracts from several Egyptian mummies of the twenty-sixth dynasty (600 BC) and the twenty-first dynasty (950 BC), as well as with Coptic and Peruvian mummies from the cemetery at Ancon when we administered the follow-up injection of human blood (see p. 335). Also, in the case of fourteen year-old human blood which we set in the sun for a long time until it had completely decayed, the precipitin reaction failed, but the anaphylactic method still succeeded in making guinea pigs hypersensitive. Moreover, our further experiments with cooked horse meat, shell-fish meat, and cooked sausage (Uhlenhuth and Händel), as well as with cooked, charred, and decayed bones, produced a positive result where the precipitin reaction and the chemical protein reaction had failed (p. 335).[38] Here, however, a strong cross reaction occurred in later testing of the guinea pigs with heterologous protein. Hailer[39] and later Bürger[40] determined in my laboratory that strongly disintegrated protein (proteolytic products) lost specificity in the anaphylactic reaction. Hailer used for his experiments material he had obtained by thorough cooking with steam or with acid, or through peptic and tryptic digestion, as well as meat extract and nutritive preparation, Bürger amino acids, pure albumoses, protamines and acid albumin.

"The protein molecules, characterized by a species-specific construction of unspecific building blocks, collapse at dissolution into their building blocks. When these are injected, they are capable of producing a sensitizing stimulus in the organism inoculated. This sensitivity is, however, not specific, that is, typical anaphylactic symptoms make an appearance after a second treatment with heterologous protein. This nonspecificity of the resulting sensitivity even appeared when protein which was still coagulatable (species specific) was present in the solution used in pretreatment along with the proteolytic products" (Hailer). Finally, it is also an interesting fact, established by my students, that organs preserved for a long time in alcohol, can sensitize 344
guinea pigs, but only when finely ground organ material is introduced into the animal's body subcutaneously or intra-

muscularly (Dold and Aoki),[41] whereas they were unsuccessful with extract (Kodama).[42] Thus Klabe[43] was still able to produce positive, partially specific reactions with alcohol preparations 25, 38, 41 and 50–60 years old.

We also extended our experiments to include plant oils and fats, where precipitin reactions are also ruled out for technical reasons. Anaphylactic symptoms appeared at the follow-up test with the corresponding native plant protein in guinea pigs sensitized to raw linseed oil, colza oil, almond oil, and coconut butter, although these symptoms were not definite in all cases. Adulterations of animal feed with *Ricinus* seeds, field mustard, and corn-cockle can also be identified in this fashion (Schern).[44] We produced similar reactions to these with animal fats (butter, lard, beef-suet, neat's foot oil) by a second injection with the homologous serum, whereby the animals, sensitized to butter, reacted to the follow-up treatment with raw and cooked milk as well as to cattle serum. The symptoms were not always so convincing in these experiments that delivering a final judgement was possible in every case. Here great caution was demanded. I must at this point give up any closer discussion of all the other attempts at differentiating human and animal hair, skin, protein of organs (lenses), hemoglobin, and sexual proteins, and I refer the reader to the relevant works.[45]

I come now to the conclusion. I hope that in considering my personal experience and some of the results of research, I have succeeded in giving an overview of the growth and development of the biological differentiation of protein. I especially wanted to emphasize the path and the thought processes which led me to discover the method of recognizing and differentiating animal from human blood.

It should emerge from my explanation that the biological
/345 process of protein differentiation has achieved fundamental importance not only for legal judgements and forensic medicine in all national states, but has also contributed to the study of animal taxonomy, of evolution, and of descent by means of its conspicuous identification of blood relationships. It has also furthered research on epidemological relationships in infectious diseases carried by blood-sucking insects. Moreover, it has proven itself indispensible for every-day meat inspection and control of food products (adulteration) and is prescribed by law. Finally it has rendered invaluable service in solving purely scientific questions in the areas of physiology, pathology, clinical medicine, anthropology, zoology, botany, and other branches of natural science.

In an effort to structure the precipitin reaction so that it is as reliable and as faultless as possible, I have worked out through technical directions and prescriptions to eliminate sources of error. By considering also the complement-binding and anaphylactic reactions, I pushed the precipitin method to the ultimate limits of its amazing capability. In my opinion one cannot contemplate any further sophistication with our present-day methods. Such sophistication would scarcely be necessary for forensic practice, since the precipitin method has shown that in the hands of an experienced expert, it has grown to meet all demands made of it. [The remainder of page 345, through page 348, consists of a lengthy Appendix, which discusses in detail the rules and regulations governing the state-controlled testing and quality control of antisera, and so forth. This appendix has been omitted from the translation.]

Notes

1. Uhlenhuth: *Dtsch. Med. Wochenschr.*, 1901, No. 7
2. For detailed information, see the book by Uhlenhuth and Weidanz, cited in footnote 4
3. Uhlenhuth and Beumer: *Z. Medizinalbeamte,* 1903, No. 5/6
4. Uhlenhuth: *Das biologische Verfahren zur Erkennung und Unterscheidung von Menschen- und Tierblut, sowie anderer Eiweisssubstanzen und seine Anwendung in der forensischen Praxis. Ausgewählte Sammlung von Arbeiten und Gutachten.* Jena: Gustav Fischer, 1905—Uhlenhuth and Weidanz: *Handbuch der Technik und Methodik der Immunitätsforschung von Kraus und Levaditi,* Bd. 2, p. 731, 1908—*Praktische Anleitung des biologischen Eiweissdifferenzierungsverfahrens mit besonderer Berücksichtigung der forensischen Blut- und Fleischuntersuchung, sowie der Gewinnung präzipitierender Sera.* Jena: Gustav Fischer, 1909; sowie entsprechende Handbuchartikel, Uhlenhuth und Steffenhagen: *Handbuch der pathogenen Mikroorganismen* von Kolle, Kraus, Uhlenhuth, 2nd ed., 1913, Uhlenhuth und Seiffert, 3rd ed., 1928—Uhlenhuth: *Über die biologische Eiweissdifferenzierung unter besonderer Berücksichtigung der forensischen Blut- und Fleischuntersuchung.* Leipzig: Akademische Verlagsgesellschaft, 1914
5. One should use uniformly thick, appropriately clean, small test tubes (precipitation test-tubes according to Uhlenhuth) with a flange, test-tubes which can be hung in a fitting rack.
6. Manteufel and Beger: *Z Immunitaetsforsch.* **33** (4/5), 1921
7. That is, a dilution of serum.
8. Uhlenhuth: *Arch. Kriminol.* **106** (5/6), 1940, and **109** (1/2) and **110** (3/4), 1942
9. Birch, beech and fir trees do not produce a false reaction.
10. Merkel: *Z. Aerztl. Fortbild.*, 1909, No. 19
11. Directions of the Federal Council for the Combatting of Cholera
12. Uhlenhuth and Beumer: *Z. Medizinalbeamte,* 1903, No. 5/6
13. *Veröffentlichung der Kaiserlichen Gesundheitsamtes, 1903,* vol. XXVII, 1, 42
14. *Works from the P. Ehrlich Institut and the Georg Speyer-Hause,* vol. 47, p. 28, Jena: Gustav Fischer, 1948
15. Nuttall: *Blood Immunity and Blood Relationship.* Cambridge: University Press, 1904
16. Mollison and v. Krogh: *Anthrop. Anz.*, 1937, no 3/4
17. Ehrhardt: *Die Verwandtschaftsbestimmugen mittels der Immunitätsreaktion in der Zoologie und ihr Wert für die phylogenetischen Untersuchungen,* Diss. Rostock, 1929
18. Mollison: Serodiagnostik als Methode der Tiersystematik. In *Abderhalden's Technik der biologischen Arbeitsmethoden,* Abt. IX, Teil 1, Heft 3, 1923
19. Uhlenhuth: *Z. Immunitaetsforsch.* **104** (2/3), 1943
20. Uhlenhuth: *Arch. Kriminol.*, in press
21. *Zuchtbuch für deutsche Schafterhunde,* vol. XXXI, No. 54984
22. Uhlenhuth, Weidanz and Wedemann: *Arb. Reichsgesdh.amt.* **28,** Heft 3
23. *Zbl. Dtsch. Reich* 1908, 60

24. Uhlenhuth and Weidanz: *Praktische Anleitung zur Ausführung des biologischen Eiweissdifferenzierungsverfahrens,* p. 161, Jena: Gustav Fischer, 1909.
Uhlenhuth: Die serologischen Untersuchungsmethoden von Fleisch- und Wurstwaren, Eiern, Fischen, etc. In *Handbuch der hygienischen Untersuchungsmethoden of Gottschlich,* p. 815. Jena: Gustav Fischer, 1927
25. Steffenhagen and Clough: *Klin. Wochenschr.,* 1910, No. 46
26. Uhlenhuth and Haendel: *Z. Immunitaetsforsch.*, 1910, 4, no. 6
27. Uhlenhuth: Festschrift für Schwalbe. *Z. Morph. u. Anthrop.*, 1914, 18
28. Zimmermann: *Dtsch. Med. Wochenschr.,* 1931, No. 6
29. Uhlenhuth: *Dtsch. Med. Wochenschr.,* 1931, No. 42
30. Uhlenhuth: *Dtsch. Med. Wochenschr.*, 1903, No. 5
31. Uhlenhuth: *Festschrift R. Koch* (60th Birthday, December 11, 1903). Jena: Gustav Fischer
32. Mezger, Jesser and Volkmann: *Dtsch. Z. Gesamte Gerichtl. Med.* **21,** No. 1
33. Uhlenhuth-Wurm: *Z. Immunitaetsforsch.* **96,** No. 2 (1939)—Hsiä: *Z. Immunitaetsforsch.* **98,** No. 5 (1940)
34. Uhlenhuth: *Dtsch. Med. Wochenschr.,* 1905, No. 14—Uhlenhuth-Remy: *Z. Immunitaetsforsch.* **79** (1933); **82** (1934); **85** (1935); **88** (1936); **92** (1938)
35. Uhlenhuth: *Kartoffelkäferforschung und bekämpfung.* Aulendorf: Editio Cantor, 1948
36. Uhlenhuth and Löffler: *Klin. Jb.* **19** (1908)
37. Uhlenhuth and Händel: *Z. Immunitaetsforsch.* **4,** No. 4 (1910)—Rhein: *Z Immunitaetsforsch.* **19,** No 3. (1913)—Scheidin: Diss., Strassburg, 1914
38. Uhlenhuth-Haendel: *Z. Immunitaetsforsch.* **4,** No. 6 (1910)—Steffenhagen-Clough: *Klin. Wochenschr.,* 1910, No. 46
39. Hailer: *Abr. ksl. Gesdh.amt.,* Berl., **47** (1914)
40. Bürger: *Z. Immunitaetsforsch.* **33,** No. 2 (1914)
41. Dold and Aoki: *Z. Hyg.* **75** (1913)
42. Kodama: *Z. Hyg.* **74** (1914)
43. Klabe: *Arch. Tierhk.* **44,** No. 3/4 (1918)
44. Schern: *Arch. Tierhk.* **36** (1910)
45. Uhlenhuth and Händel: *Z. Immunitaetsforsch.* **4,** No. 6 (1910)—Uhlenhuth: Über die biologische Eiweissdifferenzierung unter besonderer Berücksichtigung der forensischen Blut- und Fleischuntersuchungen. *Nahrungsmittelchemie in Vorträgen.* Ed. by W. Kerp. Leipzig: Akademische Verlagsgesellschaft, 1914—Clough: *Arb. ksl. Gesdh.amt.,* Berl., **31,** No. 2 (1911)

Concerning a New Forensic Method to Differentiate Human from Animal Blood*

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/187 To differentiate between human and animal blood is a
/188 problem which frequently confronts the forensic expert. If a case involves relatively fresh material, so that the morphological constituents, especially the red blood cells are still intact and a microscopical identification can be achieved with the help of a suitable solvent, then the problem is easily solved. It is then possible to diagnose human blood by measuring the size of the formed elements. But if, as usually happens, the case involves material which has dried for a considerable time on foreign bodies, on cloth, tools, walls, dishes, etc., so that the blood corpuscles, either through natural or artificial processes, have completely or to a great degree been altered in their form or completely destroyed, then it is extremely difficult to diagnose with any certainty whether such stains are of human blood or not. Indeed, in many cases the forensic experts admit that it is impossible, even with the use of blood crystal formation, to produce the desired identification. A process which would enable one, even in cases where the material was old and dried out, to determine in an unequivocal and easily executed manner whether that material came from human blood or not, must be viewed as a major step forward for forensic medicine. We have recently been busy working out such a method on which we wish to report here.

This new process grows out of Bordet's experiments on hemolysins and precipitins. Bordet demonstrated, for the first time in a systematic manner, that when red blood corpuscles of an alien animal species are introduced into the serum of an animal pretreated with those same blood corpuscles, specific substances appear which act upon the blood of the first sort in a certain fashion, and, indeed, some of these substances agglomerate these certain blood corpuscles (agglutinins) and some bring about their dissolution (hemolysins). Bordet was able to show further that these substances were specific, i.e. they acted only upon the blood which had been used for the injection. For example, a rabbit, pretreated with injections of guinea-pig blood, shows in its serum an increased presence of agglutinins and hemolysins only with regard to guinea-pig blood and not to any other type of blood. Bordet pointed out further that the reactive capacity of the animal organism to the introduction of foreign animal substances goes even further, and he showed that after the injection of certain animal fluids, new reaction products make their appearance in the serum.

Thus, he showed that, after the subcutaneous application of cow's milk to rabbits, the serum of these animals, when mixed with cow's milk, precipitated its casein (lactoserum). Tsistovitsch[2] and Bordet[3] were able to demonstrate further that also, when foreign blood serum is introduced, substances appear in the blood in the case of many animals, substances which precipitate the protein bodies of the serum used for injection. Nolf[4] repeated and confirmed these experiments. He was able to verify that the precipitated protein bodies in the blood serum were the globulins. In connection with these experiments, the animals were injected with still other types of animal protein, and the appearance of such reaction products was observed in many of them. Thus, after Myers[5] had introduced peptone, serum globulin, and crystalline albumin, and Uhlenhuth[6] egg albumin from hens' eggs and those of other birds, both saw substances appear in the serum of the animals pretreated with these proteins, substances which precipitated the corresponding protein types.

Our own experiments in this field began when we tested whether the substances which formed in the serum after the injection of animal fluids were of a strongly specific nature, i.e., whether they acted only on the fluid containing the protein which had been used for the injection. To this end, we first examined serum extracted after injections with milk. Just as C. Fisch[7] was able to do independently of us, we could demonstrate that substances appeared in the serum after injection which precipitated only the casein of cow's milk, but not that of goat or human milk, and so forth.[8] Accordingly, one of us recommended at the previous Congress for Internal Medicine[9] application of this new method to the special differentiation of different protein substances. From there we transferred our energies to working out a specific, forensic method, based on these principles, for differentiating human blood from other sorts of blood. We tried this first by using the agglutinins and hemolysins. We

*Translation of: "Ueber eine neue forensische Methode zur Unterscheidung von Menschen- und Thierblut."

in: *Berliner Klinische Wochenschrift* **38** (7): 187–190 (1901).

pretreated rabbits with defibrinated human blood, and then observed whether it was possible to make a diagnosis with the aid of the agglutinins and hemolysins, which formed in the serum of the pretreated animals. It soon turned out, however, that this process was not useful in practice, since the effects of the agglutinins and hemolysins were apparent when there was still a large number of preserved, red blood cells present in the human blood to be tested, in other words, when the blood was relatively fresh. Accordingly, we decided to use for the specific method, not the hemolysins and agglutinins which appear after injections of defibrinated blood, but rather the precipitins, which appear after injections of cell-free serum, whose specificity we had already demonstrated with lactoserum. Since, as we observed above, the precipitins[10] acted on the dissolved protein bodies of the blood, the globulins, and not on the structural elements as do the hemolysins and the agglutinins, whenever this first method is employed for diagnosis, the test object could be much older, and so altered that no morphological elements were present. The previous experimental methods were not capable of identifying older material. We proceeded in this manner: We undertook to treat rabbits with five to six subcutaneous injections each of 10 cc of cell-free, human blood serum at approximately two-day intervals. Approximately six days after the last injection, the animals were bled to death. They had done well under this treatment. The blood was placed on ice to separate out the serum. If one now adds ½ cc of this rabbit serum to a solution of human serum, diluted with physiological saline solution, or adds it to a dilute, laked solution of human blood produced with distilled
/189 water, an intense, cloudy precipitation appears almost immediately at room temperature, and even more intensely in an incubator at a temperature of 37°. A further question now was whether this precipitation was strongly specific, i.e., whether it occurred only when mixed with solutions containing human blood. To test this, we added serum from rabbits, which had been treated with human serum in the manner presented above, to the laked blood of all the animals which we encounter in daily life, as far as these were available to us, to blood from mammals, birds, fishes, altogether twenty-three different animals.[11] We then discovered that, in fact, the serum of rabbits, injected with human serum, acted in a extremely specific manner, i.e., it produced precipitation in no other blood type outside of the human, with one exception, represented by ape blood. In the laked blood of this animal we also obtained a precipitation after adding the serum of the pretreated rabbit, although only after a rather long time and to a lesser degree. This result is also of general scientific interest, in that it shows us that, in fact, the protein bodies of the ape are very close in their constitution to those of humans. For the exclusively practical goals of forensic medicine which we have pursued, this circumstance ought to cause no serious concern, since under the conditions in our land, blood stains of ape blood will scarcely come into question. Our results up to that point were still not sufficient to recommend the method at that time in practice. We had to see first whether the effect of our serum was still clearly visible, when the material to be tested was not fresh, as it had been in previous experiments, but older and transformed by time. Above all, we needed to know the thing which was most important in practice, whether in the case of such old material the process was still specific, i.e., whether or not, somehow, in old and dried types of animal blood, precipitations are produced by the specific rabbit serum, where this does not happen with fresh, animal blood types. Accordingly, in the month of October in that year we set out blood stains from humans and from all other animal species mentioned, on linen cloth; some we put on tools, for example, on a knife. These stains we produced artificially. We let these objects lie without any special care. Then, in the following January, after approximately three months, we extracted with 5 to 6 cc of saline solution one or more blood stains, each about the size of a dime, from all twenty-four test samples. These stains had, in the course of time, been transformed, and had turned brown as a result of the formation of methemoglobin. We then obtained a dirty-brown, cloudy liquid which we rendered completely clear by filtering through a paper filter. The solution must, without exception, be absolutely clear, if the result of the reaction is to be certain. Especially in the case of stains some of which from bird or fish blood it is quite often necessary to filter the fluid several times in order to remove the turbidity in the wash solution, a condition which arises from the presence of concentrated curdles, i.e., fatty impurities. We now filled each test tube with four to five cc of this solution of extract of blood stains, added to each tube ½ cc of serum from a rabbit, pretreated with human blood, and put each sample in the incubator at 37°. After twenty minutes, and occasionally sooner, the test tube in which the solution, extracted from the stain of human blood was contained, displayed a definite cloudiness; all the others remained clear, with the exception of the tube with ape blood, which showed a faint, incipient turbidity. After another fifteen minutes a definite, flocculent precipitation had been deposited on the bottom of the tube containing human blood. At this point we need not especially emphasize that the addition of normal rabbit serum, serum from a rabbit not pretreated with human blood, naturally did not produce any turbidity at all in human blood, i.e., in the solution extracted from a stain of human blood. Thus, the method enabled us easily to reach a certain decision with these blood remains, even in the case of old, dried blood substances, as to whether we were dealing with human blood or not.

For the practical application of the method we recommend the following. One should inject rabbits[12] subcutaneously five to six times in the manner described above with 8 to 10 cc of human serum. Six days after the last injection, one bleeds the animals by opening the carotids, and then places the quantity of blood extracted in the icebox to separate out the serum.[13] The experiment then proceeds in the following manner. The material to be tested is extracted as completely as possible in six to eight cc, occasionally more, of physiological saline solution. This solution, when filtered

to complete clarity, is divided into two equal portions which are poured into two sterile test tubes. To one tube is added ½ cc of the serum of a rabbit pretreated with human serum; to the other is added, as a control, ½ cc of normal serum from the same animal species, in this case, from a rabbit which was not injected with human blood. Then, four to five cc of a blood solution, laked with distilled water, or of a blood stain extract of another animal species, for example from pig blood or sheep blood, is placed into a third tube to serve as a control. To this tube is then added 0.5 cc of serum from a rabbit pretreated with human serum. All three test samples are set at a temperature of approximately 37°. If an apparent turbidity and the formation of precipitation begins within ½ to 1 hour in the tube which contains the suspected material submitted to forensic testing, and to which was added the serum of the pretreated rabbit, while the two others remain unchanged in their completely clear state, then one can make the certain diagnosis that the substance in question comes from human blood, so long as the anamnestic reaction of ape blood can be ruled out in the case of the test substance.

190 A few weeks ago, we demonstrated the method we have described here, a method which we have often carried out, to the Director of the local Royal Educational Institute for Government Pharmacology, Professor Strassmann. To what extent this method will meet the requirements of each case, which vary so greatly from one case to another, and how far the method can be perfected to meet these needs, are questions which lie far outside our area of research. Thus, in the most cooperative way, Professor Strassmann and Dr. Ziemke of the aforementioned Institute declared themselves ready to study and to develop the method further in regard to these stated goals.

References and Notes

1. Follows a demonstration given by the authors at the Physiological Society in Berlin on February 2, 1901
2. *Annales de l'Institut Pasteur,* 1899
3. Ibidem, 1899
4. Ibidem, 1900
5. *Centralbl. f. Bacteriol.,* 1900, vol. 28, No. 8-9
6. *Deutsche Med. Wochenschr.,* 1900, No. 46
7. Studies on Lactoserum and on Other Cell-Sera. *St. Louis Courier of Medic.,* February, 1900
8. Cf: *Deutsche Med. Wochenschr.,* No. 30, 1900, Vereinsbeilage, p. 178 and *Ztschr. f. Hyg.,* vol. 36, I, 1901
9. Cf: A Wassermann, Verhandlungen des Congresses für innere Medicin, 1900
10. Note added during correction: The most recent number (No. 6) of the *Deutsche Medizinische Wochenschrift* contains an article by Uhlenhuth in which the author, proceeding from the same principle, succeeds in using the precipitins for the differential diagnosis of human and animal blood.
11. Blood from the following animals was used: donkey, goat, cow, ox, calf, sheep, pig, dog, cat, ape (a small Pavian), guinea pig, rabbit, house mouse, house rat, goose, duck, pigeon, sparrow, eel, pike and tench.
12. For some time we have been testing whether, after injections of human blood, the precipitating substances also appear in the serum of other animals, larger than rabbits, since this would naturally be more convenient in practice. Thus, we are presently treating a goat with injections of human serum.
13. The human serum necessary for injection is easily obtained in these quantities from any larger hospital, where bleeding cups are often applied for therapeutic purposes. It is even easier and more convenient to get it from maternity hospitals by pressing out the placentas. Moreover, we ought to test whether the same substances appear in the serum of pretreated animals after the injection of larger quantities of human pleural transudates, or abdominal transudates, containing the same protein substances as the human serum. The action of the serum producing the reaction is stronger the sooner it is used after being removed from the rabbit. We are convinced that serum which is kept on ice still produces a reaction in a certain and prompt fashion fourteen days after its extraction. Thus, it is possible, if necessary, to dispatch the serum from a central station.

A Process for the Forensic Identification of the Origin of Blood. (Fixation of Hemolytic Complement) *

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(Director: Privy Medical Officer Professor Dr. P. Ehrlich)

/1388 The eminently important question for forensic medicine, determining the origin of blood stains, has been solved from an unexpected direction as a result of Uhlenhuth's experiments and those conducted independently by Wassermann and Schütze. The so-called biological method for forensic blood identification is known to rest on the capability of the serum from animals pretreated with certain types of blood to produce a specific precipitation in a diluted solution of that very blood type. Although the trustworthiness of this process has been thoroughly tested in theory and practice over the last few years, and we ourselves arranged many experiments in which it proved itself completely, still we cannot hide from ourselves that the technical difficulties in practice are often great and that there exists a lively desire for a control of the experiment in view of the grave importance of the results. Therefore, we would like today to publicize a method of forensic blood differentiation which we have tried to advance in close connection with the progress of serum research. We seem to have met with the best success.

Moreschi's beautiful work, conducted under R. Pfeiffer's direction, stimulated us to undertake our experiments.[1] Moreschi reported about "a type of anti-complement serum effect which forms as the result of the cooperation of two substances, the first present in the serum of the pretreated animal, the second in the serum of the animal species (or in that of a closely related species) whose serum was used for pretreatment." Without wishing at this point to delve further into the theoretical meaning of this very interesting observation, we wish only to mention that this involves the same phenomena which Gengou described a few years ago, and is connected with the presence of amboceptors which sensitize protein bodies in the blood of animals pretreated with serum protein, etc.[2] Of special importance for our goal was the fact that "the very smallest quantities ($\frac{1}{100,000}$ cc) of normal serum sufficed to produce the anti-complement effect." This fact encouraged us to apply the phenomenon described by Moreschi to the identification of the smallest quantities of human blood, which are necessarily the givens in forensic practice. Our experiments based on Moreschi have met our expectations.

The experiments, which convinced us of the usefulness of this method are the following. To carry out the experiment, for every 1 cc of 5% sheep-blood suspension 0.0015 cc of amboceptor (the serum of a rabbit, pretreated with ox blood, that reacts also with sheep blood) and 0.05 cc of fresh guinea-pig serum as complement are used. The sheep-blood corpuscles used in this system are completely dissolved by the combined action of amboceptor and complement. The serum of rabbits pretreated with human serum served as an antiserum.The addition of 1 cc of this antiserum does not influence the hemolysis. A disturbance, i.e. an inhibition of hemolysis, however, was to be expected after the above steps were followed, if a trace of normal human serum was present. On the other hand, the hemolysis would of necessity be promptly resumed, if other types of normal serum were present. The experiment confirmed the correctness of our assumption, having been carried out in the following manner: 0.1 cc antiserum and 0.05 cc complement and varying amounts of different normal sera (each brought to a volume of 1 cc in a saline solution) are mixed and are left standing at room temperature for one to two hours. Then one adds 1 cc of 5% sheep blood and 0.0015 cc amboceptor and allows the mixture to stand for one to two hours at 37°. The results of the experiment, one of many similar ones, are shown in the following table:

As the table shows, only human and ape sera effect a cessation of hemolysis; all other types of sera, which were introduced, proved to be ineffective. It should not cause amazement that the sera of humans and apes behave essentially in an analogous fashion, when we consider the close relationship between these animals, though as a rule the latter produces a clearly weaker reaction. If we do not consider the common effect of human and ape blood, we are dealing with a phenomenon which is specific for human serum as the experiments show, a phenomenon which is so extremely fine that it is easily capable of identifying $\frac{1}{10,000}$ cc, and almost always $\frac{1}{100,000}$ cc, and occasionally even $\frac{1}{1,000,000}$ cc of human serum. The extreme fineness of this method suits it especially for the forensic differentiation of blood, which process involves the identification of the smallest traces of blood. Moreover, we were able most easily to differentiate the blood of human provenance from among extracts of blood stains dried three months earlier on linen, blood stains

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*Translation of: "Ein Verfahren zum forensischen Nachweis der Herkunft des Blutes (Ablenkung hämolytischer Komplemente)."

in *Berliner Klinische Wochenschrift* **42** (44): 1388-1389 (1905).

Amounts of Normal Serum cc	Hemolysis which was begun by the addition of serum of:							
	human	ape	rat	pig	goat	rabbit	ox	horse
0.01	0	0	C O M P L E T E	C O M P L E T E	C O M P L E T E	C O M P L E T E	C O M P L E T E	C O M P L E T E
0.001	0	0						
0.0001	0	M O D E R A T E L Y						
0.00001	a trace	C O M P L E T E						
0.000001	complete							
0	complete							

which came from sheep, chickens, rabbits, guinea pigs, humans, oxen, and horses, and these stains were in a dilution in which the precipitating serum was scarcely able to produce a reaction.

Whether this method is superior to the one outlined by Uhlenhuth and Wassermann, only more experiments and practical experience can show. In any event we can immediately recommend it as a control and as a supplement to the precipitation method, and we can assert that it is equally as accurate as that method.[3] Moreover, it has certain advantages. First, the failure of hemolysis is a more apparent criterion than precipitation formation which quite often is only indicated faintly.[4] A further advantage of this system is that there is no need of clarifying a large quantity of solution for the reaction. For the Wassermann-Uhlenhuth method, this clarifying process, which is sometimes quite difficult, is absolutely necessary. In addition, the extraction of antisera to use in our experiment is easier. It is well known that it is very time consuming to obtain a high-potency serum suitable for the Uhlenhuth method, since the animals display the greatest individual variations in their ability to build precipitins. Thus, from a rather large assortment of pretreated rabbits, only a very few produce a usable serum. On the other hand, we have at our disposal examples of antisera which caused precipitation at their limit in a solution of human serum with a strength of 1:100 or 1:1000 but were capable of recognizing $\frac{1}{100,000}$ cc of human blood by using the method described. Finally the use of antisera is less restricted, in that the frequent presence of serum opalescence, which renders the observation of precipitation very difficult, is irrelevant for recognizing the hemolytic effect.

On the other hand, it might be possible that the results of the hemolytic method of identification could experience interference if unspecific, inhibiting substances are present in the objects submitted for testing. This obstacle, though unlikely must still be considered. It can be easily overcome by destroying the inhibiting effect of the human serum by cooking. In doubtful cases, a control for the test would be present in the form of a cooked solution. Regarding the technique for the process, execution of the experiment must at first be limited to those laboratories in which on-going results regarding the hemolytic effect have been collected. Presumably every hemolytic combination can be used as a reagent. It is only due to a circumstantial accident, their availability at the time, that we used the serum of a rabbit pretreated with ox blood, a serum reacting with sheep blood, as the amboceptor and guinea-pig serum as the complement. Further experiments should show whether still more appropriate combinations can be discovered.

At this point we want to say only a few words concerning the active mechanism which causes the reaction. We do not consider it essential, as Moreschi believes, that the binding of the complement and its fixation with the blood corpuscles, laden with the amboceptor, is caused by the precipitin produced by the common action of human serum and the antiserum. Rather we incline much more toward the interpretation already put forth by Gengou, that the complement fixation represents the effect of protein bodies of the blood which have been sensitized and dissolved by the specific amboceptors.

From this point of view the phenomenon which we have described can be explained without further ado. We are dealing then with the same principle which Ehrlich and Morgenroth first recognized, namely that the amboceptor, in and of itself, is incapable of binding the complement, that it must undergo an increase of its avidity by anchoring itself to the susceptible substrate so that it then is able to bind to the complement. Bordet and Gengou used this function of firmly anchored amboceptors to identify indirectly amboceptors of cellular elements in the serum. Gengou went an important step further when he transferred the effect of the antisera to the dissolved protein substances and demonstrated that one can be certain of the presence of amboceptors by means of the complement-binding function of protein solutions digested by specific antiserum. If one divides the antibodies of cells into agglutinins and amboceptors, one will be justifed in differentiating protein antibodies into precipitins and amboceptors, so long as their identity has not been proven. Consequently we must temporarily base the complement-binding function of protein bodies of the blood, laden with specific amboceptors, on the mechanism of the process as described in Ehrlich and Morgenroth's interpretation. Their

important conclusion, based on principles derived by hard laboratory work, shows how the apparently impractical and theoretical study of immunity reactions has again produced results which, when applied in practice, have proven themselves of the greatest usefulness.

Strictly speaking, this method is naturally related just as little to the identity of the blood *qua* blood as is the Uhlenhuth method. It rather makes possible only the determination of its origin. It is a method to differentiate protein types of specifically varied provenances. Therefore, the identification of blood as such must be furnished separately whenever the method is employed.

We cherish the hope that the fixation method will prove itself in later tests and will constitute a welcome increase in our aids for forensic blood diagnosis.

Notes

1. C. Moreschi. Zur Lehre von den Antikomplementen. This journal, 1905, no. 37
2. Gengou. Sur les sensibilisatrices des sérums actifs contre les substances albuminoides. *Ann. Inst. Pasteur*, Paris, Vol. XVI, 1902
3. In forensic practice it is often important because of the small amount of material at hand that one unite both methods into one experiment. One first sets up the precipitin reaction. After noting the results the complement test is appended; the mixture remains standing for a while, and then the blood and amboceptor are added.
4. Moreover, in a court case it is often desirable to be able to display the evidence at the debate. This can be done most easily by centrifuging the undissolved blood corpuscles and by preserving the residue by adding a suitable preservative. The different color of the solution (red-colorless) will represent a marked difference even for the layman.

The Forensic Differentiation of Blood Using the Antihemolytic Effect. (Second Communication)*

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/67 In issue number forty-four of this journal we recommended for forensic practice a method to identify the origin of blood by means of the fixation of hemolytic complements, a method based on the experiments of Gengou and Moreschi.[1] Since then we have collected more facts concerning the suitability of the method and its technique. Proceeding /68 from the fact that normal hemolysins, and those produced for immunity, display their effects according to the same mechanism, we have employed the hemolytic effects of normal serum to produce the reaction instead of the immune sera we used at first. Thereby the arrangement of the experiment can naturally be greatly simplified, in that in the case of normal hemolytic serum the two necessary reagents, the amboceptor and the complement, are ready to use in one liquid, while these reagents must be added separately when using artificially produced hemolysins. The hemolysin against sheep blood contained in normal rabbit serum has in our opinion shown itself to date to be the most appropriate, and this is for the following reasons. First, the rabbit is the customary laboratory animal, so that the extracting of serum should not create the least difficulty. Then, too, the hemolytic effect of different rabbit sera with respect to sheep blood is in general rather constant, so that one is not to a great degree dependent on the accidents of nature. Usually 0.25 to 0.15 cc represent the smallest doses which will lyse one cc of 5% sheep-blood cell suspension. Moreover, sheep blood is everywhere easy to obtain. If it should not be convenient to hire on a sheep at the testing center, entrusted with forensic blood differentiation, then the slaughter house can surely make the blood available. One can easily preserve this blood on ice for up to four days. Accordingly, the order of the experiment is as follows. First, in a pre-test, a completely lysing dose of rabbit serum must be established. In the experiment which is described below, this amounted to 0.25 cc. Now 0.25 cc of rabbit serum is mixed with the liquid to be tested for human blood and with the antiserum[2] (in our example 0.01 cc). The mixture is left to stand for one hour at 37°; then follows the addition of one cc of 5% sheep blood. Again the mixture stands for one hour at 37°. The reading can be taken after two hours. Failure of hemolysis indicates the presence of human blood. In a control experiment, set up in the same way, save that the solution to be tested for human blood is left out, the hemolysis must take place. A model experiment is shown in the following Table 1. Various amounts of human serum served as test objects.

Table 1

Amount of human serum cc	Amount of hemolysin (normal rabbit serum) cc	Amount of antiserum cc	hemolysis of 1cc of 5% sheep blood
1/1000	.25	.01	0
1/10,000	.25	.01	0
1/100,000	.25	.01	0
1/1,000,000	.25	.01	moderate
1/10,000,000	.25	.01	strong
1/100,000,000	.25	.01	complete
0	.25	.01	complete

As the table shows, the hemolysis is inhibited completely by the interference of $\frac{1}{100,000}$ cc of human serum, but even the presence of 0.000001–0.0000001 cc of human serum still reveals itself by clear alterations. Thus, the precision of the method leaves nothing to be desired. It seems to us that the small amount (0.01cc) of related antiserum is also noteworthy. There is at times an advantage in using smaller quantities for setting up the reaction, since in many cases a certain amount of antiserum appears to correspond to an optimum effect. In practice, it turns out that every antiserum to be used must, in any case, be tested regarding its effectiveness and then can be used in the test. We recommend 0.0001 cc as the amount of human serum to be identified. We consider it necessary for the acceptance of an antiserum for forensic purposes that it can identify at least this amount of human serum. Such a predetermination on the antiserum is exceedingly easy. It represents a reproduction of the above experiment, except that here the amount of human serum remains constant while the amount of antiserum varies. In Table 2 we present the predetermination of conditions for the antiserum used in Table 1.

*Translation of: "Die forensische Blutdifferenzierung durch antihämolytische Wirkung. II Mitteilung."

in *Berliner Klinische Wochenschrift* **43** (3): 67–69 (1906).

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Table 2

Amount of human serum cc	Amounts of hemolysin cc	Amount of antiserum cc	hemolysis of 1cc of 5% sheep blood
0.0001	0.25	0.15	a little
0.0001	0.25	0.1	a trace
0.0001	0.25	0.05	a trace
0.0001	0.25	0.025	0
0.0001	0.25	0.015	0
0.0001	0.25	0.01	0
0.0001	0.25	0.005	0
0.0001	0.25	0.0025	0
0.0001	0.25	0.0015	a little
0.0001	0.25	0.001	moderate
0.0001	0.25	0.0005	strong
0.0001	0.25	0	complete

The increase in the hemolytic effect when an excess of antiserum is used, an increase apparent from the table, is due to the hemolytic amboceptors of sheep blood which are still present in unactivated antiserum. The antiserum is extracted from a rabbit and must, therefore, also contain the normal amboceptors of such blood. Thus, an excess of hemolytic amboceptors results and, as we can report according to our relevant experiments, such an excess appears always to frustrate the demonstration of the Gengou-Moreschi phenomenon of the anti-complement effect.[3] One could easily remove the normal amboceptors of the antiserum causing the interference by absorption with sheep blood, but such measures seem superfluous for carrying out the reaction, since, as the table already shows, lesser amounts of antiserum are perfectly suited for our method. This is, moreover, a lucky circumstance, since it protects against too hastily wasting this valuable material. In order to provide an approximate basis for further experiments we would like to mention that according to our experiences with usable antisera, 0.02 cc represents as a rule the optimum quantity.[4]

/69 We would like to recommend in any case the use of normal hemolysins as the best for practice. The technique is thereby made extremely easy, and any interference from any sort of disturbing antibodies is ruled out. It would, of course, be desirable to replace sheep blood with that of a smaller animal, although obtaining sheep blood is, in our opinion, not a serious difficulty. It must be left to further experiments to demonstrate whether other hemolytic combinations of blood and serum, extracted from laboratory animals, can be recommended.[5]

Concerning the relationship of our method to the tested Uhlenhuth-Wassermann reaction we can in essence only recommend what we have already presented when we described our first experiments dealing with hemolysins produced through immunization. Just as the biological precipitin method to identify the origin of blood represents the application of the important principle of protein differentiation discovered by Wassermann, so our process, strictly speaking, is based only on the identificaiton of protein. Regarding accuracy, our method is at least as reliable as the method using precipitation. Indeed, we do not want to neglect to mention that we have had a positive result of the fixation reaction, even when no preciptation formation could be detected. In any case the strength of the precipitation and that of the fixation capability do not stand in direct proportion.[6] Therein we can see the fundamental reason for the supposition, already expressed in our first work, that our method possibly involves a different class of protein antibodies which act as amboceptors in Gengou's sense.[7]

Be that it may, it seems to us that the fixation process ought to be included with the Wassermann-Uhlenhuth method in forensic practice. We are convinced that the forensic expert will declare it a welcome change to be able to reach his decision, one so full of responsibility, by basing it on two methods, which mutually control and supplement one another. We ourselves advantageously employed this combined test in two forensic cases which were handed over recently to the institute, and we considered it of special value to be able to base the identification of human blood on a positive result in both experiments.

The first case involved a small tree leaf on which were found a few blood stains. An extract of these was produced in the least possible quantity of saline solution. 1 cc of the solution served to set up the precipitation reaction when 0.1 cc of antiserum was used. A weak but clear turbidity ensued, and finally a precipitation formation. The remaining 0.2 cc of the extract was increased ten times in volume (2 cc) with physiological saline solution. This solution, diluted to one-tenth of its strength, was still usable in the fixation reaction. Increasing amounts of this solution were each mixed with 0.25 cc of rabbit serum (as a hemolysin) and 0.02 cc of the same antiserum which was used for the precipitin reaction. These mixtures are left to stand for one hour at 37°. Then, sheep blood is added. The same experiment is simultaneously repeated, but the antiserum is not added, in order to determine whether the solution to be tested has an anti-hemolytic effect in and of itself. Table 3 shows the result.

Table 3

Amounts of the 1:10 diluted extract solution cc	1 cc of 5% sheep blood, 0.25 cc of rabbit serum	
	a) 0.02 cc antiserum	b) 0.2 cc saline solution
0.5	0	complete
0.25	0	complete
0.15	strong	complete
0	complete	complete

As the table shows, 0.025 cc of the original solution still causes a complete inhibition of hemolysis. The fixation method was thus able to identify human blood in a fortieth of the amount used for the precipitin method.

The second case which we examined involved a few small blood stains found on a wooden hammer. This experiment was set up exactly in the same fashion as indicated for the first case. While in 1 cc of the extract obtained, the addition of the antiserum resulted only in a very weak, but nonetheless apparent reaction, 0.2 cc of the same solution still produced a total inhibition of hemolysis in the fixation

experiment.[8]

Our method has proven itself not only in a laboratory test, but also under the serious conditions of real practice. Thus, we think we can recommend it as most advantageous to include in the forensic blood test next to the officially recognized reaction of Uhlenhuth and Wassermann the method of complement fixation of normal hemolysins which we have presented.

Notes

1. M. Neisser and H. Sachs. Ein Verfahren zum forensischen Nachweis der Herkunft des Blutes. *Berlin Klin. Wochenschr.*, 1905, no. 44
2. As in the Uhlenhuth-Wassermann reaction, the antiserum is rabbit serum which comes from rabbits pretreated with human serum.
3. The cause of this phenomenon certainly lies in the fact that the need for complement to produce hemolysis becomes less when the amount of amboceptor increases so that traces of free complement are still available to produce the effect.
4. The cooperation of the normal amboceptor contained in the antiserum suggested to us to utilize simultaneously the normal hemolysins to sheep blood contained in the antiserum as hemolytic reagents. In actuality this is easily possible, and accordingly, the arrangement of the experiment follows these specifications. First, one determines the dose of antiserum which will completely lyse sheep blood. Then one allows a mixture of this amount of antiserum and of the solution which is being tested for human blood to stand for one hour at 37°. Then, sheep blood is added. One must weigh another consideration against the advantage of this simplified technique for daily practice. Since the hemolytic complements of sera become ineffective rather rapidly, the hemolysins contained in antiserum will lose their effectiveness under normal preservation conditions. But, when we conserved the antisera in a frozen state at −12°, the hemolysins were preserved. At least, when we tested five-month old antisera for its hemolytic action in sheep blood, we found that the sera possessed the normal hemolytic effect.
5. We have until now been able to fulfill this need only by calling upon amboceptors obtained in the immunization process. In this respect the combination—guinea-pig blood, specific amboceptor obtained from a rabbit, and normal rabbit serum as complement—has proven itself useful in our view. The advantages of this combination lie in the fact that all of the animal sera to be used in the experiment comes from rabbits. There is only the guinea pig as a second blood donor. The use of this second method is always convenient, whereas obtaining sheep blood could be impractical. Moreover, when a case involves the identification of the blood from one of three related animal species, sheep, goats, or oxen, then sheep blood must be avoided as a reagent in the interest of clean experimental conditions.
6. Recently A. Klein also reported relevant observations (*Weiner Klinische Wochenschrift*, 1905, No. 48).
7. Wasserman and Bruck (*Med. Klinik*, 1905, no. 55) support this view in a very interesting article which appeared while this study was at the press. In ingeniously arranged experiments they use the fixation process to differentiate bacterial extracts and to demonstrate that even old bacterial extracts produce the complement binding function when the corresponding immune serum is added, although these old bacteria, as opposed to freshly obtained extracts, cannot be precipitated.
8. Note added during correction: In the meantime we have also had the opportunity to participate in a forensic blood test in which the bloodstains in question were not of human blood, but of pig blood. The use of the fixation process gave the same diagnosis (pig blood positive, human blood negative).

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