

## Section 4. Blood Grouping

Leone Lattes (1887–1954) was one of the best known medico-legal and general serologists in the first half of this century. He became involved in blood grouping in its early years, and even his earliest papers demonstrate a grasp of the subject that was not widespread at the time. His book *L'individualita del Sangue nella Biologia, nella Clinica e nella Medicina Legale* (1923) became a classic, editions of it being issued in German, French, and in 1932, in English. The Lattes papers indicate the early techniques, in which the agglutinin in the stain was sought for determination of the ABO group. Tests for isoagglutinin in blood stains are still referred to as "Lattes" tests. The 1927 paper recounts a number of his cases. This paper was written in German (he wrote papers in German and French, as well as in Italian).

Lattes' obituary appeared in *Haematologica* 38 (11) in 1954. Siracusa's paper introduced the elution procedure for detecting agglutinogens in dried blood, and it discusses the so-called absorption-inhibition method as well. The two were used side by side in these studies.

Franz Josef Holzer (1903–1974) was a well-known medico-legal blood grouping specialist who studied in this country for a time with Landsteiner. His 1931 paper introduced an inhibition procedure for grouping bloodstains which was used for many years. In 1937, he discussed the secretor characteristic as a marker in forensic investigations. The 1953 paper reviewed the current status of blood grouping, especially in its medico-legal applications. Dr. Holzer spent much of his career at the University in Innsbruck.

## On the Practical Application of the Test for Agglutination for the Specific and Individual Diagnosis of Human Blood\*

Doctor Leone Lattes

University Lecturer and Assistant

Institute of Forensic Medicine of the University of Torino

Director: Professor M. Carrara

310 The importance of the natural hetero-agglutinins for the specific diagnosis of blood, according to the method proposed by Marx and by Ernrooth, has been very restricted following the further works appearing on this subject. Without citing here works whose results are contained in the treatises, (see the chapter *Agglutination und Hämolyse* of Landsteiner in the *Handbuch der Biochemie* of Oppenheimer, and *Die forensische Blutuntersuchungen* of Leers), one can say that from all the research, it appears that the test for the hetero-agglutinins can be considered only a preliminary one to the precipitin test.

The limited significance of this test, on which many hopes were founded because of its simplicity, depends on two rules of reason. Contrary to the assertions of Marx and Ernrooth, the test can turn out negative, even when it is surely a matter of heterologous blood, either because it is a case of blood from very young individuals in which agglutinins do not exist (see Dungern, Halban, Landsteiner, Baccchi) or because the stains became dried, sometimes even for a brief period (Uhlenhuth, Martin) or finally it is because the stains were altered by some chemical or physical agent (Carrara, De Dominicis). Therefore, all authors up to now unanimously think that the unsuccessful agglutination of the human globules, in the presence of extracts of bloodstains, is not a valid reason for excluding its being heterologous blood.

311 On the other hand, not even the positive outcome of the agglutination is valuable in demonstrating that it is a matter of heterologous blood, since the reaction can occur through the intervention of the isoagglutinins that one so frequently finds in the human blood. Therefore, the test of Marx-Ernrooth does not have any precise significance, in a practical way whether the results are negative or positive and it would be absolutely necessary to reject it.

However, the authors have proposed criteria, according to which it would be possible to distinguish isoagglutination from heteroagglutination. Thus they have argued the hetero-

\*Translation of: "Sull'applicazione pratica della prova di agglutinazione per la diagnosi specifica ed individuale di sangue umano."

in *Archivio di Antropologia Criminale Psichiatria e Medicina Legale* 34 (4 ser. 5): 310-325 (1913).

Reprinted with the kind permission of Edizioni Minerva Medica, Torino, and the family of Prof. Dr. Lattes through Fiammetta Lattes Treves, Milano.

agglutination is more rapid and more intense than isoagglutination, that the isoagglutinins are much more labile and likely to disappear in a very short time, that the heteroagglutinins, unlike the isoagglutinins, lose their efficacy in the presence of a serum of the same type as those of cells that are used in the test and that heteroagglutination is regularly accompanied by hemolysis. The existence of these differential characteristics was, however, not at all confirmed. From the works of Martin, Uhlenhuth, Landsteiner and Leiner, Moss, Baccchi, etc., it is clear that they have no objective foundation and that very often, isoagglutinins exhibit behavior perfectly identical to that of the heteroagglutinins.

The only real difference that exists between the two types of agglutinin is that the heteroagglutination occurs regularly whenever the individual supplying the human cells used in the test is of inconsistent type for isoagglutination, making the selection irregular and variable according to the cells used, until human cells are found that are refractory.

Baccchi, who was recently occupied with the value of the Marx-Ernrooth test for the specific diagnosis of blood, did not hesitate to propose applying it to the practice of forensic medicine. He proposed, namely, to follow the agglutination test with the extract of a stain, not only with one variety of human erythrocytes, but with several. In that case, if all these globules became uniformly or almost uniformly agglutinated, it would be possible, with great probability, to believe that the blood under consideration is of heterologous origin, while when there are conspicuous differences in the agglutinability of the cells, it would be possible to conclude that the stain is of human blood.

It would be necessary, nonetheless, to carry out the test on a great variety of globules, since, doing it as this author does, on only four types, makes uniformity of behavior possible even for isoagglutination. 312/

Baccchi, however, apart from his perfectly theoretical justification in recommending this test, leaves us uncertain about its practical applicability, and particularly, as to the number of the types of cells it would be necessary to test before arriving at a certain diagnosis. On the other hand, the multiplicity of tests serves to rob this technique of its greatest advantage, that is to say, of its simplicity and rapidity.

In order to eliminate the inductive uncertainties in the test of the heteroagglutinins by the possible presence of iso-

agglutinins, it seems to me appropriate to confront the problem in its fundamentals, seeking to annul the action of the isoagglutinins, rather than to insist on the alleged differences of these two series of bodies. It is not possible at present to destroy the isoagglutinins in such a way as to preserve the possible heteroagglutinins, but the action of the latter can be demonstrated by recourse to a simple artifice. It is sufficient for this purpose to adopt for the reaction, not any already arbitrarily chosen human cells, but those that have been demonstrated to be refractory to isoagglutination.

All authors who have occupied themselves with this issue have noted that certain human cells are agglutinated by certain human bloods and others are not. Marx and Ernrooth also observed that certain varieties are not agglutinated by any human blood serum. The irregularity of the behavior of isoagglutination is then discussed. In reality, isoagglutination is not at all irregular, but it is characterized by certain rules, which serve to clarify the differences in behavior of single sera or single cells, and permit a choice to be made among them, when it is necessary to choose cells appropriately reactive for this diagnostic test.

From the works of Landsteiner, Langer, v. Dungern and Hirschfeld, Jansky, Moss, little known in the forensic camp, the results clearly show that isoagglutination is dependent upon the presence of certain specific groupings on the red cells, susceptible to reaction with corresponding agglutinins present in other serum, and thus manifesting the phenomenon of agglutination.

According to the above-mentioned works, the specific groupings of the human erythrocytes able to react with the isoagglutinins are only two, denoted by the letters A and B; they can be present separately or coexist, or both can be lacking. Landsteiner expressed the rule that a normal blood never contains the agglutinins able to agglutinate its own erythrocytes (and, in reality, one finds autoagglutination only in pathological circumstances), and it contains instead agglutinins able to react with the groupings that it does not have. Thus, in conclusion, human bloods can be divided into four groups: 1) groups with cells containing the grouping A, with serum containing the agglutinin called  $\beta$ , able to agglutinate B cells; 2) groups with cells with structure B and agglutinin  $\alpha$ ; 3) groups with the cells having A & B structures and lacking isoagglutinins; 4) groups with non-isoagglutinable cells, that is to say, lacking A and B, and serum containing the two agglutinins  $\alpha$  and  $\beta$ .

This division issues clearly from the above-mentioned works, but it was expressed in this form only in the works of v. Dungern and Hirschfeld.

And very notable is the fact that the percentages of the single types, quoted, independently of one another, by v. Dungern and Hirschfeld in Heidelberg, and by Moss in Philadelphia, were just about identical.

Moss, in 1600 tests carried out on the sera and cells of one hundred individuals, did not find any exceptions to the grouping scheme indicated here.

Among the four groups listed above it is the fourth that

matters to the problem occupying our particular interest.

The red cells that belong to it would have no affinity for the isoagglutinins. Therefore they would have to constitute an excellent reagent for the test of Marx-Ernrooth, for the direct interpretation of just what is necessary to eliminate the action of the isoagglutinins.

In order to find these cells easily I followed the pathway indicated by Landsteiner's rule. According to this, the bloods containing cells with no isoagglutinable groups are those whose serum contains the two agglutinins  $\alpha$  and  $\beta$ .

These bloods, according to the figures of v. Dungern and Hirschfeld and of Moss, represent about 40% of all the bloods, and are, therefore, easily encountered by examining a certain number of sera and cells chosen arbitrarily.

The technique used certainly is of great importance for the proper appreciation of agglutination, as I was able to convince myself in research done for other reasons. Moss tested for agglutination in small tubes, and perhaps this technique should be judged the most appropriate, were it not for the necessity of taking blood from a vein. The microscopical examination in hanging drops often gives uncertain results with agglutination, given the possibility that the cells can collect upon one another by the simple action of gravity.

It does not seem appropriate to me to introduce an agglutination test on too minute a scale, since too many positive reactions would be required, often masking the reality of the situation. In my opinion the "traces" cannot be accepted as indicative of a positive reaction. According to Baccchi, "The formation of small and few groups of corpuscles are observed in the most peripheral parts of the drop, given simply stacking of these same corpuscles at their borders." On the other hand, I would, like Baccchi, call such reactions negative. These facts correspond quite well to the data obtained in tubes by considering as positive agglutination (by analogy to bacterial agglutination), only cases in which the cells are really agglutinated, that is to say, reunited in an irregular accumulations of many globules, with superposition, and not merely their collecting upon one another. It is undeniable that sometimes one has small, clear diamond shapes according to this criterion, since often not all of the corpuscles become agglutinated. In my records I assigned three degrees of agglutination: the first—positive—in which the formation of the accumulations is evident, but there remain free corpuscles; the second—strong—in which the majority of the corpuscles are agglutinated; and the third—total—in which all or almost all the corpuscles are within the same mass.

The tests were done in hanging drops with red cells washed twice and suspended at about 5% in physiological saline solution. I allowed one loop of these cells to react with two loops of serum, diluted by half. In order to obtain the necessary serum and cells easily, I aspirated seven to eight drops of blood from the ball of the finger in pipettes constructed especially for the purpose, and containing many glass beads. Shaking these pipettes defibrinated the blood, and the globules were then separated from the serum by centrifugation. Only the cells being necessary, I collected a

		RED CELLS																			
		O				B				A						AB					
		L.L.	G.E.	L.J.	MAZ	R.V.	RIC.	R.R.	R.P.	C.V.	G.C.	P.L.	ET.	ALF.	M.C.	R.S.	Z.G.	C.C.	C.M.	L.C.	
$\alpha\beta$	L.L.	-	-	-	-	-	N	+	+	+	+	+	+	+	+	+	+	+	+	+	N
	G.E.	-	-	-	-	-	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	L.J.	-	-	-	-	-	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+
$\alpha$	RIC.	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	N			+
	R.R.	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	N			+
	O.E.	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
	R.P.	-	-	-	-	-	-	-	-	-	+	+	N	N	N	+	+	+	+	+	+
$\beta$	P.L.	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+
	ET.	-	-	-	-	-	N	+	+	+	-	-	-	-	-	-	-	-	-	-	+
	ALF.	-	-	-	-	-	N	+	+	+	-	-	-	-	-	-	-	-	-	-	+
	M.C.	-	-	-	-	-	N	+	+	+	-	-	-	-	-	-	-	-	-	-	+
	R.S.	-	-	-	-	-	+	+	N	+	-	-	-	-	-	-	-	-	-	-	+
O	G.B.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	L.C.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

N=unexpected negative (see text) [Designated in the original text by heavy type minus signs]

315 drop of blood directly in 1 cc of physiological saline solution. The observation of the reactions were made after 1/2 to 1 hour of constant temperature incubation.

In order to identify the non-agglutinable cells I allowed the cells and the sera of a certain number of normal individuals (twenty-four in all) to react so as to distribute them in the various groups on the basis of their behavior in the agglutination test.

316 I report in a table (see above) the results of these tests indicating for every serum and cell the category to which the results belong, omitting from the table only a few of the tests conducted, which gave patterns with cells and sera that were too incomplete.

From this table the division of the bloods, examined in the way indicated above, is very clear. There are two classes of bloods in which the sera reciprocally agglutinate each other's cells and these have, respectively, the cells A and the cells B.

These two classes do not occur with the same frequency, and according to the nomenclature of v. Dungern and Hirschfeld, the one that is encountered most frequently can be designated A, and the less frequent one B.

We have then a group with A cells and agglutinin  $\beta$  able to agglutinate the B cells (blood A $\beta$ ), another with B cells and  $\alpha$  agglutinin (blood B $\alpha$ ). Another still, with cells lacking A and B namely the non-isoagglutinable, and with serum containing  $\alpha$  and  $\beta$  agglutinins (blood O $\alpha\beta$ ). A last class, representable by AB $\alpha$ , has a serum not provided with isoagglutinins, and its cells are agglutinated by sera from the other three classes.

The twenty-four bloods that I examined were divided in the following way: 6 O $\alpha\beta$ ; 5 B $\alpha$ , 11 A $\beta$ , 2 AB $\alpha$ .

In the table are reported in heavy type some reactions which do not correspond as one would have thought to the above-mentioned division. [Heavy type is replaced in the translation by the letter N.] Certain cells containing the groups A or B were not agglutinated by sera containing the corresponding agglutinins  $\alpha\beta$ .

I found only negative exceptions, that is to say, in certain cases the agglutination did not occur where it could be expected; but I never observed abnormal, positive agglutinations in opposition to the division of the bloods into four groups. It is not impossible that in the cases in which agglutination was absent, it would have occurred eventually, but was so weak as to be unappreciable with the technique used. Still, even v. Dungern and Hirschfeld have noted that not all the homologous bloods behaved identically. Thus, certain agglutinins  $\alpha$  did not react with all the A cells but only with a part of them. By absorbing, moreover, a serum  $\alpha$  with A cells (namely, by treating it with an excess of A cells and by separating it by centrifugation), there often remain agglutinins for the other A cells. So that the biological configurations A, B,  $\alpha$ ,  $\beta$ , cannot be considered as unitary entities, but as corresponding to series of substances that can assemble according to certain relationships.

V. Dungern and Hirschfeld admit, therefore, that all the bloods of one and the same group are not identical, but that a further subdivision exists among them; and even with the use of animal sera "absorbed" with human cells of single groups (experimental conditions rendered much more complex by a partial superposition of the groups) there are differences that can more properly be called individual, and theoretically permit the diagnosis of the single individuals.

The possibility that certain agglutinations are unexpectedly negative does not have great importance for the choice of the globules to be used in the Marx-Ernrooth test. Since, for this, one must use non-isoagglutinable globules, only the eventuality of exceptional positive isoagglutinations would invalidate the results of the test, causing one to be able to confuse isoagglutination with heteroagglutination. Instead, the O cells presented perfectly uniform and identical behavior with respect to the isoagglutinins, not having been agglutinated by any of the sera tested. It is, therefore, possible to identify human cells in the way described above, which are both theoretically and experimentally refractory to isoagglutination.

317

They are, therefore, very well suited to serve as reactive cells for the reaction of Marx-Ernrooth. The positive value of the reaction under these experimental conditions is not being able to attribute agglutination to isoagglutinins. The results of all the previous work show that the heteroagglutinins act uniformly on all the human cells, and furthermore, this characteristic has been considered by Baecchi as the one which can differentiate them from the isoagglutinins. I tried a simple control; since the experiments of other authors were already decisive on the matter of the action exercised by the animal bloods on the non-isoagglutinable O cells.

The tests were carried out by treating the cells of LL and GE in the table with extracts of the bloodstains of the more common animals (rabbit, guinea pig, dog, chicken, etc.) and unanimously showed positive agglutination.

318 These results coincide with the analogous tests of v. Dungere and Hirschfeld. These authors have, nevertheless, observed that the serum of the anthropoids (chimpanzees) can, in some cases, fail to agglutinate the A or B cells. This analogy between the serum of the anthropoids and that of man, is not surprising nor does it in any way change the value of positive agglutination of the O globules, which often occurs even with blood of higher apes.

Negative results from the reaction carried out on these cells cannot, as is clear, have any decisive significance. In fact, as was mentioned above, it can happen that even heterologous bloodstains are inactive on human cells.

The reaction of Marx-Ernrooth carried out on non-isoagglutinable human cells reacquires value for its precious properties of rapidity and simplicity. It can be objected that the identification of the needed cells is rather complex and requires testing of a number of bloods. But even if one wanted to examine as many bloods as I myself did (that does not seem necessary), suffice it to say that this set of tests has been accomplished once and for all. It may be noted that, in the blood of adult individuals, the relative properties of agglutination are maintained unaltered for an indefinite time period, such that once an individual carrier of O cells is identified, one can employ these globules for the reaction at once, eventually carrying out a control test from time to time.

As long as the test was done in that way, it had notable simplicity and certainty, but it cannot, nevertheless, compete with the precipitin test. The latter is, in fact, of much more general applicability, and especially has the advantage of permitting the direct recognition of human blood, while agglutination cannot directly demonstrate neither the presence nor the absence of human blood, but only the presence of non-human blood. The agglutinin reaction, performed with known cells, can lend notable service, nonetheless, when confirmed by the precipitin reaction, and especially in the cases which it is appropriate to orient oneself to the nature of the bloodstain rapidly, before having set up the precipitating sera. Besides, the positive outcome of the agglutinin test can indicate directly, whenever the precipitin test with

anti-human serum is by itself positive, the simultaneous presence of non-human blood in the human bloodstain. 31

To the agglutinin test is, therefore, attributed a value in itself, different from and better than the simple criterion of confirmation.

Isoagglutination has also been used, as is known, for the individual diagnosis of human blood. The authors who proposed this application, namely Landsteiner-Richter and Biffi, based it on the fact that autoagglutination is a completely exceptional phenomenon in healthy individuals; therefore, when a stain shows positive agglutination with the cells of a particular individual, it is possible to exclude that bloodstain from having come from that same individual.

Everyone agrees that if, instead, agglutination does not occur, one must stop and declare that no response can be given.

Baecchi justifiably thinks that one must not stop at this point, because if a stain does not agglutinate certain corpuscles, that does not mean that it cannot very well agglutinate some others. Therefore, he thinks that comparing the behavior of the stain with that of the blood of the individual suspected of crime and to a series of erythrocytes of diverse origin, one can arrive at two orders of conclusions. First of all, if one finds a type of corpuscle to which the two bloods react entirely differently, it cannot be doubted that their differentiation is indisputably established. Since, according to this author, it can be supposed that all the bloods are capable of isoagglutination, it would always be possible, following this indirect method, to exclude a given stain as having come from a particular person, given that, with a sufficiently large series of corpuscles, it is practically impossible to find two stains that resemble one another. Therefore, it would always, or in most cases, be possible to decide on the non-correspondence of a given bloodstain with a given person. In the second place, it would be possible in favorable circumstances to arrive at a direct individual diagnosis of a stain, when the stains furnished by an individual demonstrate a behavior completely identical to that of the stains to be diagnosed, using a suitable series of corpuscles of various origin.

Well, now, these clear, new facts notably restrict the practical value of these conclusions. 320

Above all, there exists in every series of bloods a percentage which is destitute of isoagglutinating capacity, and this is equal to about 10% according to the experiments of v. Dungere-Hirschfeld and Moss and also according to mine. In the series of normal individuals studied by Baecchi the percentage is the very same (7 in 63) if, for the above-mentioned reasons, the "traces" are considered negative. Baecchi has said that these bloods would have demonstrated agglutinating properties, if tried with a larger series of cells than he employed. This is, in reality, very improbable.

The two o sera of my table (GB and LC) were tried on twenty-two and twenty-three different types of human cells, respectively, and none of them ever agglutinated. One can,

therefore, affirm that bloods exist which lack isoagglutinins.

Therefore, when a stain is constituted by a blood of that kind, unless circumstances are very favorable for freshness and preservation, the diagnosis of exclusion of a particular individual will turn out completely uncertain, even if this blood were endowed with isoagglutinating properties, since these would be able to disappear in the stain.

But there is still another more important possibility, namely that the two bloods which are under consideration for identification or differentiation belong to the same group. Then they can resemble each other to such degree as to lead to more dangerous errors. The perfectly analogical behavior that can exist between two different bloods renders very problematical the assertion that one can always diagnose exclusion. Even in Baecchi's tests, one finds that there are groups in which the smallest quantitative differences of the agglutination of four varieties of corpuscles would not permit exact differentiation of the single individuals. But also, adopting a very extensive series of corpuscles, as in the tests I followed, we can observe the same behavior in different bloods belonging to the same group. The possible existence of exceptional reactions (those in which agglutination is absent), those which distinguish a blood from the others of the same group, allows one to suppose that, by multiplying the number of tests still further, it would be possible finally to find a cell that differentiates two similar bloods. But it does not seem to me necessary to take account of such a theoretical possibility in practice. In the first place, these exceptional reactions can be verified equally with different bloods, as my table clearly shows. In the second place, the number of red cells that would have to be examined in order to lend certainty to finding a difference between two bloods of the same group in every case would have to be considerable, such as to remove from this procedure every element of practicability. In the third place, the imprecision of the number of cells on which the investigation would have to be based would not allow the results to have the degree of certainty indispensable for forensic judgments. If we think, in fact, of obtaining from a stain and from a suspect's blood identity of behavior with respect even to an extensive series of cells, we can conclude that the two bloods belong to the same group; but at the state of our knowledge we will not be able to assert that the two bloods belong to the same person, or even to different persons. We can always think that, multiplying again the number of cells tested, we might find some difference, and that, therefore, the bloods can be different. Thus one cannot with any argument establish that the absolute identity of behavior excludes the possibility of another blood of the same group. In my experiments I obtained at various times absolute identity, even in the exceptions, of the behavior of different bloods with respect to a non-negligibly large series of erythrocytes.

By this I do not intend to deny that there can exist and does exist an individuality in the human blood, but I believe only that it is not manifest through these characteristics so clearly that we can demonstrate it at present by means of

isoagglutinins.

I think, therefore, that to speak now of direct individual diagnosis is premature. This does not exclude the possibility of searching thoroughly and going beyond the simple diagnosis of human blood, and it is actually possible to make a distinction directly between the different bloods, which has very notable forensic importance. In addition to the prudent negative diagnosis of a stain (not belonging to a given individual) of Landsteiner-Richter and Biffi, a positive, direct diagnosis seems to me perfectly warranted, not yet of the individual, but rather of the group. Having established that the stain is human (preferably by means of the precipitin test) one can find some value in the isoagglutinins, whose resistance to different agents was established in preceding work, in order to circumscribe the number of individuals to whom the stain can belong, and assigning to it one of the four groups of which we spoke above. This can be attained by allowing the extract of the stain to react with A cells and with B cells, or better yet, having seen the possibility of exceptions, on several varieties of both A cells and B cells. If only the A cells become agglutinated, it is a matter of a  $B\alpha$  blood; if only B cells, of  $A\beta$ ; if both, of  $O\alpha\beta$ . When neither A nor B become agglutinated, the diagnosis will have to remain inconclusive, since it could be a matter of  $AB_0$  blood, but the destruction of the agglutinins which might have been present could also have occurred.

Only when blood is very fresh and manifestly unaltered, will one be able to presume that it should be assigned to this last group.

One must, in addition, establish the group to which the blood of the suspected individuals belong, and one can then make the timely comparisons easily and possibly draw conclusions from them.

I carried out some of these tests with recent bloodstains, setting up conditions under which I would not be subject to any preconceived notions or suggestions, and I obtained favorable results.

A colleague brought me a stain on blotting-paper, three days old, with a request to indicate to which of seven persons it belonged. The extracts of the stain were tested with A cells (GC) and with B cells (CV) which never showed exceptional reactions. The extract of the stain agglutinated the B cells and not the A ones. The sera and cells of the seven persons indicated were examined. One of these bloods belonged to the group  $O\alpha\beta$ , two to the group  $B\alpha$ , four to the group  $A\beta$ . Therefore I judged the stain as belonging to one of these four, which matches exactly.

This result is already notable, having succeeded in excluding three persons out of seven; but when the number of persons to be distinguished is smaller, the response can be completely individual.

I was given a stain on blotting-paper that was five days old, with an offer to state to which of three people it belonged. The extract of the stain agglutinated the A cells as much as the B cells. Of the three persons, the first has  $O\alpha\beta$  blood, the second  $A\beta$ , the third  $B\alpha$ ; I judged, therefore, that

the stain belongs to the first of these, the one which matches exactly.

323 One cannot give a definitive value to this laboratory research, the conditions being much more complex in practice. Nevertheless, in the expectation that other studies will establish the limits of applicability of this group diagnosis, the following points do not seem doubtful to me. First of all, although more modest, an individual direct diagnosis is more certain. On the other hand, the value of this research surpasses that of the simple reaction between the stain and the cells of the individual suspects, since, whenever that reaction gives negative results, it permits no other conclusions; but the investigation of the groups can be made to distinguish whether this negative result depends: 1) on similarity of blood group of the stain with that of the suspect; 2) on the fact that the stain does not contain isoagglutinins; or 3) on the fact that the suspected blood belongs to a non-isoagglutinable group, (Oab). This distinction, obviously, has considerable importance.

If one succeeds in establishing that the blood of the stain and that of the suspect belong to different groups, the response will be easy, since it certainly cannot be a matter of the same blood.

When, instead, it turns out that the stain and the suspected blood belong to the same group, this coincidence can have, as is clear, very great judicial importance.

An individual diagnosis of exclusion can be indicated within the limits of the same group (and only when it is not a matter of the group ABo lacking isoagglutinins) on the basis of different behavior with a variety of erythrocytes from the same agglutinable group; and as is stated above, and can be seen from the table, it will perhaps be possible in some favorable case to be done. But a direct and sure individual diagnosis of identity between the two bloods cannot, for now, be achieved, since whenever they have identical behavior with respect to a long series of erythrocytes of diverse origin, the possibility that they belong to different individuals cannot be excluded.

I conclude as follows:

1. Among the four groups into which the bloods can be divided on the basis of their capacity to participate in isoagglutination, there is one in which the red cells are refractory to isoagglutination.

324 2. The study of these cells is very timely as is their use for the specific, negative diagnosis of human blood according to Marx-Ernrooth, the interfering action of the isoagglutinins being eliminated in this way.

3. For the identification of the individual origin of a bloodstain, its assignment to one of the above indicated groups can be very useful.

4. It cannot always be considered possible to establish by means of agglutination that a stain does not belong to a given person. This negative diagnosis will almost always be impossible when the blood of the stain does not contain isoagglu-

tinins, and it will be extremely difficult and uncertain when the stain and the blood of the individual suspect belong to the same group. The assignment of the stain and of the suspected blood to some one of the groups can illuminate the individual origin of the stain more than the absence of the simple reaction between the stain extract and the cells of the suspect blood.

5. The one positive, direct diagnosis obtainable by means of isoagglutination up to now is the group diagnosis. It is premature to speak of individual, direct diagnosis, since different bloods belonging to the same group can agglutinate in an identical way a lengthy series of test cells.

### Works Cited

1. Baccchi, Sulla diagnosi individuale di sangue umano. *Arch. di Psich. etc.* 31, 4-5, 1910
2. Baccchi, Ricerche sulla prova biologica di Marx e Ernrooth per la diagnosi specifica di sangue umano. *Arch. intern. de med. leg.* 4, 270, 1910 (bibliografia)
3. Biffi, Sulle emoagglutinine del sangue umano etc. *Ann. d'igiene sperim.* 1930, II, 232; *Bol. de la Ac. nac. de med. de Lima*, 3-2, 1930 (*Se-maine Med.*, 1903, 424)
4. Carrara, Di un nuovo metodo per la diagnosi specifica di sangue umano. *Arch. di Psich. etc.* 25, 332, 1901
5. De Dominicis, Sulla reazione di Marx-Ernrooth. *Giorn. di Med. Leg.* 11, 1904
6. v. Dungern, Ueber Nachweis und Vererbung biochemischer Strukturen und ihre forensische Bedeutung. *Meunch. Med. Wochenschr.*, 1910, No. 6, 293
7. v. Dungern und Hirschfeld, Ueber Nachweis und Vererbung biochemischer Strukturen. *Zeitschr. f. Immunitaetsforsch.* 4, 531, 1910
8. Idem, Ueber Vererbung gruppenspezifischer Strukturen des Blutes. *Ibid.* 6, 284, 1910
9. Idem, Ueber gruppenspezifischer Strukturen des Blutes. *Ibid.* 8, 526, 1911
10. Halban, Agglutinationsversuche mit mütterlichem und kindlichem blute. *Wien. Klin. Woch.* 545, 1909
11. Jansky, Haematologische Studien bei Psychotikern. *Kliniky Sbornik*—1907, No. 2. *Jahresbericht f. Neurol. u. Psych.* 1907 (da Moss)
12. Landsteiner, Ueber Agglutinationserscheinungen norm. menschl. Blutes. *Wien. Klin. Wochen.* 1901, 1132
13. Idem, Agglutination und Haemolyse. *Hdb. d. Biochemie Oppenheimer* II, 1, 413
14. Landsteiner und Richter, Ueber die Verwertbarkeit individueller Blutdifferenzen fuer die forensische Praxis. *Zeits. f. med. Beamte* 1903, No. 3
15. Landsteiner und Leiner, Ueber Isolysine und Isoagglutinine in menschlichen Blute. *Zentr. f. Bakter.* 38, 548, 1905
16. Langer, Ueber Isoagglutinine beim Menschen etc. *Z. f. Heilk.* 24, 111, 1903
17. Leers, *Die Forensische Blutuntersuchungen*, Berlin, 1910
18. Martin, Isoagglutination beim Menschen nebst Bemerkungen Unterscheidung von Menschen- und Säugetierblut. *Zentr. f. Bakt.* 39, 704, 1905
19. Marx und Ernrooth, Eine einfache Methode zur forensischen Unterscheidung von Menschen- und Säugetierblut. *Muench. Med. Woch.* 1904, No. 7, 293 and No. 16, 296
20. Moss, Studies on isoagglutinins and isohemolysins. *Bull. of the Johns Hopkins Hosp.* 21, 63, 1910
21. Uhlenhuth, Der forensische Blutnachweis. *Wiener Med. Wochenschr.* 1904, No. 43-44. *Fortschr. d. Med.* 1904, No. 3

## Two Practical Cases of Individual Diagnosis of Human Blood\*

**Doctor Leone Lattes**

University Lecturer and Assistant

The Institute of Legal Medicine  
University of Torino

Director: Professor M. Carrarra

/298 The results of the experiments that I have been conducting for many years on the individual diagnosis of human blood have an application in two practical cases which are, to my knowledge, the first of those kinds of diagnoses to actually be performed. The publication of these cases, demonstrating the resolution of one of the more important problems of judicial practice, will undoubtedly interest everyone involved in forensic medicine. Simultaneously, the technique used in this diagnosis, indicated in a general way a previous publication of mine† will show that it is necessary to submit to the exigencies of the particular case in point, and show some flexibility according to the circumstances.

The first case was not of a judicial nature, but a purely private matter.

The fifty-year old worker, R. G., appeared at the Institute for Forensic Medicine in order to explain a situation that had gravely disturbed the tranquility of his family for a good three months. He came for advice to a colleague, Dr. Bertola, who indicated to him that the Institute of Forensic Medicine was the only place where he might perhaps be able to clear up this affair. At R's first explanations, and having seen his interest in the thing and its relationship with the studies we had been pursuing, I accepted gladly the assignment.

/299 R. told how on a Sunday, he had put on a dress shirt and gone to a town near Torino where he stayed until late at a country inn of some friends. The next day he took it off in order to wear it again the next Sunday; he then noticed that there were blood stains on the shirt, but he ignored it. The next day his wife, A. G., a woman of high character, very jealous, who habitually accused her husband of disloyalty, asked him for an explanation of those spots, proving in her mind that he had once again been disloyal and that the stains surely demonstrated that he had had relations with other women during his stay outside Torino. (This had been fur-

ther confirmed by a fortune-teller!) The husband claimed, on the other hand, to be innocent and had intended from the beginning on the contrary to keep the stained shirt in the hope of being able to demonstrate his true innocence with it.

The discussions surrounding this matter became continual and so harsh so as to render life impossible for every member of the family, so much so that even strangers in the neighborhood were implicated. R. was extremely desirous of clearing up the mystery of the stains so that peace could return to the household. While the wife explained the stains in the above-mentioned manner, he advanced the following hypotheses as to their origin:

(1) Being in the habit of going to buy meat from the butcher, he thought it possible that on the Sunday on which he wore the shirt he soiled his fingers with blood in choosing the meat, and then pulling out the shirt to urinate he soiled it.(??)

(2) On the Monday on which he left the shirt, he came to find his wife's friend, the one who helped her to make the beds. This friend was menstruating and remained for some seconds alone in the bedroom. He supposed that she was able to use the shirt to dry the menstrual blood.

(3) It was more probable that the suspicious wife, not having ever been able to prove his supposed adultery, had made the stains with real blood, seeking to provoke a confession from him.

These last two hypotheses aroused anger and incredible resentment.

The shirt, of the finest linen, shows in the first part, on the anterior area near the edge, an oval and irregular spot about 5 × 2 cm, rather dense; other similar ones, 2 × 2 cm., are near the edge; other thinner stains, with the appearance of having been rubbed, are located at a distance of 10 to 25 cms from the edge. Given the appearance of the stains and R's age, I suspected that that man could be afflicted with prostatic hypertrophy and that the blood came precisely from a hemorrhage of the urethra. He said that for a year and a half he had had to get up two or three times almost every night to urinate; he never had any retention of urine nor observed any hemorrhaging; the outflow of urine was somewhat diminished. He often experienced sensations of fullness in the rectum.

By rectal exploration was noted a rather hard prostatic gland, protruding noticeably in the rectum. On the sacrum

\* Translation of: "Due casi pratici di diagnosi individuale di sangue umano."

in *Archivio di Antropologia Criminale Psichiatria e Medicina Legale* 37 (4 ser. 7): 298-308 (1916).

Reprinted with the kind permission of Edizioni Minerva Medica, Torino, and the family of Prof. Dr. Lattes through Fiammetta Lattes Treves, Milano.

† See in this journal, 1915. In a previous communication (*Giorn. dell'Acc. di Med. di Torino*, 1916) I indicated further useful modifications of the technique, which will be reported in a forthcoming issue.

and the buttocks were found a large and itchy eczema. The wife many times observed blood stains on the sheets, but she always attributed them to the scratching of the eczematous skin. She never saw blood on the shirts.

Summing up all the possible origins of the blood stains, excluding, for obvious reasons, the hypotheses of the wife, the diagnosis had to be made among bovine blood, urethral blood of R. G., menstrual blood of her friend T. E., and blood of the wife A. G.

With a small trace of stain one performs the species diagnosis with high titer anti-human precipitating serum, according to the usual technique, with the following controls: human serum, human stain, ox blood, physiological saline solution. It showed very evidently that the stain is human blood; it immediately showed an opaque ring just as the one within the human controls.

This limited it, therefore, to the individual diagnosis of three people, and I was prepared to carry on with it.

As a result of my previous work, the first objective was to obtain an extract of about the same volume as the blood from which the spot was constituted. Since the spot was not crusted on, but impregnated the material, it was convenient to obtain the piece of dried blood by removing from the stained piece a similar, clean piece of the same surface. In order to do a similar comparison with the greatest possible exactness, after various tentative experiments, I went back to the following method. Having chosen the largest and most dense stain, I delimited the smallest area of linen that contained it, I counted the threads in the one direction and in the other with the help of a strong lens; the threads showed 180 in one direction and 65 in the other.

Then in the clean part of the shirt I cut 10 pieces of cloth with the same number of threads. To do this, I cut with the scissors somewhat larger pieces, then with a thin needle I separated the excess threads, and finally, with straight cutting scissors and with the help of the lens, I cut the edges of the protruding threads from the edges of the rectangular pieces of linen. Dried in a thermostat, the 10 pieces were weighed with maximum precision with the following results:

1. 0.0900 g	6. 0.894 g	
2. 0.0900 g	7. 0.893 g	
3. 0.0898 g	8. 0.893 g	Average
4. 0.0898 g	9. 0.0890 g	0.0895 g
5. 0.0898 g	10. 0.0885 g	

301 The stained material, cut in the same way, weighed 0.0944 grams. Therefore, deducting the average, the maximum and the minimum weights of a similar piece of unstained material from this number, the weight of the dried blood was, on the average, 0.0049 grams, but it could vary between 0.0059 and 0.0044. Considering that the residue of dry blood is 20%, in round numbers, of the total weight (Hammarsten), it was necessary to add 0.0176 grams of distilled water in order to renew the volume of blood (basically, that amount small enough to avoid hypotonicity). Then to increase the volume of the extract in a way that would have certainly diluted it by half, taking away, therefore, from the maximum weight

of the blood, it was necessary to bring the extract to a volume of 0.0472 cc with physiological saline solution. As a practical matter, I rounded the figures, and in small closed weighing bottles made of emery I put the cut out stain, following its contours, adding to it 0.02 cc of distilled water and 0.03 of physiological saline solution, leaving it to steep for twelve hours in an ice box and squeezing it repeatedly with a glass rod. Then, squeezing the piece of cloth between two pieces of glass I could, with a small capillary pipette withdraw a dark brown extract, to be used for the tests; its dilution with respect to the blood could vary from 1:2 to 1:3, and therefore can be regarded as appropriate. This extract was collected in a small tube, closed with a rubber stopper.

According to the proposition of Landsteiner and Richter, they would test the agglutinating property of this extract with respect to the red cells of the three suspected persons, mixing two loops of extract in a hanging drop with one of a 5% suspension of washed cells. All three of the tests turned out negative. I went on then to the agglutination tests, according to the same technique, of running test erythrocytes with the extract and with the sera of the individual suspects, diluted 1:3 with physiological saline solution. Besides these, some other complementary tests were done, letting the cells and sera of the three individuals react with one another. These were distinguished by a simple mark inserted by my colleague, Romanese. As test cells I used a pair of A cells and a pair of B cells, which I had already noted in my previous research. The results obtained were the following (23-25 February 1915) [as shown in Table I].

I did not keep track of quantitative differences in the agglutination, since in the three month old stain the agglutinins could be weakened compared to the fresh sera.

The meaning of these designations was the following:

- I.—R. G. (husband)
- II.—T. E. (friend)
- III.—A. G. (wife)

The above tests show that the husband and the friend belong to group  $\beta A$ , the wife, instead, to group  $\alpha\beta 0$ . And since the stain belongs to group  $\beta A$ , the results exclude the possibility that the blood could have come from the wife. Having arrived at this point, it was necessary to distinguish the blood of the husband from that of the friend, although the hypothesis that the blood came from the latter was somewhat improbable, and vigorously denied. At this point it would have been necessary to carry out tests with long series of B cells to see if the two bloods behaved differently with respect to any one of them. But as I explained elsewhere, the outcome of this difficult investigation is quite problematical, and the experiment would have been justified only if no other way could have been found. Since among the elements of the problem was the circumstance that the friend's blood could only have been menstrual blood, it was much simpler here to distinguish the source of the blood, and thus the investigation became a matter of determining whether the stain was from menstrual or common blood.

At this time, the diagnosis of menstrual blood based on the

Table I

1. Serum	Cells	Agglutination
I	Cab. (A)	-
I	Gra. (A)	-
I	Cav. (B)	+
I	Ro. (B)	+
I	II	-
I	III	-
L.L. ( $\alpha\beta$ )	I	+
Cav. ( $\alpha$ )	I	+
2. Serum	Cells	Agglutination
II	Cab (A)	-
II	Gra. (A)	-
II	Cav. (B)	+
II	Ro. (B)	+
II	I	-
II	III	-
L.L. ( $\alpha\beta$ )	II	+
Cav. ( $\alpha$ )	II	+
3. Serum	Cells	Agglutination
III	Cab. (A)	+
III	Gra. (A)	+
III	Cav. (B)	+
III	Ro. (B)	+
III	I	+
III	II	+
L.L. ( $\alpha\beta$ )	III	-
4. Stain extract	Cells	Agglutination
"	Cab. (A)	-
"	Gra. (A)	-
"	Cav. (B)	+
"	Ro. (B)	+
** { "	I	-
"	II	-
"	III	-

\*\* Test of Landsteiner and Richter

glycogenic reaction of the vaginal cells, according to Wiegmann, had become widespread. Numerous experimental tests done on ordinary blood, and on both fresh and old menstrual blood for the past seven years, had convinced me of the specificity, certainty and practicality of that reaction. I found the technique proposed by Brandino particularly suitable; a great number of vaginal cells could be observed even on small and very old stains. In no case did the reaction fail.

Having taken small pieces of the stained shirt in different places, I subjected them to discoloration in 2% hydrogen peroxide for twenty to thirty minutes. After then rinsing them in physiological solution, I treated them on a microscope slide with Lugol solution. At no point could I observe glycogenic cells. A diffused light violet color appeared on the cloth and small violet spots, which must have been due to starch from the ironing. Besides direct observation of the material, scrapings of the bloodstain were also examined; but even in this way I could not observe the glycogenic cells. Given the consistency and the certainty of the reaction, one could conclude that the stain was not one of menstrual blood, nor could it have belonged to T.

The definitive conclusion of the above inquiries was, there-

fore, that the stain was of human blood and it was the blood of R. G.

This result restored the peace to family G.; not only were the components of this case indeed in concert with an appreciation of biological principles, but, from the response of a physician completely disinterested in the question, a new and plausible origin of blood was also brought to light, namely, the hypotrophic prostrate which, naturally, they had not thought of before. Having seen this sequence of events more than once, they observed blood stains on the sheets, being able to exclude eczema as their origin, and this served to confirm the reality of much that I had explained.

In another practical case I had occasion to apply the individual diagnosis. This time it was a case of great judicial importance. An individual was suspected of a most serious homicide. Besides the general capacity to commit a crime, the judicial circumstances seemed to be singularly stacked against him. Especially important among them was the presence of numerous blood stains on the overcoat that he wore. Now, he explained these stains as having come from a heavy nose bleed resulting from a blow he received to the nose. There were, however, strong reasons to believe that the blood of the stains could have belonged to the victim. Thus, circumstances were present in which only the individual diagnosis could be valuable, since it was a matter of human blood beyond question.

During the autopsy of the body of the victim, I withdrew some blood from the heart. From this I prepared serum, lightly rose-colored, a part of which I dried at low temperature, and a part I kept fresh in an ice box. I also attempted to keep the red cells for the agglutination test, but by the time I accomplished this, they had changed too much and thus could not be used. The time that had elapsed between the death and the autopsy was about forty-eight hours. After forty-eight hours I did the agglutination test. The stains were at least four days old, irrespective of the differing versions of their origins. The blood of the suspected individual was taken the same day as the test. The serum was diluted by half, the cells prepared in a 5% suspension.

Because I was examining the serum of the victim, I was motivated to make an observation which was important for the proceeding, and on which I initiated other investigations. Using the usual technique (two loops of serum and one loop of 5% cell suspension) with this serum, diluted by half, I consistently observed the phenomenon of true and proper rouleaux formation, roughly simulating an agglutination. And this was visible while using cells, so that from many tests there were no isoagglutination results. The dilution of the serum by half was not enough, therefore, as it is in the case of sera taken from the living, to eliminate pseudo-agglutination. The pseudoagglutination from rouleaux, is, as shown by my previous work, the consequence of an excessive concentration of the serum employed. Well, the determination of the dry residue of serum taken from the cadaver showed that it had acquired a noteworthy concentration.

In fact, 7.5 cc of serum gave a dry residue (at 100°) of

1.1880 g. According to the tables of Hammarsten, the dry residue of serum corresponds to about 1/11 of the weight of water (= volume). However, in this serum it was a little less than twice this value. Since the serum was only lightly tinted rose, this increase of dry residue is, in all probability, attributable to a diffusion of water from outside of the vascular system, exactly the phenomenon I was to study. The fact remains that diluting the serum of the cadaver at 1:4 rather than at 1:2 inhibits pseudoagglutination, proper agglutination remaining unaltered.

Thus, the conclusion formulated in my previous work, namely, that in order to obtain certain results in the isoagglutination test, it is necessary to dilute the serum 1:2 (that becomes 1:3 in following the proportions of added cell suspensions used by me) is applicable only when the serum was taken from the living. In serum drawn from a cadaver a greater dilution will be necessary, according to the degree of concentration of this serum, to be determined on a case by case basis where it is possible. In general one will be able to avail oneself of the criterion that the dilution must be such as not to provoke pseudoagglutination by rouleaux in cells that do not show isoagglutination. In my case the serum of the cadaver was used in the dilution of 1:4.

The extract of the stains was made in the following way. The stains were crusted on smooth woolen cloth, so that they could be cut away with a pointed bistoury, almost without damaging the material. Most of the stains were collected in a weighing bottle and dried at 37°. The aggregate weight was 0.0656 g. Not all of this weight was blood, there still being present much woolen cloth. The stains were then treated with a quantity of solvent, presumed the minimal amount necessary according to the above stated criteria, namely, with 0.2 of distilled water and 0.2 of physiological saline solution. It was kept for six hours in an ice box, macerated and pressed repeatedly with a small glass rod. The blood readily dissolved, and there remained a mass of threads. These were gathered into a small ball that was, as much as possible, squeezed out with a wide pincers. After this, the threads were washed repeatedly with distilled water, dried out, and weighed. They weighed 0.020 grams. Therefore, the real weight of the dry blood from the stains was 0.0656 - 0.0200 = 0.0456, corresponding to approximately 0.18 cc of fresh blood. This blood would have had to be diluted to a volume of 0.36 cc. The extract already prepared with 0.4 cc of solvent was, therefore, a slightly greater dilution, but was still perfectly appropriate for the tests.

The investigation directed at the individual diagnosis was naturally preceded by the demonstration of the human origin of the stains by means of the precipitin test, in the manner indicated in the above-mentioned case.

The test of Landsteiner and Richter followed successively, that is to say, the agglutination of cells from the individual suspect by means of the stain extract, and turned out negative.

I went on, therefore, to the agglutination tests with test cells of known group, proceeding, as in the previous case,

with a pair of A test cells and a pair of B test cells. I report now all the results obtained, neglecting, for the reasons mentioned above, the simple quantitative differences. [See in Table II].

Table II

Serum of the suspect, diluted 1:2, with red cells		Agglutination
Cab. (A)		+
Ros. (A)		+
Ro. (B)		+
Ov. (B)		+
of the suspect		-
Liquid serum of the victim, diluted 1:4, with red cells:		Agglutination
Cab. (A)		-
Ros. (A)		-
Ro. (B)		+
Ov. (B)		+
of the suspect		-
Dried serum of the victim, redissolved, with red cells:		Agglutination
Cab. (A)		-
Ro. (B)		+
of the suspect		-
Stain extract with red cells:		Agglutination
Cab. (A)		+
Ros. (A)		+
Ro. (B)		+
Ov. (B)		+
*** of the suspect		-

\*\*\* Test of Landsteiner and Richter

Besides these tests others were done as controls with my blood, which has non isoagglutinable cells and serum containing the two agglutinins  $\alpha$  and  $\beta$ . The sera in the dilutions employed did not agglutinate my cells at all; besides, my serum, which strongly agglutinated the four examples of test cells used, was completely inactive with the cells of the individual suspect.

The blood of the suspect belonged, therefore, to group O  $\alpha\beta$ , that of the victim, to group A $\beta$ .

The extract of the stain reacted like the blood of the individual suspect; and it was differentiated distinctly from that of the victim by its isoagglutinating power with the Ro. and Ov. cells. Therefore, one could conclude: (1) that the stains found on the cape did not belong to the victim; (2) that it was perfectly credible that they came from the suspect.

With this answer the positive, indirect diagnosis was achieved and the medico-legal inquiry was finished. Certainly, such a response could not, of course, be assumed to be a decisive element of proof, considering the novelty of the investigation. (Much time was needed before probative value was attributed to fingerprints in trials!) Therefore it is appropriate to take note that the course of the inquiries on the case had to abandon definitively the original theory, and to diminish the suspicion of that particular individual; from that came the greatest demonstrative value of the biological, medico-legal inquiry.

(In another assessment on blood stains which I did with Professor Carrara in a homicide case, the magistrate asked us if a stain on the lining of the jacket of the accused came from the blood of the person accused (as he maintained), or from that of the victim, already looked at by the other experts. We withdrew blood from the accused in order to at-

tempt the test.

However the scantiness and the small size of the stain scarcely allowed the specific diagnosis, and would not give a sufficiently concentrated extract of high enough strength, so that all the tests gave negative results and the inquiry failed).

## On the Technique of the Isoagglutination Test for the Individual Diagnosis of Blood\*†

Doctor Leone Lattes

Institute of Forensic Medicine  
University of Torino  
Director: Professor M. Carrara

400 The technique of the practical individual diagnosis of blood that I described in my recent work (1) is founded directly upon the results of experimental inquiry. Having thus demonstrated the essential importance of serum concentration for the certainty of the test, there followed the necessity of knowing the titer of the stain extract and, therefore, the weight of the dried blood to be dissolved. Now, inasmuch as this technique shall be retained as the most scientific and rigorous, and has furnished me with excellent results in two particularly favorable cases (2), it remains a fact that it cannot be considered applicable in most medico-legal circumstances.

A technique more suited to general use, and particularly for the investigation of small stains, in which the possibility of weighing the blood is quite problematical, would therefore be highly desirable.

401 In the recent experiments of De Dominicis (3), having confirmed the practical interest in the isoagglutination test, has been proposed a technique, reminiscent of Landsteiner and Richter (4), directed precisely at the investigation of common bloodstains.

He proposed placing a drop of cell suspension on the surface of the microscope slide, and to tease apart a pair of threads of the stained material, about 4 or 5 millimeters long, and then putting on the cover slip. Under these conditions agglutination can be observed which would otherwise have been very weak or absolutely negligible.

One observes the agglutination proper where the liquid in the preparation appears more distinctly hemoglobin-colored.

This technique undoubtedly has the value of great rapidity and simplicity, besides that of being applicable to very small stains. But, these advantages and the technique's adaptability do not suffice, and, to the contrary, they have an entirely subordinate value. The first indispensable require-

ment is that the test be able to demonstrate isoagglutination with certainty, consistency and, especially, specificity.

From my previous research of a general nature, which must be kept in mind when considering attempts at practical application, it is clear that the isoagglutination test cannot have any value at certain concentrations of agglutinating serum; more precisely, it must be kept within the broad limits of 1:2 to 1:25, and, practically, it can be said, within 1:3 and 1:10. At lower dilutions, true isoagglutination can be simulated by non-specific pseudoagglutination; at greater dilutions, the agglutination is no longer appreciable.

Now, in the technique of De Dominicis, the concentration is not calculated, and the test is so devised that the same concentration is employed to meet the widest variations in conditions. And precisely, if during the unravelling of the thread material, the blood readily dissolves in the drop of cell suspension, the concentration will become more than 1:15 and the agglutination will not be visible, as with a blood that would not show it under other circumstances. If, instead, the blood diffuses slowly around the edge of the thread, it will be able to bring about a concentration sufficiently elevated to cause pseudoagglutination. De Dominicis recognizes that with concentrated blood solutions, as well as with the concentrated bloodstains, one can have agglutination, which is as much as saying pseudoagglutination. 40

In the test proposed by him, the concentration of the serum at every point in the procedure depends on too many uncontrollable factors: the unravelling, the rapidity of dissolution and diffusion of the blood, the movements of the preparation, and even the thickness of the stained thread upon which will depend the thickness of the compressed liquid layer between the microscope slide and the cover slip.

I wanted to introduce controls into the proposed technique, using serum and cells whose relative behavior in isoagglutination was perfectly known to me from hundreds of experiments, while adhering strictly to the specifications of De Dominicis. It is incontestable that in certain cases, blood and cells capable of giving an agglutination reaction do so in a distinctly observable way. On the other hand, the test very often shows negative results, probably because the blood diffuses into the drop during the unravelling process.

These negative results, obtained with blood-red cell combinations with which agglutination would have to be posi-

\* Translation of: "Sulla tecnica della prova di isoagglutinazione per la diagnosi individuale del sangue."

in *Archivio di Antropologia Criminale Psichiatria e Medicina Legale* 37 (4 ser. 7): 400-408 (1916).

Reprinted with the kind permission of Edizioni Minerva Medica, Torino, and the family of Prof. Dr. Lattes through Fiammetta Lattes Treves, Milano.

† A summary was communicated to the Acc. di Med. di Torino April 7, 1916.

tive, invalidate the consistency of the test.

I ignore that which De Dominicis understands exactly as "indicative agglutination", since he qualifies it by saying that under other conditions "it would have to be considered very slight or as downright negligible". As I have already stated explicitly elsewhere, in order to be able to admit that agglutination has occurred, one must see true, irregular clumping of the cells; the simple groups formed by contact are not enough to demonstrate it. Even less is it so in the test of De Dominicis, since in it one can easily see that the cells might amass themselves around all of the artifacts of the preparation, air bubbles, threads of the material, heterogeneous granules, and the edges of the preparation, by the simple physical action of capillarity and without the intervention of agglutination. Such more or less irregular masses and incidental contact of cells with debris are observed also with blood-red cell combinations which would certainly be negative for agglutination.

Experimenting with the same combination of blood and red cells both agglutinable and non-agglutinable, respectively, it was possible at times to "guess" partly the difference, which included looking at the order of magnitude of the "general outlines", and recognizable only because the result had been previously known.

403 But I maintain, in contrast to such a view, and in speaking not of blood and red cells of known type, but of a case to be resolved, that no one would have dared to risk a diagnosis of positive agglutination.

However, this would not be the dangerous aspect of the De Dominicis test, since the negative results at least have the value of not compromising anything, it being possible to admit that a previously agglutinable blood has lost this property. As I indicated in the work already cited, only positive agglutination has diagnostic value, never the negative.

Unfortunately, however, the test of De Dominicis can give rise to pseudoagglutination.

It happened often that in using either threads of material from a dense stain, or even a thinly crusted blood which I did many times, one could obtain all around the trace of blood a picture of sufficiently intense agglutination, even experimenting with a combination of blood and red cells in which isoagglutination could not possibly occur, and at other times with stains and red cells of the same person.

The excessively high serum concentration around the blood trace can, therefore, sometimes cause pseudoagglutination.

Besides, during the unravelling of the stain, proposed by De Dominicis, one can spread around in the cell suspension clumps from the blood that constituted the stain. These clumps are easily confused with a true and proper agglutination result, and in any event they impede a certain appreciation of the results of the test.

Therefore, the door remains open to grave errors of judgment.

On the whole, the test of De Dominicis is inconsistent; at certain times it yields a result that actually conforms to one

obtained when applying the more rigorous scientific techniques. But, on the contrary, it can yield the two opposite defects: that of not revealing a true and proper agglutination or else that of simulating a completely non-existent one. Without a doubt, this happens principally because in certain cases, the concentration of serum remains so low, while in others, it becomes too high.

It being truly important in forensic medical practice to obtain a simple and reliable method for the individual diagnosis of common bloodstains, he sought to eliminate the two above-indicated causes of error and to reconduct the test under the conditions demonstrated to be necessary by scientific inquiry, by avoiding the preparation of a titrated extract of the blood, frequently impossible.

The difficulty to be resolved resided in the contrast between the necessity of having a strong enough serum concentration to see agglutination well, and the danger that pseudoagglutination might manifest itself as the result of an excessive concentration. Now it seems to me very difficult with a purely empirical approach to the dissolution of the blood to obtain, in a consistent way, that serum concentration optimum which the theoretical inquiry has shown to be around 1:2-1:3.

More easily attainable in fact is the intentional elimination of the pseudoagglutination, not *prior to* but *after* the reaction is terminated.

The tests on microscope slides and in small tubes demonstrated that once the cellular clumps, characteristic of true agglutination, were constituted, a very considerable dilution was not enough to make them disappear. On the other hand, with excessively high serum concentration, one can have a regular or irregular mass of cells (pseudoagglutination), but a small dilution suffices in this case because the cells immediately separate one from another.

I report some of the tests carried out, which I repeated many times with various dilutions, and always with the same result.

(1) 0.05 of  $\alpha$  serum is mixed in a tube with 0.05 physiological saline solution and 0.1 of a 5% suspension of red cells of Group A. After 15 minutes, a few loops of the liquid are withdrawn, after shaking, and examined in a hanging drop. Virtually all the cells are agglutinated in a large clump. If 1 cc of physiological saline is added, the clump persists. If another 1 cc of physiological saline is added (a dilution of the serum at this point of about 1:44), the clump still persists.

(2) 0.05 of  $\alpha\beta$  serum is mixed in a tube with 0.05 physiological saline and 0.05 of a 5% suspension of red cells of Group A. Examining the material in a hanging drop after 10 minutes: large clumps of cells. 0.1 of the liquid is withdrawn and mixed with 0.9 of physiological saline: the clump persists. If another 1 cc of physiological saline is added (dilution of about 1:60), the clump still persists.

(3) A small drop of  $\alpha\beta$  blood is mixed in with  $\alpha\beta$  serum, and the mixture agitated. Examination on a slide or in a hanging drop shows almost complete pseudoagglutination

(also identifiable were numerous, short little masses). 0.05 of physiological saline is added to 0.1 of this liquid. Upon examination, just about all the cells are free, there being some limited pseudoagglutination. If 0.1 of physiological saline is added to 0.1 of this liquid, all the cells appear free.

The same results are obtained by carrying out these dilution experiments on slides or in hanging drops, just as well as in tubes.

From these tests it can be concluded that the clumps of true agglutination persist even at a dilution of the serum at which agglutination would not occur in any appreciable way. But the clumps from pseudoagglutination (masses) disappear as soon as the dilution is such that pseudoagglutination is no longer manifest (1:2).

On the basis of these facts, the previous dilution of the serum does not appear indispensable to the reliability of the isoagglutination test, at least for most practical purposes. But with reference to stains, those from which a titrated extract cannot be obtained, one can proceed in such a fashion that the maximum concentration of blood is possible. With the agglutination that eventually occurs, a successive appropriate dilution will permit the determination of whether it is a true or simulated agglutination.

Thus one can practically apply this method.

It is necessary, as done by Landsteiner-Richter and De Dominicis, to add a portion of stained material directly to the cell suspension.

Therefore a thin crust of blood is placed on the slide, or in dealing with material, a small square of a couple of millimeters on a side. This form is preferable to that of the thread, since with it, one can obtain a stronger concentration of serum, either all around, or also in the network of the material. If the material is thick, it can be advantageous to cut a small hole in the center of the fragment, where the cell suspension can sit enclosed, in a little cell, as it were.

It can be necessary instead to unravel the threads of the material, as De Dominicis proposes, because this unravelling very often brings about a rapid dissolution of the blood into the suspension. It is convenient to proceed in such a way as to make the bloody material as compact as possible, collected together at the same point, but without causing an excessive gap between the slide and the cover slip, with a corresponding large increase of the quantity of liquid interposed between them. One, therefore, adds a small drop of cell suspension and covers it instantly with a cover slip, taking care not to displace the blood trace, then eventually fills up the preparation with other drops of cell suspension.

So far the hanging-drop method is without a doubt better for a scientific study of isoagglutination, for in this case one must simply evaluate a simple microscopic preparation. In these preparations the flow within the liquids is almost impeded, and because of that, the serum dissolves only in the zone of the liquid adjacent to the bloodstain, reaching there its maximum concentration. There is, nevertheless, often a problem, namely that the cells move only very little and struggle to reunite in order to agglutinate; it is appropriate

for that reason to have some minimal lateral movements back and forth under the cover slip, in order to make the interaction of the cells easier but carefully avoiding, however, the blending of the liquid.

The preparation is then left to sit in a humid chamber, and it is examined two or three times, displacing it as little as possible, and finally after a half hour it is perfectly useless to prolong the observation further. Positive agglutination is usually visible in a few minutes: it is manifest by the clumping of the cells in the liquid zone immediately adjacent to the blood trace. Sometimes, instead, the cells unite on the glass plate, especially when one has stains on materials, in such a way that the agglutination is not easily recognizable in a direct way, but makes it necessary, therefore, to displace the preparation, as will be further indicated below.

Often (not always however!) in working with a blood-red cell combination that cannot give agglutination, one observes a clumping, even an intense one, because of pseudoagglutination; so that, although true agglutination is usually more intense than pseudoagglutination, one cannot make a diagnosis using this criterion.

In order to distinguish the two phenomena it is necessary to proceed with the dilutions. Since the serum is concentrated only in one small zone surrounding the blood trace, where, precisely as noted by De Dominicis, the hemoglobin color is observed, it is sufficient to mix it with the rest of the suspension contained in the preparation in order to obtain a considerable dilution of this zone. To accomplish this, the cover slip is removed, and the liquid material that is far from the edge of the fiber or the thin blood crust residue is blended using the edge of the cover slip, and the preparation then covered again using another, larger cover slip (18 × 18 instead of 15 × 15). With this maneuver, which must be done in every case, a duplicate result is obtained. First of all, if the agglutination had taken the form of being spread out, or if the clumps were concealed in the network of the material, the blending of the liquid renders very evident, and thus verifies the agglutination, if it exists. In the second place, a homogeneous dilution of the serum of the stain is obtained throughout the preparation.

The dilution obtained in this way is certainly completely empirical; but it greatly surpasses that needed for the verification of pseudoagglutination. In order to get a rough idea of it, I left drops of blood (about 0.05 cc) to dry on a pane of glass for further testing. The round drop dries there, dividing itself into many sections. Withdrawal of a portion of a section is adequate. For the experiment, I withdrew a section of about 1/20 of the drop and divided it into halves, each one of these corresponding to 1/40 of 0.05 of blood or, in round numbers, to 0.001–0.002 cc of blood.

At least 0.02 cc of cell suspension was necessary for the preparation, and thus, if the blood was completely dissolved, its dilution would not have been less than 1:10. Usually, the dilution is even higher because the blood is not completely dissolved, and because the quantity of suspension is greater.

The mixing of the liquid makes the clumps of cells, pre-

viously limited to the vicinity of the blood trace, spread out into the entire preparation. If it is a question of true agglutination, it could easily be distinguished among the numerous isolated cells. If instead, it were pseudoagglutination, the clumps, also voluminous, completely break down and are no longer observed among the isolated cells.

Following this technique, it was always possible in many tests to successfully eliminate the cause of error due to pseudoagglutination, which is certainly the one to be feared most in this case. Failure to break the bloodstain into small bits causes the spreading out of the clumps from the same bloodstain in the preparation to become inevitable, and renders the judgment uncertain.

408 The test yields results nearly consistent with small blood crusts (of course the blood on which the systematic examination of the serum was done had previously demonstrated its power to agglutinate the cells which were used), at least under the conditions I tested, those being dried blood, unaltered, and no older than ten months. Agglutination is usually indicated by large clumps and does not lend itself to doubts.

The results with blood impregnated materials are less consistent; sometimes, specifically in experiments with isolated threads, an agglutination that would have been expected is not seen with certainty. Obviously, the "compactness" of the bloody substance being less under these conditions, a sufficient concentration of serum in the surrounding liquid

for bringing about a certain agglutination is not reached. In this sense, the test presents the same problem as that of De Dominicis, though more rarely; and the greater consistency is attributable to having omitted the unravelling of the fabric, and also of having sought every device to augment the concentration of the serum.

As disagreeable as the possibility of these negative results are, they are not, as I already stated above, of great importance, because one does not in any case attribute probative value to negative agglutination.

I believe that these above-proposed modifications of the technique, based on the laws governing isoagglutination, and which permit the carrying out of the test in a simple and certain way on small blood traces (particularly in the form of small crusts) make a further contribution to the attempts at individual diagnoses.

Given the great practical importance of the problem, I hope that other investigators will want to take these methods under consideration, in order to affirm their value and improve them in the points where they might be wanting.

#### References

1. See in this journal, 1915
2. See in this journal, 1916, the preceding issue
3. Sulle indicazioni individuali del sangue. *Cesalpino* 11-437, 1915
4. *Z. f. Med. Beamte* 16-85, 1903

## Practical Experience Concerning Blood Group Determination in Stains\*<sup>1</sup>

Prof. Leone Lattes

Modena, Italy

402<sup>1</sup> Italian legislation does not at present allow tests concerning paternity except in very special cases.

In Germany blood group determination has already been widely used for the very important medico-legal goals of recognizing, or better, of excluding paternity. While scientific experiments in this field have developed fruitfully in Italy under Mino, we have not had at our disposal practical experiences for the reason given above.

The forensic cases, which we have had to examine, concern, therefore, the individual identification of blood stains.

The number of cases which I have examined is, indeed, modest in comparison to the frequency with which the question should be submitted to testing. Without a doubt, this results from the fact that the judges inquiring into the cases, as well as the forensic physicians, are not sufficiently familiar with the possibility of making determinations or, at least, of obtaining useful, individual indications. Although I have only done about ten cases, in fact, no single expert, so far as I know, has at his disposal equal *practical experience*.

I, therefore, think it useful to present all of these together, even though some of them have already been individually presented.<sup>2</sup>

I hope that this representation of these real cases, taken from actual practice, almost all of which were favorably resolved, will convince the forensic physicians of the importance of this new examination method and of the necessity of introducing it into regular forensic medicine.

403 In addition, I would like to indicate some technical processes which broadens the possible applications of the method, and simplifies the process of carrying it out. In the older cases, of course, I could not yet use the new processes, which were in need of further study.

I shall now enumerate the individual cases.

1. Dried blood stains, approximately three months old, on a shirt (not a court case).

It was necessary to determine whether the blood came (a) from a cow, or (b) from a human being; and if human, whether from a certain man, from his wife, or from a friend

of these two (in this case menstrual blood was involved).

*The Method Used:* Production of a titrated blood extract by indirect weighing of the blood (average difference between a particle of blood and a series of several other particles of the same size). Test for isoagglutinins; microscopic method in the hanging drop.

*Results:* The precipitation caused by rabbit antiserum to humans showed that it was a question involving human blood.

The Landsteiner-Richter test (hereinafter abbreviated L.-R. V.), applied to the three persons, was negative.

The grouping proved that the stain contained the agglutinin  $\beta$ ; it belonged, therefore, to Group II ( $A\beta$ ).

The grouping of the three persons by means of serum and blood corpuscle testing demonstrated that the man belonged to Group II ( $A\beta$ ), the wife to Group I ( $O\alpha\beta$ ), and the friend to Group II ( $A\beta$ ). The absence of menstrual glycogen cells eliminated the friend as a possible source.

*Judgment:* The blood stain came from the man.

2. Four day old, dried blood stains, on a piece of cloth. A court case (murder). It was necessary to determine whether the stains came from the suspected perpetrator of the crime (nose bleed) or from the victim.

*The Method Used:* Production of a titrated blood stain extract by weighing. Testing for isoagglutinins by means of the microscopic method in the hanging drop.

*Results:* L.-R. V. against the blood corpuscles—negative. The grouping showed that the stain contained both isoagglutinins  $\alpha$  and  $\beta$ ; it belonged, accordingly, to Group I ( $O\alpha\beta$ ).

The grouping of the accused (by means of serum and blood corpuscles) showed that he belonged to the same group I ( $O\alpha\beta$ ); the grouping of the victim (obtained by using the serum extracted during the coroner's examination) showed that he belonged to Group II ( $A\beta$ ).

*Judgment:* The blood stain comes from the accused and not from the victim (accused was released).

3. Very thin blood stains, dried for about one month on linen cloth. Court case (murder). It was necessary to determine whether the stains came from the accused (previous abrasion).

*Method Tried:* Empirical extract preparation, in this case of an extremely small quantity of blood, too small to weigh. The extract was ineffective against the blood corpuscles of the accused [who belonged to Group II ( $A\beta$ )], as well as against the test blood corpuscles A and B. No isoagglutinins

\* Translation of: "Praktische Erfahrungen über Blutgruppenbestimmung in Flecken."

in: *Deutsche Zeitschrift für die Gesamte Gerichtliche Medizin* 9: 402-410 (1927).

Reprinted with the kind permission of Springer Verlag, Heidelberg and New York, and the family of Prof. Dr. Lattes through Fiammetta Lattes Treves, Milano.

could be demonstrated.

*Result:* Negative.

4. Fifteen to eighteen month old blood stains adhering to a silk cap. A court case (murder). It was necessary to determine whether the blood stain corresponded to the blood of the victim (preserved in a dry state).

*Method Used:* Direct microscopic method (cover-slide method). Control by means of elective agglutinin-absorption.

*Result:* In the stain, the isoagglutinin  $\alpha$  was demonstrated (with weak reaction); the absorption reaction showed that the blood from the stain removed the agglutinin  $\beta$  from a serum  $\alpha\beta$ ; it thus contained the agglutigen B. It, therefore, belonged to Group III ( $B\alpha$ ). The blood of the victim (well-preserved) contained strong agglutinin  $\alpha$  and the agglutigen B; it belonged, therefore, to the same group.

*Judgment:* The blood from the stain corresponded to the blood of the victim.

5. Small, scaly blood stains, more than three weeks old, found in a trouser pocket. Court case (murder). It was necessary to determine whether the blood from the stain came from the accused. The accused could offer no explanation regarding the origin of the stain.

*Method Used:* Direct cover-slide method.

*Result:* The L.-R. V. was negative. The grouping demonstrated the presence of agglutinin  $\beta$  in the stain; it belonged, therefore, to Group II ( $A\beta$ ).

The grouping of the accused (with serum and blood corpuscles) showed that he belonged to the same Group II ( $A\beta$ ).

*Judgment:* The blood from the stain corresponded to that of the accused. A microscopical examination of the sediment of the extract produced for serum preparation showed fragments of a crushed flea. The stain was thus caused by crushing the hemophagous insect (accused set free).

6. Numerous, thick blood stains on a shirt, two or five months old depending upon the proffered explanation. Court case (murder). It was necessary to determine whether the stains came from the accused (nose bleed). (The victim's blood was not available).

*Methods Used:* 1. Stain extracts titrated by weighing. 2. Cover-slide method with dried extract sediment. 3. Elective absorption.

*Results:* The use of extracts in the hanging drop resulted in uncertain results. The cover-slide method, undertaken with dried-extract sediment, showed with complete certainty the presence of both isoagglutinins  $\alpha$  and  $\beta$ . The L.-R. V. was negative. The absorption experiment, carried out with two successive portions of the stain, removed absolutely no agglutinin; there was thus no isoagglutigen present. The blood from the stain belonged to Group I ( $O\alpha\beta$ ).

The grouping of the accused (by means of serum and blood corpuscles) demonstrated that he belonged to the same Group I ( $O\alpha\beta$ ).

*Judgment:* The blood from the stain corresponded to that of the accused.

7. Numerous blood stains, two and a half months old and

dried on smooth stones. Court case (murder). It was necessary to determine whether the stains came from the accused. The accused had a small, festering wound on one finger which the judge interpreted as the result of a bite, but which the accused claimed was a small work injury. The wound, it was alleged, had bled and thus stained the stones along the path that the murderer used to get away from the scene of the killing.

*Methods Used:* Direct, cover-slide method (the case was especially suited for this method). Control by means of elective absorption.

*Results:* L.-R.V. negative.

The grouping demonstrated the presence of very active agglutinin  $\beta$  in the stain. By means of the absorption test it was shown that the blood from the stain removed the agglutinin  $\alpha$  from a test serum  $\alpha\beta$ . Thus, agglutigen A and agglutinin  $\beta$  were contained in the stain; it, therefore, belonged to Group II ( $A\beta$ ). The blood of the accused was tested (serum and blood corpuscles), and it belonged to the same Group II ( $A\beta$ ).

*Judgment:* The blood from the stain corresponded to that of the accused (As a result, an indictment was issued in this case against the accused).

8. The examination of this notable case was requested not by the officials, but by the ophthalmologists and psychiatrists who were to answer the important forensic question regarding simulating the effects of accidental injuries on the job.

A girl (with pronounced hysterical symptoms) was injured in the eye with iron clips which harmed only the conjunctiva outside the cornea. The chips were removed with a magnet, and the small wound was on the point of healing cleanly. In the eye clinic, however, it was noticed during the morning round that the bandage was plentifully soaked with pure blood, which had partially trickled down; what is more surprising, this took place more than one month after the accident. The ophthalmologists were unable to find any source of the profuse bleeding, either from the conjunctiva which had almost completely healed, from the eye lids, or, even less, from the completely sound cheeks. Moreover, no one was able to observe the bleeding. As a result, they consulted with the psychiatrists with the idea that it could be some mysterious, hysterical bleeding. The psychiatrists, however, were skeptical regarding the situation; they turned to me, asking that I test the blood from the bandage and compare it with that of the girl's.

*Methods Used:* The bandages were examined a few hours after their removal; they were soaked with blood that had run to some extent, and in the deep layers the blood was so fresh that I was able to produce from it suspensions of well-preserved blood corpuscles. Thus, the agglutigen content could be directly determined by measuring capability of the blood corpuscles to agglutinate. The agglutinin determination was carried out by means of the cover-slide method with extract sediment dried on the slide itself.

The grouping of the girl's blood with serum and blood

corpuscles showed that it belonged to Group II (A $\beta$ ).

**Results:** a) The first bandage, removed without any special attention, produced the following results.

	<u>Agglutination</u>
Bandage blood and girl's blood corpuscles (L.-R.V.)	strong
Girl's serum and bandage blood corpuscles	"
Test serum $\alpha$ and bandage blood corpuscles	"
Test serum $\beta$ and bandage blood corpuscles	"
Bandage blood and test blood corpuscles A	"
Bandage blood and test blood corpuscles B	"

Of course, the sera were used in the dilution 1:3, and the observations were carried out at a temperature of 25°. The results of the reaction demonstrated the following: 1) that the bandage blood did not come from the girl; 2) that the blood was possibly from an animal since the reactions, all positive, belonged to the category of heteroagglutination. Although, from the outset, the situation did not indicate this, it was necessary to carry out a species diagnosis: namely, by means of sero-precipitation and the test of O blood corpuscle agglutination. The precipitation reaction, carried out under strict controls with anti-human serum from rabbits, produced a *negative result*.

**Judgment:** The blood which had soaked the bandage we were given did not come from the girl, but from an animal.

b) After I had communicated these results indicating deception to my colleagues, the girl was strictly isolated. Nonetheless, the blood-soaking occurred again six days later. I now examined the bandage which was sent to me with the same test sera and blood corpuscles we used in the first test; i.e., I repeated in parallel the tests on the first bandage (with unchanged results), though the blood-corpuscle suspension from the first bandage, having been preserved in the icebox, appeared in a somewhat altered state.

/407 With the second bandage I obtained the following results:

	<u>Agglutination</u>
2nd bandage blood and girl's blood corpuscles	negative
2nd bandage blood and test blood corpuscles A	negative
2nd bandage blood and test blood corpuscles B	strongly positive
2nd bandage blood and test blood corpuscles O	negative
Test serum $\alpha$ and 2nd bandage blood corpuscles	positive
Test serum $\beta$ and 2nd bandage blood corpuscles	negative
Girl's serum and 2nd bandage blood corpuscles	negative

The parallel precipitation tests conducted with the same antiserum from rabbits (at a dilution value of 1:1000) produced the following results: the failure of any disturbance to appear in the case of the first bandage; on the other hand, a strong precipitation ring in the case of the second, a ring which was still very clear, when one further diluted the serum by half (1:2000).

**Judgment:** The blood of the second bandage was certainly different from the blood of the first; it was human blood and surely belonged to Group II (A $\beta$ ), the same as that of the girl. (Further experiments showed that it was not a question of menstrual blood). I still have not found out what practical conclusions were drawn from my experiments. Concerning this case, Professor A. Sacerdote and I will bring out a publication.<sup>3</sup>

This case points up how the source of errors resulting from heteroagglutination possess not only a theoretical value, but they must in practice always be kept in mind, even when there is no suspicion. Even when I have not specially mentioned it, the precipitation reaction with anti-human serum was performed in every case as an indispensable test, and so too the agglutination test with O-blood corpuscles was carried out in almost every case. Only when the blood demonstrated a differential effect on the human blood corpuscles, usually the ones used in the test, and when, in addition, the amount of blood was too small (cases 1, 4, 5), did I sacrifice the test.<sup>4</sup>

Regarding the technical aspects, I consider it unnecessary to repeat everything here which I have dealt with separately in other works.<sup>5</sup>

No matter how great the preference of some authors, and especially of the great expert, Dr. Schiff, may be for the microscopical method, it remains completely barred from the area of forensic, individual diagnosis of blood stains for obvious reasons. 41

In the context of the microscopical technique, the process which I worked out (called by Schiff "the Lattes cover-slide method"), merits the first place because it is so easy, as even Dr. Schiff admits; one adds the smallest traces of dried blood to a fitting suspension of blood corpuscles in a usual microscopical preparation; in carrying out the process, one protects against the threat of error: 1) by diluting through mixing of the preparation; 2) by the use of lecithin suspensions (spherical blood corpuscles); and 3) by keeping the temperature between 20° and 25°.

This process can be used straightaway if the stain is found in a crusted state (even if it is very small), as in my cases 4, 5, and 7. On the other hand, in the cases in which the blood has thoroughly soaked into some material, no good results are produced when a piece of the material is added directly to the preparation, especially on account of the excessive thickness of the layer of liquid which is contained between the slide and cover slip. The production of titrated extracts can sometimes lead to good results (cases 1 and 2); in other cases this method fails on account of its unavoidable inaccuracy. Moreover, it is complicated and time-consuming, despite the useful application of the torsion scale to weigh the blood and the dilution liquid. According to my latest experience, it is preferable to substitute the extraction procedure with the cover-slide method, suited for blood crusts, by using a special technique which I conceived and perfected for my case 6, and which functioned outstandingly in the many tests of case 8.

If no blood crusts are present, but only blood-soaked material or substratum, I produce for myself artificial crusts. This is done most easily in the following manner (provided the blood is not insoluble). The stains are first cut and are macerated for a few hours in the icebox with a very small amount of distilled water (so that there is not present an excessively low salt level); with a capillary tube, the extract is absorbed from the material between two pieces of glass;

Case	Type of Bloodstain	Age	Microscopical Method Used	Individuals Used for Comparison	Possibilities of Origin	Diagnosis of the Stain	Judgment Concerning the Individuality of the Blood
1.	Soaked into linen	3 months	extract; hanging drop	1. cow 2. man II (A $\beta$ ) 3. woman I (O $\alpha\beta$ ) 4. woman II (A $\beta$ ) (menstrual blood)	1. Accidental staining 2. Urethral bleeding 3. Willful staining	Human, not menstrual blood group II (A $\beta$ )	Belongs to man II
2.	Soaked into scarf	4 days	extract; hanging drop	victim II (A $\beta$ ) accused I (O $\alpha\beta$ )	1. Nose bleed 2. Murder	Human blood I (O $\alpha\beta$ )	Belongs to accused
3.	Soaked into linen	1 month	extract; hanging drop	accused II (A $\beta$ )	1. Previous bruise 2. Murder	Not feasible	Negative
4.	Dried on silk	15-18 months	1. cover-glass method 2. elect. absorpt.	accused III (B $\alpha$ )	1. Previous head wound 2. Murder	Human blood III (B $\alpha$ )	Agreement with the accused
5.	Dried on scarf	3 weeks	cover-glass method	accused II (A $\beta$ )	1. From accused (?) 2. Pocket stains through bloody hand	Human blood II (A $\beta$ )	Agreement with the accused
6.	Soaked into linen	2 or 5 months	1. extract; hanging drop 2. indirect cover-glass method 3. elect. absorp.	accused I (O $\alpha\beta$ )	1. Nose bleed 2. Murder	Human blood I (O $\alpha\beta$ )	Agreement with the accused
7.	Dried on stone	2-½ months	1. cover-glass method 2. elect. absorpt.	accused II (A $\beta$ )	Bleeding from a wound of the murder near the scene of crime	Human Blood II (A $\beta$ )	Agreement with the accused
8a.	Bloody bandages	fresh	1. indirect cover-slide method	suspected girl II (A $\beta$ )	1. Hysterical bleeding	Animal blood	Does not belong to suspect
8b.	Bloody bandages	fresh	2. blood cell agglutinability		2. Simulation of accident injury	Human blood II (A $\beta$ )	Agreement with the suspect
9.	1. Dried on straw 2. Soaked into scarf 3. Soaked into wood	4 months	1. cover-glass method 2. indirect cover-glass method	accused II (A $\beta$ )	See note on page 407 [footnote 4]	1 and 2 human blood II (A $\beta$ ) 3. not feasible	Agreement with the accused

using a fan, very small drops of the extract are then dried at low temperatures on a slide. This process is repeated so that four to six droplets are placed on the same spot. Thus, one obtains a series of slides on each of which is a thick bloody crust, about the size of a pinhead.

To this crust one adds the corresponding blood corpuscle suspension and covers it with the cover slide without mixing the crust and the suspension. The agglutination, when positive, manifests itself clearly on the edges of the encrustation.

410 I can warmly recommend this process since it has produced for me outstanding results. The possible applications of the cover slide method to a variety of blood stains ought to be disseminated to all forensic physicians. Of course, if the stain is not soluble or produces no isoagglutinins, one will

have to employ the absorption tests to aid in the examination. The absorption tests are in any case to be evaluated as a control, so that one can determine the agglutinogen as well as the agglutinin, i.e., can obtain an integral representation of the blood group. The elective absorption, however, has a much narrower field of application than does the direct agglutination test, since it demands much greater amounts of blood.

From all my cases, one can see that in some of these (cases 4, 6, 7, 8b) it was possible to produce only one demonstration of agreement, though one very important for the forensic goal in the cases; in other cases, given the state of affairs before the court, it was possible to answer the forensic question most specifically, in that one could either offer an indi-

## Blood Grouping

vidual diagnosis of exclusion (negative), as in cases 1 and 2; or one could utilize a secondary finding (cases 5 and 8). Only in a single case was it impossible to obtain a usable result.

I hope that these practical cases will awaken general interest and encourage the systematic use of individual blood stain diagnosis.

### Notes and References

1. Delivered at the 15th Meeting of the German Society for Legal and Social Medicine in Düsseldorf, September, 1926.
2. *Arch. di antropol. crim. e med. leg.* 37, 3, 1916: 44, 1923; 45, 1925; *Rass. internaz. di clin. e terap.* 72, 192
3. In the *Arch. di Antrop. crim. e med. leg.* 1927

4. Note added during correction: At the request of Prof. Goroncy (Königsberg in Prussia), I recently dealt with another case of murder (I do not know the legal circumstances).

Age of the stain: About 4 months

Cover-slide method to identify agglutinins:

Dried blood of the criminal	Group II (A $\beta$ )	(Pronounced reaction)
Small crusts on straw	Group II (A $\beta$ )	(Pronounced reaction)
Bloodstained vest	Group II (A $\beta$ )	(weak reaction)
Blood spotted wood chips	Not able to be determined	

5. See my contribution: "Methoden zur Bestimmung der Individualität des Blutes" in *Abderhalden's Handbuch der Biol. Arbeitsmethoden*, 1927

## The Isoagglutinable Substance of Blood and its Demonstration for the Individual Diagnosis of Stains\*†

**Doctor Vittorio Siracusa**

Assistant

Institute of Legal Medicine of the R. University of Messina  
Director: Professor Leone Lattes

362 The procedure followed until now for attaining the individual identification of human bloodstains consisted of the demonstration of the isoagglutinating power of the stain under examination with fresh, appropriately selected cells. The isoagglutinins, contrary to what was asserted for years by various authors, resist harmful influences such as drying, putrefaction, moderate heating and exposure to air quite well; thus, in a good number of cases they can be identified easily enough in the stains, resulting in the direct assignment of the blood to a blood group (group I: O cells; serum a & b; group II: A cells; serum b; group III: B cells; serum a; group IV: AB cells; serum o).

363 Still, in other cases it is not possible to demonstrate the existence of any isoagglutinating power in a stain. This negative result can be due above all to the circumstances of the blood under consideration, which, even if still very fresh, can belong to the group distinguished by the absence in serum of both the human isoagglutinins. But even the existing isoagglutinins in fresh blood can be altered and destroyed by harmful influences (age of the stain, excessive heating, chemical influences, etc.) as is verified by stains which have become insoluble in water.

A negative result in an investigation on isoagglutinins in a stain is, therefore, without significance, and the method does not permit, in that case, any conclusion about individual origin.

Even in the first attempts at individual diagnosis consideration was given (Biffi) to utilizing for the diagnostic reaction not the isoagglutinins, but the cells contained in the stain. Biffi believed that it was possible to restore them to the original condition in order to test the specific isoagglutinability. But, given the delicacy of the reaction and the practical impossibility of reconstituting the dried out cells in their integrity, the idea could not be applied. It resulted further from other investigations that iso-

agglutination is a complex phenomenon, in which a specific element could be distinguished by the selective absorption of the agglutinins, and a non specific element and the reunion in clumps of red cells and the flocculation (Lattes)<sup>1</sup>.

It would be sufficient therefore, for diagnostic purposes, that the first could be conveniently ascertained.

The selective absorption, already studied by Landsteiner and his collaborators, and then by several other authors, is a reversible process (according to the temperature) in such a way that the greater part of the agglutinin bound to the cells can be recovered in solution; it would be possible for this property to acquire practical value.

Professor Lattes has, for the record, advised me to carry out investigations directed at the eventual utilization of this phenomenon for diagnostic, medico-legal purposes; namely, to verify abstractability of a true and proper agglutinability from the capacity of the cells in a stain to selectively absorb isoagglutinins and yield them up again at a higher temperature, and this, even when the stain has undergone alterations so as to render the demonstration of the agglutinins impossible (this test because of its simplicity is always the method of choice when it can be done).

Encouraging experiments in this direction have already 36 been accomplished by Schütze<sup>2</sup> (in his experiments for other reasons) showing the agglutinin-absorbing capacity of the residue of distilled water extracts of stains, in other words, of the globular stroma.

As for the possibility, thus far not studied, that the agglutinable substance of blood, even though altered or denatured, is in a position to absorb the isoagglutinins, it prompted us to examine the analogous situation in bacterial agglutination. It turned out, in fact, from various investigations that germs whose typhus or proteus bacilli are able to absorb specific agglutinins to the very same extent after cooking as in the fresh state (Scheller, Friedberger-Pinczower, Kumagai, Dessau, Lange)<sup>3</sup>, or else after treatment with dilute acid without showing the phenomenon of agglutination (Eisenberg and Volk<sup>4</sup>, Wassermann<sup>5</sup>).

On the other hand, numerous investigations exist from which it emerges that bacteria treated with various chemical agents are modified little or not at all in their agglutinability. They could still be able to conserve the property of absorbing specific agglutinin.

\* Translation of: "La sostanza isoagglutinabile del sangue e la sua dimostrazione per la diagnosi individuale delle macchie".

in *Archivio di Antropologia Criminale Psichiatria e Medicina Legale* 43 (4 ser. 14): 362-384 (1923).

Reprinted with the kind permission of Edizioni Minerva Medica, Torino.

<sup>1</sup> The results of this investigation were communicated to the R. Accademia Peloritana of Messina in May, 1922. See the Atti of the Academy, vol. 30, 1922.

Thus, cholera vibrios, killed with chloroform vapor, can still be agglutinated (Bordet)<sup>6</sup>.

Bacteria subjected to the action of various bacteriocidal substances conserve agglutinability (Van de Velde)<sup>7</sup>.

365 Typhus bacilli treated with formaldehyde can be substituted for living ones in the reaction of Widal (Widal and Sicard)<sup>8</sup>. They conserve the sensitivity to the agglutinins for several months (Nicolle)<sup>9</sup>.

Thus pure carbolic acid, the colloidal silver and sublimate (Righ)<sup>10</sup> does not modify the intensity of the agglutination of living bacteria.

Silver nitrate (1%), sublimate (1%), potassium dichromate (1/2 N), osmium tetroxide (1%), acetone (50%), ether, chloroform, formalin (10%), and hydrogen peroxide (25%) do not exercise any influence on typhus bacilli (Busacca)<sup>11</sup>.

Other substances would increase the affinity of the bacteria for the agglutinin: weak solutions of osmium tetroxide (Imai).

Others, on the other hand, would destroy it: sodium hydroxide (Porges)<sup>12</sup>, 95° alcohol (Busacca)<sup>11</sup> or they would limit it: pure acetone and acids (Busacca)<sup>11</sup>, alkali (Dreyer and Blake)<sup>13</sup>. According to Busacca, the behavior of the typhus bacteria to the alkali, is different from that of the second substance employed: there was no change with ammonia, a notable reduction with sodium hydroxide.

366 I carried out various experiments both with blood altered by physical or chemical agents, and with blood in which isoagglutinins could not be demonstrated for other reasons (nature of the group, old age).

## I. Blood Treated with Physical Agents

In this series of investigations I tested the capacity for absorption of isoagglutinin either of blood simply dried, from five to twenty days before, or of blood rendered insoluble by heating at 100° for 5–10 min., or also of red cells washed and baked. I did not think it necessary to repeat the absorption experiments on fresh cells, the results of which I am by now very certain.

The samples of blood, belonging to a determined group, carefully pulverized (in small tubes by means of crushing with a small glass rod), or else the cooked cells were left for several hours at ordinary temperature in close contact with an appropriate quantity (sometimes little, at other times an excess) of fresh serum providing the two isoagglutinins (serum a & b); after that the material was centrifuged and subjected to two types of investigations:

1. The residual isoagglutinating properties of the serum, separated by centrifugation, was tested.

The preparations were made in hanging drops and observed after about 15 mins, shaking the microscope slide frequently; the serum was used in a 1:3 dilution in order to avoid every possibility of pseudoagglutination that would confuse the results.

2. The sediment was suspended in an excess of cold phys-

iological saline (preferably at 0°) and washed two or three times; following incubation in a small quantity of new physiological saline for 15 min at 45°–50° (a temperature which from experience purposely created the most favorable opportunity for the extraction of the agglutinins fixed to the cells) and rapidly centrifuged with a water mantle at 45°.

I then tested the isoagglutinating properties of the supernatant fluid (containing the agglutinins yielded up in the heat from the sediment) on fresh A and B cells.

It would be superfluous to report all the experiments done, inasmuch as they yielded concordant results, although quantitatively somewhat different. I maintain that a reason for these differences, of however small importance, must be sought in the fact that the absorption power of a stain varies 367 by reason of the fineness of its pulverization, the degree of which cannot easily be assessed in a single test.

I will report some of the more significant experiments.

### 1. Dried blood

0.05 cc of blood with group A cells (S. V.) dried for three weeks, is pulverized and suspended in 0.1 cc of serum a & b (L.L.) freshly diluted by half. After half a day the material is centrifuged and separated and the serum is strongly colored by hemoglobin:

Absorbed serum + fresh A cells (S.V.) = no agglutination

Absorbed serum + fresh B cells (C.C.) = total agglutination

The sediment was washed at 0° and extracted for 15 min at 45° in 0.03 cc physiological saline solution and centrifuged while hot:

Extract from the sediment + fresh A cells (S.V.) = intense agglutination

Extract from the sediment + fresh B cells (C.C.) = negative agglutination.

Other experiments with B blood gave concordant results. In addition to suspending the dry blood in excess of serum (1 cc), the successive test with the extract from the sediment gave still very rapid and intense specific agglutination.

(The sera ab (L.L. and S.G.) used in these and in the succeeding experiments agglutinate the A cells as intensely as the B cells).

### 2. Heated blood

0.025 cc of blood with type A cells (S.V.) dried and pulverized, was added to 0.1 cc of physiological saline solution in a small tube that was stoppered and then immersed 5 min in a boiling water bath. The tube was cooled and 0.1 cc fresh serum a + b (L.L.) is added and mixed up thoroughly. After half a day at ordinary temperatures it is centrifuged:

Absorbed serum + fresh A cells (S.V.) = negative agglutination

Absorbed serum + fresh A cells (S.E.) = negative agglutination

Absorbed serum + fresh B cells (C.C.) = total agglutination

The sediment, washed at 0°, is extracted for 15 min at 45° with 0.1 cc of physiological saline solution and centrifuged

while hot:

Extract from the sediment + A cells (S.V.) = moderately positive agglutination

/368 Extract from the sediment + A' cells (S.E.) = positive agglutination

Extract from the sediment + B cells (C.C.) = negative agglutination

In other experiments the absorption is not complete, at least with certain cells, with a first portion of stain, but only with a second.

0.05 cc of A' blood (S.E.) dried and suspended in physiological saline solution was baked at 100° for 5 min, then treated with 0.1 cc of fresh serum ab (L.L.):

Absorbed serum + fresh A' cells (S.E.) = weakly positive agglutination

Absorbed serum + fresh A cells (S.V.) = positive agglutination

Absorbed serum + fresh B cells (C.C.) = strongly positive agglutination

The serum is absorbed further with 0.025 cc of blood A' (S.E.) dried and baked, as above, in physiological saline solution:

Absorbed serum + fresh A' cells (S.E.) = negative agglutination

Absorbed serum + fresh A cells (S.V.) = negative agglutination

Absorbed serum + fresh B cells (C.C.) = strongly positive agglutination

In all the experiments in which the stain and the absorbing serum were mixed in proportions similar to those indicated, the absorption of the agglutinins appeared complete and wholly specific, if not in the first test then at least in the second. The subsequent test of the agglutinin extraction on the other hand, often gave less clear and sometimes even negative results, clearly because of the scarce quantity of agglutinins recovered, due either to the small quantity of serum employed (precisely with the intention of obtaining the complete absorption of one of the two agglutinins contained in it), or to the procedure of washing the first sediment from the extraction, or perhaps to the temperature during extraction, which in some experiments surpassed the indicated 45°-50°.

Such negative results were obtained, for example, in the following experiment:

0.05 cc of pulverized B blood (C.C.) was suspended in 0.1 cc of physiological saline solution and baked 5 min at 100°. 0.1 cc of serum ab (L.L.) was then added, and after incubation for several hours the mixture was centrifuged:

/369 Absorbed serum + fresh A cells (S.V.) = total agglutination

Absorbed serum + fresh A' cells (S.E.) = total agglutination

Absorbed serum + fresh B cells (C.C.) = negative agglutination

The sediment is washed twice with physiological saline solution at 0°, and afterwards extracted with 0.1 cc of it for

15 min at 45°-50°, then centrifuged while hot:

Extract from sediment + fresh A cells (S.V.) = negative agglutination

Extract from sediment + fresh B cells (C.C.) = negative agglutination

(only 3-4 cells appear doubtfully associated with one another).

In order to better investigate the possibility of recovering the absorbed agglutinins even from baked stains, I conducted other experiments using greater quantities of serum, so that the agglutinable substance would be maximally exposed to agglutinin. Thus in the following experiment, the proportions of serum were such that absorption did not turn out to be complete.

To 0.05 cc of B blood suspended in physiological saline solution and baked above 100°, was added up to 0.3 cc of fresh serum ab (L.L.) After twenty-four hours, the material was centrifuged:

Absorbed serum + fresh A cells (S.E. and S.V.) = total agglutination

Absorbed serum + fresh B cells (C.C.) = slow, weak agglutination

The sediment was washed twice with physiological saline solution at 0° and extracted with 0.05 cc of it at 45°-50° for 15 min:

Extract of the sediment + fresh A cells (S.E. and S.V.) = negative agglutination

Extract of the sediment + fresh B cells (C.C.) = evident agglutination (persisting to a 1:3 dilution of the extract).

Very clear results have been obtained using red cells, rather than dried blood, baked at 100°.

0.2 cc of A blood (S.V.) is suspended in physiological solution and washed twice; the liquid is removed as much as possible and the cellular sediment sprinkled with boiling physiological saline solution, then the small tube is immersed into a boiling water bath, where it is left about 10 min.

A shiny, homogeneous suspension is obtained, in which, however, the microscopical examination shows, moreover, the presence of small amorphous lumps and of a few recognizable cells. This suspension is centrifuged and the sediment resuspended in 0.1 cc of fresh ab serum (L.L.).

After an hour it is centrifuged and prepared in the usual way: 370/

Absorbed serum + fresh A cells (S.V.) = negative agglutination

Absorbed serum + fresh A' cells (S.E.) = negative agglutination

Absorbed serum + fresh B cells (C.C.) = intense agglutination

The sediment was washed and extracted at 45° in the usual way with 0.1 cc of physiological saline solution:

Extract of the sediment + fresh A cells (S.V.) = strong agglutination

Extract of the sediment + fresh A' cells (S.E.) = strong agglutination

Extract of the sediment + fresh B cells (C.C.) =  
negative agglutination

## II. Blood Treated with Chemical Agents

Having withdrawn blood of a determined group, I allowed the washed red blood cells to be in contact with various chemical agents for a time, not less than twenty-four hours, and at an ordinary temperature of 22°. Then I removed by washing or neutralization the substance employed and I proceeded to the tests of absorption and of extraction of agglutinins from the cellular sediment, using the same technique employed in the preceding experiments.

*Substances employed:* Hydrochloric acid, acetic acid, sodium hydroxide, ammonia, mercuric chloride, silver nitrate, potassium permanganate, potassium dichromate, osmium tetroxide, formaldehyde, ethyl alcohol, ethyl ether, chloroform, acetone.

### 1. Hydrochloric acid

0.05 cc of blood with A cells (S.V.) are twice washed; the sediment is added to 2 cc of HCl solution at 3.7% (1N). Macroscopical homogeneous suspension: a clear solution is reobtained by centrifugation. After twenty-four hours in HCl, the cells, washed three times, appear shrivelled under a microscope and present the features of discrete agglutination. The addition of agglutinating serum a and b in hanging drops did not modify this appearance.

The absorption test on such cells, twenty-four hours in HCl, washed three times and kept for forty-eight hours at 0° with 0.1 cc of fresh serum ab (S.G.), and centrifuged, gave the following results:

Absorbed serum + fresh A cells (S.V.) = negative agglutination

4371 Absorbed serum + fresh B cells (P.G.) = strong agglutination

Another 0.2 cc fresh serum ab (S.G.) is added. After twenty-four hours at 0° it is centrifuged:

Absorbed serum + fresh A cells (S.V.) = weak agglutination

Absorbed serum + fresh B cells (P.G.) = total agglutination

The sediment is washed three times with physiological saline solution at 0°, resuspended in 0.02 cc of physiological saline solution, and extracted for 15 min at 45°, then centrifuged while hot:

Extract from the sediment + fresh A cells (S.V.) = rapid, almost total agglutination

Extract from the sediment + fresh B cells (P.G.) = negative agglutination

### 2. Acetic Acid

(a) 0.05 cc of blood with A cells (S.V.) is twice washed; to the sediment is cautiously added dropwise 2 cc of glacial acetic acid (rapid addition provokes complete, instantaneous hemolysis) and one gets partial hemolysis with the formation of small reddish-brown membranes and of a few white

masses (cellular stroma).

After twenty-four hours the material is washed repeatedly until the odor of the acetic acid is no longer present, and reduced to minute fragments by crushing in the same small tube with a small glass rod. The sediment used in the single tests was about  $\frac{1}{2}$  or  $\frac{1}{3}$  of the initial cellular sediment. To it is added 0.1 cc of fresh serum ab (S.G.). After twenty-four hours at 0° it is centrifuged:

Absorbed serum + fresh A cells (S.V.) = strong agglutination

Absorbed serum + fresh B cells (P.G.) = very strong agglutination

The decanted serum is absorbed again for twenty-four hours at 0° with 0.05 cc of other A blood (S.V.) from the acetic acid. After centrifugation:

Absorbed serum + fresh A cells (S.V.) = moderate agglutination

Absorbed serum + fresh B cells (P.G.) = very strong agglutination

The decanted serum is, for a third time, absorbed for twelve hours at 0° with 0.05 cc of other A blood (S.V.) from the acetic acid. After centrifugation:

Absorbed serum + fresh A cells (S.V.) = weak agglutination

Absorbed serum + fresh B cells (P.G.) = slow, moderate agglutination

The three sediments are combined and 0.4 cc of fresh serum ab (S.G.) is added. After some hours at 0° the sediment is washed three times with physiological saline solution at 0°, resuspended in 0.02 cc of the same, extracted for 15 min at 45°, and centrifuged while hot:

Extract from the sediment + fresh A cells (S.V.) = very strong, rapid agglutination

Extract from the sediment + fresh B cells (P.G.) = negative agglutination

(b) 0.05 cc of blood with A cells (S.V.) is dropped onto a microscope slide and kept suspended for twenty-four hours in a closed container containing acetic acid. It acquires a red-brown yellowish color. Then, it is left 1-2 days to dry at ambient temperature. This results in a small, rough, thin layer that smells slightly of acetic acid. It is put in a small tube, and crushed carefully for a long time with a small glass rod, but one obtains a coarse material rather than a powder. afterwards it is washed repeatedly with physiological saline solution and 0.1 cc of fresh serum ab (S.G.) is added. After an hour at 0°, it is centrifuged:

Absorbed serum + fresh A cells (S.V.) = medium agglutination

Absorbed serum + fresh B cells (P.G.) = slow and strong agglutination

The decanted serum is absorbed again for some hours at 0° with 0.05 cc of A blood (S.V.) as above. After centrifugation:

Absorbed serum + fresh A cells (S.V.) = weak agglutination

Absorbed serum + fresh B cells (C.C.) = slow, mod-

## erate agglutination

The decanted serum is absorbed for a third time for some hours at 0° with 0.05 cc of A blood (S.V.), as above. after centrifugation:

Absorbed serum + fresh A cells (S.V.) = negative agglutination

Absorbed serum + fresh B cells (P.G.) = slow, weak agglutination

The three sediments are combined and 0.4 cc of fresh ab serum (S.G.) is added. After some hours the sediment is thrice washed with physiological saline solution at 0°, resuspended in 0.02 cc of the same and extracted for 15 min at 45°, than centrifuged while hot:

Extract from the sediment + fresh A cells (S.V.) = rapid, very strong agglutination

Extract from the sediment + fresh B cells (P.G.) = negative agglutination

## /373 3. Sodium hydroxide

(It is impossible to have cellular sediment from concentrated or dilute solutions of NaOH, whether in water or in physiological solution, since red blood corpuscles are hemolyzed, if not immediately (concentrated solution), then during the washing operations).

0.05 cc of blood with A cells (S.V.) are dropped onto a microscope slide and 0.05 cc 0.1N NaOH is overlaid and lightly mixed. It is left twenty-four hours to dry out at ambient temperature. Then the NaOH is neutralized by overlaying a drop of 0.1N HCl and the preparation left to dry out for another twenty-four hours at ambient temperature. This results in a small, hard, fragile layer that is placed in a small tube and pulverized, then is washed repeatedly with physiological saline solution (that of the first wash is slightly colored by hemoglobin. 0.1 cc of fresh ab serum (S.G.) is then added. After few hours, it is centrifuged:

Absorbed serum + fresh A cells (S.V.) = negative agglutination

Absorbed serum + fresh B cells (P.G.) = rapid, very strong agglutination

Another 0.1 cc of fresh ab serum (S.G.) is added and after twenty-four hours at 0°, the sediment is thrice washed with physiological saline solution at 0°; resuspended in 0.02 cc of physiological saline solution, extracted for 15 min at 45°, and centrifuged while hot:

Extract from the sediment + fresh A cells (S.V.) = very strong agglutination

Extract from the sediment + fresh B cells (P.G.) = negative agglutination

## 4. Ammonia

(It is impossible to have cellular sediment from concentrated or dilute ammonia solutions, whether in water, or in physiological saline solution, because the red blood corpuscles are hemolyzed.)

0.05 cc of blood with A cells (S.V.) are dropped onto a microscope slide and kept suspended for 3-4 days in a closed receptacle containing ammonia. The stain acquires a brown color and smells of ammonia. Then it is kept for twenty-five

hours to dry out at ambient temperature. One obtains a small, hard, fragile layer that no longer smells of ammonia. This is placed in a small tube, and is pulverized. It is then washed repeatedly with physiological saline solution and 0.1 cc of fresh ab serum (S.G.) is added. After twenty-four hours at 0° it is centrifuged:

Absorbed serum + fresh A cells (S.V.) = negative agglutination

Absorbed serum + fresh B cells (P.G.) = total agglutination

0.1 cc of other fresh ab serum (S.G.) is added and after twenty-four hours at 0°, the sediment is thrice washed with physiological saline solution at 0°, resuspended in 0.02 cc of physiological saline solution, extracted for 15 min at 45°, then centrifuged while hot:

Extract from the sediment + fresh A cells (S.V.) = strong agglutination

Extract from the sediment + fresh B cells (P.G.) = negative agglutination

## 5. Mercuric Chloride

0.05 cc of blood with A cells (S.V.) is washed twice; the sediment is treated for four days with 2 cc of 5% sublimate. Macroscopically, brown homogeneous suspension. Under the microscope the cells show altered form: lance-shaped biscuits; but they are all isolated from one another. The sediment is washed until all trace of reaction of the Hg disappears from the washing liquid, and is then suspended homogeneously in physiological saline solution. This suspension treated with sera a and b shown to contain the specific isoagglutinable property.

The absorption test on 0.05 cc of such A blood (S.V.) from the sublimate, kept for twelve hours at 0° with 0.1 cc of fresh ab serum (S.G.) and then centrifuged, gave the following result:

Absorbed serum + fresh A cells (S.V.) = negative agglutination

Absorbed serum + fresh B cells (P.G.) = total agglutination

0.2 cc of the same ab serum is added and after twenty-four hours at 0°, and being centrifuged:

Absorbed serum + fresh A cells (S.V.) = almost negative agglutination

Absorbed serum + fresh B cells (P.G.) = total agglutination

The sediment is washed twice with physiological saline solution at 0°, resuspended in 0.03 cc of the same, kept for 15 min at 45° and centrifuged while hot:

Extract from the sediment + fresh A cells (S.V.) = rapid, very strong agglutination

Extract from the sediment + fresh B cells (P.G.) = negative agglutination

Identical results were obtained in absorption tests with cells having remained for two months in 5% sublimate solution, and with *fresh ab serum* (S.G.).

In other experiments in which I employed cells that had

been left four days in sublimate, and *old ab serum* (L.L.), kept in a vial, but still strongly agglutinating for A and B cells in control experiments, the results were a little bit different in that they revealed a diminution in the specificity of the absorption, not, however, confirmed by the test for extraction of the agglutinins. In fact the A cells showed an absorption of the b agglutinin (for which they do not normally have any affinity) but to a minor degree, without, however, yielding it up in the absorption test.

/375

6. Silver Nitrate

0.05 cc of blood with A cells (S.V.) are washed twice; to the sediment is added 2 cc of 1% silver nitrate. Macroscopically: shiny, coarse suspension. Under the microscope: spherical cells reunited in large clumps.

After repeated washing, first with distilled water, then with physiological saline solution, is added 0.1 cc of fresh ab serum (S.G.). After twenty-four hours at 0°, it is centrifuged:

Absorbed serum + fresh A cells (S.V.) = negative agglutination

Absorbed serum + fresh B cells (P.G.) = slow, medium agglutination, more marked at the borders

0.1 cc of fresh ab serum (S.G.) is added and after some days at 0°, the sediment is thrice washed with physiological saline solution at 0°, resuspended in 0.02 cc of physiological saline solution, extracted for 15 min at 45°, and centrifuged while hot:

Extract from the sediment + fresh A cells (S.V.) = very strong agglutination

Extract from the sediment + fresh B cells (P.G.) = negative agglutination

7. Potassium permanganate

0.05 cc of blood with A cells (S.V.) are washed twice; to the sediment is added 2 cc of 2.4% potassium permanganate: coarse suspension.

After repeated washing with physiological saline solution is added 0.1 cc of fresh ab serum (S.G.), and after twenty-four hours at 0° it is centrifuged:

Absorbed serum + fresh A cells (S.V.) = negative agglutination

Absorbed serum + fresh B cells (P.G.) = negative agglutination

0.1 cc of other fresh ab serum (S.G.) is added and after some hours at 0°, it is centrifuged:

Absorbed serum + fresh A cells (S.V.) = negative agglutination

Absorbed serum + fresh B cells (P.G.) = negative agglutination

/376

24 hours at 0°, the sediment is thrice washed in physiological saline solution at 0°, resuspended in 0.02 cc of the same, extracted for 15 min at 45°, and is centrifuged in the heat:

Extract from the sediment + fresh A cells (S.V.) = negative agglutination

Extract from the sediment + fresh B cells (P.G.) = negative agglutination

8. Potassium dichromate

0.05 cc of blood with A cells (S.V.) is washed twice; to the sediment is added 2 cc of 6.7% potassium dichromate (in other experiments, 3% was used with identical results). Macroscopically, a shiny, finely granulated suspension. Under the microscope, spherical cells are reunited into large clumps, simulating agglutination. After repeated washing with physiological saline solution is added 0.1 cc of fresh ab serum (S.G.). After 12 hours at 0°, it is centrifuged:

Absorbed serum + fresh A cells (S.V.) = negative agglutination

Absorbed serum + fresh B cells (P.G.) = very strong agglutination

0.1 cc of other fresh ab serum (S.G.) is added, and after some hours at 0° the sediment is washed three times with physiological saline solution at 0°, resuspended in 0.02 cc of the same, extracted for 15 min at 45°, and centrifuged in the heat:

Extract of the sediment + fresh A cells (S.V.) = strong agglutination

Extract of the sediment + fresh B cells (P.G.) = negative agglutination

9. Osmium tetroxide

0.03 cc of blood with fresh A cells (S.V.) is washed twice; to the sediment is added 2 cc of 1% osmium tetroxide. Macroscopically homogeneous suspension of brownish color after a few minutes, and a few clumps. After 24 hours, the cells are washed three times with physiological saline solution. Under the microscope the cells appear in various forms: Lance-shaped and faceted but perfectly isolated from the other. Treated separately in hanging drops with serum a and serum b, loss of the specificity of isoagglutination is observed, in that both the sera agglutinate the cells intensely, though the a serum does so in greater measure. The absorption test, however, of 0.05 cc of such blood with A cells (S.V.), treated as above, kept for 24 hours at 0° with 0.1 cc of fresh ab serum (S.G.) and centrifuged, gave the following results:

Absorbed serum + fresh A cells (S.V.) = negative agglutination

Absorbed serum + fresh B cells (P.G.) = total agglutination

0.1 cc of other fresh ab serum (S.G.) is added, and after 24 hours at 0°, the sediment is thrice washed with physiological saline solution at 0°, resuspended in 0.02 cc of the same, extracted for 15 min at 45°, and centrifuged in the heat:

Extract from the sediment + fresh A cells (S.V.) = negative agglutination

Extract from the sediment + fresh B cells (P.G.) = negative agglutination

I obtained identical results after fractional absorption

with two equal portions of cells, each corresponding to 0.05 cc of blood, using blood fixed with osmium tetroxide vapors, according to the following technique:

0.05 cc of blood with fresh A cells (S.V.) is dropped onto a microscope slide, kept suspended for twenty-four hours in a vessel containing some 1% osmic acid then allowed to dry out for twenty-four hours. This gives a thin layer that is ground up in a small tube, washed three times with physiological saline solution and added to the absorbing serum.

#### 10. Formaldehyde

0.05 cc of fresh blood with A cells (S.V.) is twice washed; to the sediment is added 2 cc of 5% formalin in physiological saline solution. Macroscopically, homogeneous suspension of brownish color after a few minutes. The cells thus fixed for six hours, and then washed three times with physiological saline solution, appear under the microscope to be perfectly preserved in their form. Treated separately in hanging drops with a serum and b serum, the specific isoagglutinability has been preserved, and is of an intensity similar to that of fresh cells. For the absorption test, 0.05 cc of formalin-treated blood with A cells (S.V.), three times washed, is added to 0.1 cc of fresh ab serum (L.L.). After some hours at 0°, it is centrifuged:

Absorbed serum + fresh A cells (S.V.) = negative agglutination

Absorbed serum + fresh B cells (P.G.) = rapid total agglutination

After some hours of incubation in another 0.2 cc of the same serum, by the end of which the a agglutinin is completely absorbed, the sediment is washed twice with physiological saline solution at 0°, resuspended in 0.02 cc of the same, extracted for 15 min at 45°, and centrifuged in the heat:

Extract from the sediment + fresh A cells (S.V.) = almost total agglutination

Extract from the sediment + fresh B cells (P.G.) = negative agglutination

I obtained identical results with cells kept in fixing solution for twelve days, or else fixing the cells with vapors of formaldehyde in the following way:

0.05 cc of blood with fresh A cells (S.V.) is allowed to coagulate on a microscope slide that is kept suspended for three days in a vessel containing some formalin. Then it is dried out for 24 hours, in order to facilitate the pulverization of the stain. This done, it is washed twice with physiological saline solution and the absorption and extraction of agglutinins carried out using techniques and obtaining results the same as the preceding.

#### 11. Ethyl Alcohol

0.05 cc of blood with A cells (S.V.) is twice washed; to the sediment is added 2 cc of alcohol at 95°. Macroscopic appearance: large clumps. To this sediment, having remained in alcohol 48 hours and then having been washed three times with physiological saline solution is added 0.1 cc of fresh ab

serum (S.G.). After 60 hours at 0°, it is centrifuged:

Absorbed serum + fresh A cells (S.V.) = strong agglutination

Absorbed serum + fresh B cells (P.G.) = very strong agglutination

The decanted serum is added to another 0.05 cc of A blood (S.V.) which was washed twice and kept for four days in 2 cc of alcohol, then washed three times with physiological saline solution. After 24 hours at 0°, it is centrifuged:

Absorbed serum + fresh A cells (S.V.) = moderate agglutination

Absorbed serum + fresh B cells (P.G.) = very strong agglutination

The decanted serum is added again to another 0.05 cc of A blood (S.V.) which was twice washed and treated for six days in 2 cc of alcohol, then washed three times with physiological saline solution. After 24 hours at 0°, it is centrifuged:

Absorbed serum + fresh A cells (S.V.) = moderate agglutination

Absorbed serum + fresh B cells (P.G.) = very strong agglutination

The three sediments are combined and added to 0.2 cc of fresh ab serum (S.G.). After twenty-four hours we proceeded with the usual extraction:

Extract from the sediment + fresh A cells (S.V.) = 37  
very strong agglutination

Extract from the sediment + fresh B cells (P.G.) = negative agglutination

Such experiments were repeated even with cells which remained in alcohol for a month and prolonging for up to several days the contact of the absorbing serum with successive fractions of cells. The results were the same as those mentioned above, in the experiments in which fresh ab serum (S.G.) was employed. In two other experiments, on the other hand, in which old serum (L.L.) was employed, like that used in the experiments with the sublimate (see above), a diminished degree of specificity of absorption was also shown, the A cells having absorbed and yielded up both the agglutinins, but in different measure: the a agglutinin totally but the b agglutinin only partially, as was shown by the marked difference in the intensity of agglutination with the A and B test cells.

#### 12. Ethyl ether

0.05 cc of blood with A cells (S.V.) are washed twice; the sediment is added to 2 cc of ether. Macroscopically, the cells appear attached, in amorphous clumps of a pale brown color, to the walls of the small tube. After 24 hours, it is washed repeatedly with physiological saline solution, and 0.1 cc of fresh ab serum (S.G.) is added. After 24 hours at 0°, it is centrifuged:

Absorbed serum + fresh A cells (S.V.) = negative agglutination

Absorbed serum + fresh B cells (P.G.) = very strong agglutination

There is added 0.1 cc of fresh ab serum (S.G.) and, after

some hours at 0°, the sediment is thrice washed with physiological saline solution at 0°, resuspended in 0.02 cc of the same, extracted for 15 min at 45°, and centrifuged in the heat:

Extract from the sediment + fresh A cells (S.V.) = almost total agglutination

Extract from the sediment + fresh B cells (P.G.) = negative agglutination

I obtained identical results with cells exposed in the following way to the vapors of ether:

0.05 cc of blood with A cells (S.V.) is dropped onto a microscope slide and kept for three days in a closed container containing some ether. Then it is left to dry 24 hours at ambient temperature; a thin, hard, fragile layer is obtained, which is placed in a small tube and pulverized. It is washed repeatedly, and the experiments on the absorption and extraction of the agglutinins are carried out by the usual technique.

### 13. Chloroform

0.05 cc of blood with A cells (S.V.) is washed twice; the sediment is treated for twenty-four hours with 2 cc of chloroform. Macroscopically, coarse suspension. It is washed repeatedly with physiological saline solution, and 0.1 cc of fresh ab serum (S.G.) is added. After 24 hours at 0°, it is centrifuged:

Absorbed serum + fresh A cells (S.V.) = negative agglutination

Absorbed serum + fresh B cells (P.G.) = rapid, very strong agglutination

0.1 cc of other fresh ab serum (S.G.) is added and after some hours at 0°, the sediment is thrice washed with physiological saline solution at 0°, resuspended in 0.02 of the same, extracted for 15 min at 45°, and centrifuged in the heat:

Extract from the sediment + fresh A cells (S.V.) = strong, agglutination

Extract from the sediment + fresh B cells (P.G.) = negative agglutination

I obtained identical results with cells exposed to chloroform vapors in the following way:

0.05 cc of blood with A cells (S.V.) is dropped onto a microscope slide, and kept for twenty-four hours in a closed container containing chloroform. Afterwards it is dried out for four days at ambient temperatures yielding a thin, fragile layer that is placed in a small tube and pulverized. It is washed repeatedly and the absorption and extraction of agglutinin experiments are performed in the usual way.

### 14. Acetone

0.05 cc of blood with A cells (S.V.) are twice washed; the sediment is added to 2 cc of acetone. Macroscopically, coarse suspension. After 24 hours, it is washed repeatedly with physiological saline solution and 0.1 cc of fresh ab serum (S.G.) is then added. After 24 hours at 0°, it is centrifuged:

Absorbed serum + fresh A cells (S.V.) = negative agglutination

Absorbed serum + fresh B cells (P.G.) = total agglutination

0.1 cc of other fresh ab serum (S.G.) is added and after some hours at 0°, the sediment is thrice washed with physiological saline solution at 0°, resuspended in 0.02 cc of the same, extracted for 15 min at 45°, and centrifuged in the heat:

Extract from the sediment + fresh A cells (S.V.) = very strong agglutination

Extract from the sediment + fresh B cells (P.G.) = negative agglutination

I obtained identical results with cells exposed to acetone vapors in the following way:

0.05 cc of blood with A cells (S.V.) is dropped onto a microscope slide and kept for twenty-four hours in a closed container containing acetone. Afterwards it is dried out for four days at ambient temperature: a fragile, reddish-white, thin layer is obtained, which is placed in a small tube and pulverized. It is repeatedly washed and the absorption and extraction of the agglutinins is then carried out with the usual technique:

## III. Blood Devoid of Isoagglutinins

Finally I carried out experiments on dried blood containing the two agglutinable substances A and B, namely, belonging to the group which never shows any isoagglutinins in fresh stains.

0.05 cc of dried AB blood is pulverized and suspended in 0.3 cc of fresh ab serum (L.L.). After ten hours:

Absorbed serum + fresh A cells (S.V.) = negative agglutination

Absorbed serum + fresh B cells (C.C.) = negative agglutination

The sediment is thrice washed with physiological saline solution at 0°, and extracted at 45° with 0.05 cc of physiological saline solution:

Extract from the sediment + fresh A cells (S.V.) = strong agglutination

Extract from the sediment + fresh B cells (C.C.) = moderate agglutination

As controls for some of the various experiments described, I even tried to keep dried A blood in contact with serum b, and I could not, as was to be expected, observe any absorption; and even from the washed sediment I could not recover any trace of agglutinin.

## IV. Old Blood

I had the opportunity to apply these experiments to a practical case of determining the isoagglutinable substances in old human bloodstains seen in connection with a crime.

In these stains, dating back 18 months, Professor Lattes had been successful, some months before, in demonstrating the a agglutinin, now strongly attenuated. The demonstra-

tion of a specific isoagglutinable substance would constitute in this case an effective, appropriate control.

The multiple stains were crusted on the material of a beret: several of them were scraped off, carefully pulverized, and suspended in 0.1 cc of fresh serum (L.L.), diluted 1:3 in physiological saline solution. After ½ hour, it is centrifuged:

Absorbed serum + fresh A cells = total, immediate agglutination

Absorbed serum + fresh B cells = nearly negative agglutination, with some small groups from cells lying on one another

(The unabsorbed ab serum immediately and intensely agglutinated the A and B cells).

The test of heat extraction of the sediment, washed at ordinary temperatures, did not yield results; nevertheless, with only the above-mentioned test the presence of a substance in the stain able to absorb the b agglutinin could be demonstrated, consistent with the report of a agglutinin previously obtained. The blood under examination could thus be assigned to group aB.

## Conclusions

From the above experiments results the biologically interesting fact that the antigens of the blood, to which the isoagglutinins are bound, preserve this property, unaltered, after baking at 100° (by analogy to certain microbial antigens with respect to specific agglutinins), and even when the dried out blood has undergone the prolonged action of time.

Further, in examining the action of various chemical agents, such power is conserved. Thus it is so with the following substances: hydrochloric acid (1N solution), sodium hydroxide (0.1N), ammonia (vapor), mercuric chloride (5%), silver nitrate (1%), potassium dichromate (3-6.7%), osmium tetroxide (1% and vapor), formaldehyde (5% and vapor), chloroform (100% and vapor), ethyl ether (100% and vapor), acetone (100% and vapor).

Some substances (acetic acid and ethyl alcohol) although not to completely abolishing the selective absorption, weakened it, although with the acetic acid it was not possible to stabilize (see the experiments) that part of the decay due to the modification of the isoagglutinable substance nor the cellular destruction (acetic acid in the liquid state) nor the coarseness of the pulverized material (vapors).

Still other substances (2.4% potassium permanganate) completely abolish all selective absorption.

The diminution of the degree of specificity of absorption recounted in some experiments (sublimite, alcohol) is not attributable to the denaturing treatment on the cells, but seems more properly to have to do with the serum.

In fact, the phenomenon occurs consistently every time old serum is employed. In the very few cases in which the same fact is seen with fresh serum (silver nitrate and acetic acid vapors) it could be due rather to true absorption with damage to the agglutinins, due to the prolonged contact with cells loaded with denaturing substance.

Indeed, completely negative results were always obtained

in such experiments (in spite of the accuracy of the technique and the previous addition of ab serum in excess) with the opposite test, that of the extraction of those agglutinins, apparently fixed in part to the cells in the absorption test in a way contrary to the group specificity.

From a practical point of view, the possibility emerges of being able to identify the isoagglutinable substance in dried blood, which has been rendered insoluble by age, excessive heat or the influence of numerous chemical substances.

The best results are obtained by allowing the least possible quantity of a fresh ab serum to act upon the pulverized blood substance (most appropriately for several hours, shaking frequently) and sampling, after centrifugation, the residual isogglutinating properties. Blood A absorbs the a agglutinin, blood B the b, blood AB, both. When, after prolonged absorption for twenty-four hours, the two agglutinins were still active, but to a different degree (this a sign of incomplete absorption) it was possible, where there is enough of the stain, to render the test completely specific, by a second absorption with a new dose of pulverized blood. A confirmation of the specific absorption, though less constant for the sensitivity of the technique, can be achieved by extracting the agglutinins at 45°, which were fixed in the substance of the stain in the previous test, and by then determining their nature by reaction with fresh cells of a specific group (A and B). In order to facilitate this test it is necessary to suspend the pulverized stain in an excess of ab serum and to carry out the necessary washing with physiological saline solution at 0°. It is advisable to use fresh ab serum, with which more precise and specific results are obtained.

In conclusion, even when one does not find isoagglutinins in the stains, either because of the group to which the blood belongs, or because of their destruction, the demonstration of their individual origin, it is nevertheless very often possible to demonstrate their individual origin (their group), by means of identification of the isoagglutinable substance contained in the stains.

I thank Professor Lattes for the suggestions he gave me and for the constant assistance rendered me during the execution of this research.

## Literature

1. Lattes. Sui fattori dell'isoagglutinazione nel sangue umano. *Haematologica* 3, 401, 1921
2. Schütze. Hämagglutination and its medico-legal bearing, with observations upon the theory of isoagglutinins. *Brit. J. of exp. Path.* 2, 26, 1921
3. V. Lange. Zur Frage der Hitzebeständigkeit der gebundenen Antikörper. *Z. f. Immun. frosch.*, Orig. 32, 449, 1921
4. Eisenberg and Volk. *Zeitschr. f. hygiene*, 1902
5. Wassermann. Ueber agglutinine und Praecipitine. *Zeitsch. f. Hyg.*, 1903
6. Bordet. Cited by Widal and Sicard
7. Van de Velde. Influence de la chaleur, des métaux lourds et d'autres antiseptiques sur les cultures de b. typhiques etc. *Semaine Médicale*, 1897
8. Widal and Sicard. Etude sur le sérodiagnostic etc. *Ann. Inst. Pasteur*, 1897

*Blood Grouping*

- Idem. La réaction agglutinante sur les bacilles morts. *Comp. rendus Soc. Biol.*, 1897
9. Nicolle, Ch. Suite d'expériences relatives aux phénomènes de l'agglutination des microbes. *Annales Inst. Pasteur*, 1904
10. Righi. Su alcune modalità dell'agglutinazione del bacillo di Ebert. *Riv. di Igiene e Sanità Pubblica*, 1910
11. Busacca. Sulle modificazioni determinate da vari agenti chimici sulla agglutinabilità dei batteri e sulla capacità agglutinante dei sieri. *Annali di Clinica Medica*, Anno XI, fasc. 4
12. Porges. Ueber die Folgen der Veränderungen des Bakterien-proteins für die Agglutination und Präcipitation. *Zeitsch. exp. Pathol und Ther.*, Vol. 1
13. Drever and Blake. On the agglutination of bacteria. *Journal of Path. Bact.*, 1906

## A Simple Procedure for the Determination of Groups in Dried Blood by Agglutinin Binding\*†

Dr. Franz Joseph Holzer  
Assistant

Institute for Forensic Medicine of the University of Innsbruck  
(Chairman: Prof. Dr. Karl Meixner)

Whenever one writes or speaks about the importance of blood groups in forensic medicine, it immediately gets down to the question of determining blood groups from dried blood traces. In fact, that ought to be one of the most important problems for the forensic physician. Though the problem is solved now, the situation is unfortunately still bad. This results from the fact that the number of communications concerning this problem is in crying disproportion to its importance, and many of these studies misunderstand the other problems in the area of blood group research.

We ought not to assume that few researchers have tackled this question; the explanation lies rather in the old observation that one does not report on his failures. We have earnestly striven here to make some progress, and we believe that we can report today on some essential successes.

The identification of the characteristics of blood corpuscles and serum usually causes no difficulties in the case of fresh blood. If the dried blood is not too old, the substances are indeed present on which the peculiarities of the groups are based, but here we are faced with the difficulty that the encrusted blood corpuscles can no longer be separated as the unsuccessful experiments which Biffi set up in this direction have demonstrated. Thus, we are no longer able to detect agglutination.

In order to base a blood group identification on these blood spots, a whole series of processes have been offered, all of which rest on two fundamental ideas. The one is to get the agglutinins in solution by dissolving the blood spot and then to test this liquid in the same manner as serum with test blood cells known to belong to a certain group. On this principle rest the processes of Landsteiner and Richter, de Domenicis, and Lattes. Landsteiner and Richter have claimed that, with this test, the blood group can be determined from a dried blood stain up to four months old.

Though the picture of clumping about the blood flake is a neat one, nevertheless, in the majority of cases this method has failed. Even with blood stains no more than a week old, clumping failed to take place in half the cases. With older stains we had only isolated cases of success.

The assertions of Müller of the Zurich surgical clinic, together with Brunner, concerning the agglutinin enrichment process seemed to us most enticing.

He produced extracts stronger in concentration than in the original blood and thought thereby that he could extract a smaller quantity of more powerful substances from a larger quantity of inferior-grade initial substances. According to Goroncy's report, Müller leached the blood stain at a temperature of 0° in order to eliminate the autoagglutination. Dried swabs served for the experiment.

To dissolve the dried blood he recommended a weak, saline solution of 0.2 to 0.3 percent, more than distilled water, observing that better leaching was to be obtained with a saline solution. He took the solution in an ample quantity and concentrated it in a vacuum at 16° to 19° to the consistency of syrup. He stored the concentrated residue in an icebox until he was ready to carry out the test. If the concentration goes too far, as can easily happen, the syrup can be diluted again somewhat in order not to have a salt content which could disturb the test. Müller especially stresses that only lecithin blood corpuscles are to be used. Since in the case of weak agglutinins the clumping often makes its first appearance after a long period, Müller keeps the slides in a moist chamber. Experiments to free extracts from hemoglobin by means of animal charcoal were unsuccessful in that Müller thereafter produced no clumping. In order to test the authenticity of the clumping, Müller depends not on a cover slip as Lattes did, but he tests with O-blood corpuscles whose clumping proves autoagglutination.

According to their report Müller and Brunner have been able to establish the original blood group in seventy percent of eighty dried samples which were up to eighteen months old. Thus, age definitely plays no role in this test. As Popoff reports, Serebrjaikow, whose work was not available to me, has described a procedure very similar to that of Müller.

With great hopes we tried Müller's process with our collection of swabs. Though we followed exactly the prescriptions we were unsuccessful.

447

\* Translation of: "Ein einfaches Verfahren zur Gruppenbestimmung an vertrocknetem Blut durch Agglutininbindung".

in *Deutsche Zeitschrift für die Gesamte Gerichtliche Medizin* 16: 445-458 (1931).

Reprinted with the kind permission of Springer-Verlag, Heidelberg and New York.

† Delivered at the 19th meeting of the German Society for Legal and Social Medicine in Königsberg, November 12, 1935.

We returned again to the properties of the blood corpuscles. Since in the agglutination of blood corpuscles agglutinins are taken out of the blood fluid, it is a closely related concept that the agglutigen in dried blood can be identified by its ability to bind with a specific agglutinin.

There exist a number of reports, especially in the treatises, concerning the methods to identify groups, though, as mentioned by way of introduction, very little is said about the employing of such processes. Schiff and Higuchi provided the most exact prescriptions.

Compared to the processes of distinguishing the blood group of fresh blood, these are certainly intricate and lengthy so that false notions about the difficulty of the undertaking and about the quantity of blood required for the test frightened us at first, as it probably did many others.

The process is based essentially on the following: a serum of group O with the agglutinins  $\alpha$  and  $\beta$  or a serum  $\beta$  and a serum  $\alpha$  are treated separately or in a mixture with the dried blood stain whose group identity is unknown. Afterwards the sera are tested to see if their agglutinin content has remained the same or has altered. If agglutinins are used up, then the corresponding agglutigen was present in the blood spot. In this process the strength of the agglutinins of the test serum, a strength which varies extraordinarily from person to person, plays a decisive role.

After we had learned in experiments with fresh blood corpuscles about the necessary quantitative relationships to produce a sufficient absorption, we went on to experiments with dried blood. Blood in a dried state is estimated in general to weigh about  $\frac{1}{5}$  that of fluid blood. Schiff estimated that, to absorb an agglutinin,  $\frac{1}{5}$  of its weight in fresh blood is necessary. If we hold to his estimate, then, given that blood dries to approximately  $\frac{1}{5}$  of its weight (Hammarsten's and our own experiments) and given that the agglutigen does not essentially decrease in the drying process, a quantity of dried blood from  $\frac{1}{5}$  to  $\frac{1}{10}$  of the serum mass would be sufficient for absorption, i.e., for 0.1 cc serum, approximately 0.01 g of dried blood. This hypothesis, derived from calculations, was completely confirmed by experiments.

From pulverized dried blood  $\frac{1}{5}$  of the serum mass (0.02 g dried blood to 0.3 cc serum) is sufficient to take up almost all the agglutinin. Even  $\frac{1}{20}$  to  $\frac{1}{30}$  are still clearly able to reduce the agglutinins.

After many experiments the following process proved to be the best to determine blood groups from dried blood samples. Finally we use it exclusively.

In small test tubes 6 cm long and 5 to 6 mm wide, 10 mg of dried blood are placed. If blood on swabs is involved, then from a selection of thoroughly blood-soaked swabs, the same amounts are weighed out in the same fashion—the weight of the fiber is not to be considered at all. Then the serum, which has been measured out before the experiment, is introduced into the test tubes with a graduated syringe. Using a syringe has the advantage of speed over filling with a graduated pipette.

All the test tubes in the test series are shaken hard once

and then placed for twenty-four hours in the icebox. Thereafter, these are centrifuged and a small amount of the liquid is siphoned off with fine, hand-drawn pipettes for the new evaluation. As the best test sera, we select such serum as contains almost exactly the same quantity of agglutinins  $\alpha$  and  $\beta$  compared to our constant test blood sample. The essential part of our method is the type of evaluation of the serum and the preparation of the dilutions which are required for such evaluation.

The evaluation was carried out on glass plates with eight concave depressions such as those introduced for the evaluation of sera in the institute by my director, Professor Meixner.\*

In carrying out these experiments our need for plates increased greatly. We were able sometimes to meet our needs by cementing to a standard  $9 \times 12$  glass plate four microscope slides, each with two concave depressions, which also reduced our costs considerably. It is easier and cleaner, of course, to work with ready-made plates.

Dilutions of the serum, proceeding according to the power of two, is produced in the depressions of the glass plates in the following way. First, in all the cavities four drops of physiological saline solution are added with the same fine pipette. Then four drops of the serum to be evaluated are dropped into the first niche and are subsequently mixed carefully by repeatedly drawing up and ejecting the fluid with the pipette, while at the same time stirring the fluid. Then we carry over four drops of this mixture into the next tray; the rest is ejected back into tray one. So, in the same way, the dilution is carried through until, in the eighth cavity, we have reached a dilution of 256. Thereupon, from each niche of this first plate, half of the contents, i.e., two drops, are transferred into the corresponding cavities of the second plate. The one plate serves for the test with A-blood corpuscles, the other for the test with B-blood corpuscles. From a two-percent suspension of these we add a drop to each depression. We label the one plate with A, the other with a large B. We have accustomed ourselves always to place the A plate on the right in order to avoid mix-ups. Having reached a level of dexterity through practice, I now require five minutes at the most to set up the evaluation experiment from the introduction of the saline solution to the addition and stirring of the test blood corpuscles. With good pipettes I need only three minutes.

Immediately after mixing in the test blood corpuscles, the reading times are notes in ink or wax crayon on the plate.

The first reading is taken after ten minutes, reckoning from the time the test blood corpuscles were added, the second after 30 minutes (Fig. 1—[not reproduced in the translation]).

In the determination of endpoint titer the same process is followed. Naturally, the same blood corpuscles must be used, since blood corpuscles also display a widely varying sensitivity, so that some are easily agglutinated while others

\* The plates can be obtained from the Siebert Co. in Vienna for 5 schillings.

are not, a fact which numerous experiments have confirmed.

Occasionally the final yield is too small, forcing an experiment with half the quantity of serum. Quite often the tubes were placed again into the icebox and the experiment repeated after several days. In the icebox, very little of the fluid is lost through evaporation. We also noticed that an overgrowth of germs, which was indeed present in the dry samples, impaired the results.

In every test series, a tube filled only with serum was also placed in the icebox and was evaluated together with the others in order to reveal any reduction in titer from another cause. By this means we found in a few cases an unimportant reduction, at the most a single dilution. Such a small reduction, however, was not sufficient to prove agglutinin binding.

Our experiments were conducted at first with samples of dried blood whose blood group had already been determined beyond a doubt as fresh blood. The blood samples came for the most part from the clinics where in most cases we immediately soaked a swab with blood; some samples I collected myself. 330 such tests were undertaken. In addition, we examined old, dried blood samples whose blood group was not established in the fresh blood, among which were samples

over fifty years old. We have conducted 100 such experiments to date. We will discuss these later. Here let us emphasize that a clear reduction in binding capacity could not be established in cases of these old blood stains.

Table I shows a section from an experimental series. The cases have not been selected for any particular reason, but they are reproduced as they were examined. We are dealing here with samples, all of which were at least eight months old. The series was set up with a serum of group O of small and unequal titer. The bold face numerals<sup>†</sup> indicate a reading taken after thirty minutes, those enclosed in parentheses the reading taken under the microscope, a process carried out with all the samples, producing values one dilution higher. The decision regarding the blood group was reached only through a comparison of the final titration with the initial titer, and afterwards, the results were compared with the blood group determined by tests with fresh blood. The table shows that the group determined with dry samples is in complete agreement in these cases.

<sup>†</sup> Values in boldface type in the original article are italicized in the translation.

Table 1

O Serum, Reinstadtler, Alois Sept. 3, 1929						Initial Titer			Reduction in titer in dilution steps		Conforms to blood group
						10	4(8)	8(32)			
						30	4(16)	16(32)			
Dried Blood	Serum (cc)	Person's age (yr)	Age of blood (mo)	Weight (mg)	Blood Group (determined in fresh blood)	Time of reading (min)	Final Titer		with		
							A	B	A	B	
Josef H.	0.1	30	8	15	AB	10	0(0)	0(0)	2	3	AB
						30	0(2)	2(4)			
Albin V.	0.1	—	9	15	B	10	4(8)	0(4)	0	4	B
						30	4(16)	0(2)			
Franz K.	0.1	51	8	15	O	10	8(16)	16(32)	0	0	O
						30	8(16)	16(64)			
P . . . (app.)	0.1	5	8	15	A	10	0(0)	16(32)	2	0	A
						30	0(2)	16(64)			
Müller J. .	0.1	30	8	15	A	10	0(0)	16(32)	2	0	A
						30	0(4)	32(64)			
Johann Ko.	0.1	—	8	15	B	10	4(8)	0(0)	0	4	B
						30	4(8)	0(4)			
Siegfried H.	0.1	16	8	15	O	10	4(8)	16(32)	0	0	O
						30	4(16)	32(32)			
Adolf N.	0.1	16	8	15	A	10	0(2)	16(16)	2	0	A
						30	0(4)	16(16)			
Frau X.A.	0.1	—	8	15	A	10	0(2)	16(32)	2	0	A
						30	0(2)	32(64)			
Wilhelm St.	0.1	34	8	15	A	10	0(0)	16(32)	2	0	A
						30	0(2)	32(32)			
Wilhelm Tö	0.1	—	9	15	O	10	4(8)	16(16)	0	0	O
						30	4(16)	16(32)			
Franz Wi. .	0.1	46	8	15	B	10	4(8)	2(4)	0	3	B
						30	4(16)	2(4)			
Franz E	0.1	81	8	15	O	10	4(8)	16(16)	0	0	O
						30	4(8)	16(32)			
Johann Dob.	0.1	45	8	15	O	10	8(8)	8(16)	0	0	O
						30	8(16)	16(32)			

Table 2

		O-Serum, Müller, Fr. October 8, 1929					Initial Titer		Reduction in titer in dilution steps <sup>2</sup>		
					10	16(32)	16(32)				
					30	32(32)	32(64)				
Dried Blood	Serum (cc)	Age of person (yr)	Age of blood (days)	Weight (mg)	Blood Group (determined in fresh blood)	Time of reading (min)	Final Titer		with		Conforms to blood group
							A	B	A	B	
Olga K	0.1	—	8	10	A	10	0(0)	16(16)	5	1	A
						30	0(0) <sup>1</sup>	16(32)			
Heinr. Pr.	0.1	—	8	10	O	10	16(16)	32(64)	1	1	O
						30	16(32)	32(64)			
Marcia Gr.	0.1	—	8	10	B	10	16(16)	2(4)	1	4	B
						30	16(16)	2(4)			
Maria Hirz.	0.1	23	8	10	B	10	8(16)	0(2)	1	5	B
						30	16(16)	0(2)			
Maria K..	0.1	21	8	10	B	10	16(16)	2(4)	1	4	B
						30	16(16)	2(4)			
Herbert Ph.	0.1	—	8	10	A	10	0(0)	8(16)	5	2	A
						30	0(0)	8(16)			
Josef Kner.	0.1	—	8	10	O	10	16(16)	16(16)	1	1	O
						30	16(16)	16(32)			
Schreyer	0.1	—	8	10	B	10	16(16)	2(4)	1	4	B
						30	16(16)	2(4)			
Kohlh.	0.1	—	10	10	A	10	2(2)	32(32)	4	0	A
						30	2(2)	32(32)			
Johanna Gr.	0.1	—	10	10	O	10	8(16)	32(32)	1	0	O
						30	16(16)	32(64)			
Gretl B	0.1	—	10	10	A	10	0(0)	16(32)	5	0	A
						30	0(2)	32(64)			
Sauter	0.1	—	10	10	B	10	8(16)	4(4)	1	3	B
						30	16(16)	4(8)			
Mttre	0.1	—	10	10	O	10	16(16)	32(32)	1	0	O
						30	16(32)	32(64)			
Karl H.	0.1	—	10	10	A	10	0(0)	4(8)	5	2	A
						30	0(0)	8(8)			
Unterg	0.1	—	10	10	O	10	16(16)	16(32)	1	0	O
						30	16(32)	32(64)			

<sup>1</sup> Since we started with a dilution of one-half, zero means that clumping was no longer visible by the second dilution step  
<sup>2</sup> Reading by naked eye after 30 min.

Table 2 comes from an experimental series conducted with dried blood in its second week. Here the experiment was carried out with higher-potency serum having almost identical titers for A and B corpuscles. When using lower-grade serum the binding sites of a dried blood sample are frequently not completely saturated. When more potent serum is used, the binding sites can be completely saturated, and thus more decisive results are obtained, thereby bringing about greater certainty of interpretation. Even if the weak sera were completely deprived of agglutinins and the stronger serum, either in 1:2 dilution or undiluted, still agglutinated, the lowering in the case of the latter was still more pronounced than in the case of the weak serum with its binding capacity completely used up. Still more exact experiments were set up in the following manner. Eight drops of sera with various high titers (unfortunately no especially potent sera were at my disposal) were each mixed with one drop of washed blood corpuscles which were as free as possible of liquid. These are shaken and left to stand for a certain

length of time at room temperature. They are then shaken 45 once more and centrifuged. Then the serum is measured against the identical quantity of the same test blood corpuscles. Here again it is clear that extremely low-potency sera 2(4), 4(8), were as a rule completely deprived of agglutinins, and were no longer capable of causing agglutination. Sera, however, with higher titers, 16(32), 64(64), were after saturation 45 so exhausted that they only agglutinated further at full or half strength. With weak sera the binding capacity of the blood corpuscles added does not fully come into play. This is shown by special experiments where blood corpuscles, treated with weak serum and then added to fresh serum, still extracted agglutinins from the fresh serum.

By combining the results of all the binding experiments, the following picture emerges. Agglutinins of titer 8 were reduced by a blood corpuscles on the average 2.7 dilution steps, by B blood corpuscles on the average 2.4 dilution steps; agglutinins of titer 16, on the other hand, were reduced on the average 3.5 degrees of dilution by A and 2.8 degrees of

dilution by B corpuscles. It is, therefore, recommended that high-potency sera be used for agglutinin binding, though, of course, the quantity of serum in these cases ought not to be too large.

As is apparent from the above figures, we found a stronger agglutinin binding produced by A than by B. Several unsuccessful attempts with samples of dried blood also corroborated this finding. Thus, out of seven samples of dried blood which Professor Lattes kindly gave to us, we incorrectly identified the 2 B bloods as O. Even repeating the experiment, this time leaving the samples for a longer time in the icebox, produced only a small weakening of  $\beta$ .

We achieved better success with eight samples which were sent to us from the Vienna Institute of Forensic Medicine. Of these eight we were able to identify all of them correctly.

With 56 samples on filter paper which also were kindly given to us by the Vienna Institute, we made twelve incorrect identifications, i.e. 21.4%. In one of these cases the binding of  $\beta$  by B failed to occur, whereas eleven times a false binding of  $\alpha$  without A occurred, and one time a false binding of  $\beta$  without B.

The most unwelcome false reaction was the binding which made one think of a binding caused by the substratum. We undertook experiments to examine this problem, using various types of flour, potatoes, rice starch, other parts of plants, sand, cloth, cotton, different types of paper, and similar objects. Using filter paper, these experiments, in fact, produced insignificant binding of the agglutinin. Recently we obtained relatively strong binding with blood-soaked mud, while less contaminated samples of the same blood did not produce false reactions. Cloth and cotton-wool have so far shown themselves to be harmless. In all cases one must be cautious and should always test the substratum for its binding capacity.

All together we tested 387 cases, including the dried samples of Lattes and those from Vienna. We correctly identified 366 samples, which represents a success rate of over 90%.

454 Finally, with the method here presented we tested old blood stains which were found among the *corpora delicti* of our institute. These blood stains were on tools of every sort, and also on bullets, clothes, parts of plants, and earth. Finally, there was dried blood which had been stored for other reasons years and even decades earlier. Here, too, the results of the experiments were surprisingly good in that we obtained, in a large number of cases, a clear binding of one or of both agglutinins. Naturally, it was not possible to show whether this binding corresponded to the blood group. One should notice, however, that the binding occurred in the same strength and in the same time as with samples which were not as old. Moreover, though only 100 cases were tested, the comparative numbers of the blood groups determined by the test agreed pretty well with the distribution of blood groups in our population (see in Table 3).

Table 3. Distribution of Blood Groups

Results of examination of 100 old samples		Percent in roughly 2000 diagnoses in Innsbruck based on double determinations
group	percent	percent
O	38	41.95 (42)
A	28	43.15 (43)
B	12	10.22 (10)
AB	10	4.68 (5)
uncertain	12	—

The examination of several blood stains on a cudgel which was used forty years earlier in a robbery-murder, produced a varied binding, which indicated blood of different groups. This result was explained without further ado since two criminals, one of whom carried this club, had killed four people in the attack.

In agreement with Siracusa who reported that it is still possible to demonstrate blood groups when dealing with blood treated with alcohol and formalin, we were successful in using our procedure on pieces of a collection which had, for the most part, been treated following Kaiserling's method. We intend to follow this line still further by checking the blood group diagnosis of old *corpora delicti* by determining the blood group of the corresponding cadaver sections which have been preserved.

An especially important question in the absorption process is the significance of the test results. We repeatedly saw that even blood corpuscles of group O reduced their titer by a small amount, although usually only one degree of dilution. So too we saw that blood corpuscles of group A and B were able to weaken slightly the other agglutinin, which was confirmed by the experiences of Thomsen and Worsaae.\* Thomsen not infrequently observed a reduction of the other agglutinin by half through the action of the opposite agglutigen, especially in the case of sera of not very high titer.

This relationship likewise struck us. One can easily explain this, as Thomsen and Worsaae indicate, in the following manner. To reduce the agglutinin content by a half in the case of a high-potency (agglutinin-rich) serum, much more agglutigen is necessary than with weak (non-agglutinin-rich) serum. For this reason high-potency serum is certainly to be preferred, serum in which the binding of the opposite agglutinin makes a difference of only a fraction of a degree of dilution. By examining this relationship, Thomsen and Worsaae have found that the "connection of the two agglutinins in the O serum is only an apparent one, that rather the heterologous agglutinin is secondarily bound to the complex of blood corpuscle and homologous antistubstance".

A small reduction in titer, therefore, allows no conclusion. Occasionally, we have not taken notice of a two-dilution step reduction, especially when the other agglutinin was greatly reduced.

If one has enough blood, one can endeavor to clarify the

\*Oluf Thomsen and E. Worsaae, Über die Möglichkeit eines Zusammenhanges zwischen den im Serum der O-Gruppe enthaltenen Isoagglutininen Anti-A ( $\alpha$ ) und Anti-B( $\beta$ ). *Z. Rassenphysiol.* 2, No. 1 (1929).

results in doubtful cases by repeating the experiment with different sera or by allowing the mixture to stand for a longer time.

If the determination of the blood group based on the binding to agglutinin is possible in the case of fresh blood only with a certain error rate, we ought not to be surprised that the error factor is greater with altered blood. Our goal must naturally be to get at the agglutinin in old blood. In a large number of cases, therefore, we have also carried out the Lattes-test along side of the process described here. Unfortunately, as we have already stated, this test was mostly without success.

Hôdoy maintains that faeces binds agglutinin in a similar way as blood does, which would naturally be of great significance. Experiments which we set up to examine this effect, however, have shown no correspondence with the blood; rather they have indicated an irregular and non-specific binding, which constantly changes even in the case of stool samples from one and the same person. Sometimes they have displayed a total disturbance of the agglutinins.

In any case we want to examine this question still further.

Several investigators, Schiff, Lattes and others have suggested a confirmation of the binding test by means of an elution experiment, and Schiff is reporting successes with dried blood. We have had no success with it. This failure seems understandable to us. An elution such as that after the binding by fresh corpuscles is only conceivable, if the agglutinin is fixed for the most part to the reticulum of the blood corpuscles and remains bound to the reticulum even in the case of altered blood. Schütze has observed binding with stroma, and there are also claims made concerning the binding capability of hemoglobin. I myself was able to obtain binding peculiar to a group with hemoglobin, which had been dissolved and freed from the stroma, just as I could with dried blood. I succeeded in doing this also with pure hemoglobin which had been dried and stored for a long time.

In separated serum, however, we always have after absorption hemoglobin which had dissolved. This could explain the failure of the separation experiments. If namely the binding of the agglutinin with dissolved hemoglobin does not take place accompanied by precipitation, then the separation of the agglutinin through the usual separation process is understandably not possible. The question of whether, in the case of the binding of the agglutinin by the agglutininogen, a precipitation takes place, is not easy to answer. First, it is possible to separate out precipitate from apparently pure sera with high-speed centrifuges; on the other hand, it is very difficult, especially when working with small quantities, to obtain blood solutions to produce binding which are completely free of corpuscular constituents. Experiments, which we set up to clarify this problem, argue for precipitation.

The agglutinin which is bound by fresh blood corpuscles, can be completely freed again at a temperature of 45°. When dried blood is added to the serum, however, the hemoglobin goes into the solution. The hemoglobin does not now precipitate out when the agglutinin is bound as do the blood

corpuscles. Even when it is possible by warming to separate the agglutinin from the dissolved hemoglobin, we cannot separate again the dissolved hemoglobin by means of centrifugation as we can the blood corpuscles, and at a temperature at which the agglutinin can be identified by agglutinating of the test blood corpuscles, it would most probably be bound immediately a second time by the hemoglobin present.

One should think of the possibility of separating the hemoglobin at a temperature of 45° by using chemicals and thus again of obtaining the agglutinin.

In summary, we ought to say that the receptors A and B are constant, in any case much more permanent than the agglutinins, and they make possible an identification of the blood groups through binding of the agglutinin even in the case of old, dried blood stains. The process we have used and described above is relatively simple and demands no special preparations. It fails in a very small percentage of cases; we were clearly successful in over 90% of our attempts.

Errors result most easily through false binding or through the failure of binding in which case, again, the receptor B is more likely to go unrecognized than the receptor A. Still in the area of the natural sciences there are scarcely any experiments which do not contain some possibility of error.

In any case the method we have utilized has produced significantly better results than all those processes to date concerning which there have been rather exact reports available.

A principal advantage is that it can still be used with very old samples as well as with blood stains as small as two mg.

Concerning the success in experiments with cadaver sections which have been preserved in the ordinary fashion as exhibit samples, we are in need of a comprehensive overview, though here the process can also be used.

## Literature

- Dold, H. & Rahel Rosenberg, Nachweis von Isopräcipitinen im menschlichen Blut. Nachweis der 4 menschlichen Blutgruppen durch Isopräcipitation. *Klin. Wschr.* 7, No. 9 (1920)
- Goroncy, Curt, Zur Frage der individuellen Blutdiagnose. *Dtsch. Z. gerichtl. Med.* 5, 178 (1925)
- Über die Bedeutung der Temperatur für die Differenzierung der echten und falschen Isoagglutination. *Dtsch. Z. gerichtl. Med.* 6, (1926) Blutgruppenbestimmungen in der gerichtsarztlichen Praxis. *Dtsch. Med. Wschr.* 1929, 306-308
- Haraguti, J., Blutgruppennachweis an Zigarettenstummeln und Zahnstochern. *Bulteno de la Medicina Nagasaki* 1, No. 1
- Higuchi, S., Über den Nachweis der 4 menschlichen Blutgruppen in Blutflecken. *Z. immun. Forschng.* 60, 246 (1928)
- Hirschfeld, I., Über den Einfluss der Temperatur auf die agglutinable Substanz. *Arch. Hyg.* 60 (1907)
- Konstitutionsserologie und Blutgruppenforschung.* Berlin: Julius Springer 1928
- Hodyo, H., Untersuchungen der Blutgruppen durch Menschenkot. *Bulteno de la Medicina Nagasaki* 1 No. 1
- Karsten, Kettel and Oluf Thomsen, Quantitative Untersuchungen über die menschlichen Isoagglutinine Anti-A and Anti-B. *Z. Immun. Forschng.* 65, No. 3-4 (1930)
- Krainskaja-Ignatowa, Über die Gruppeneigenschaften des Spermas. Zur Frage der individuellen Zugehörigkeit von Samenflecken. *Dtsch. Z. gerichtl. Med.* 13, No. 6 (1929)

- Kritschewski, L. and L. A. Schwarzmann, Die gruppenspezifische Differenzierung der menschlichen Organe. *Klin. Wschr.* 6 No. 44 (1927)
- Landsteiner, Karl and Max Richter, Über die Verwertbarkeit individueller Blutdifferenzen für die forensische Praxis. *Z. Med.-beamt.* 16 (1903) *Wien. klin. Wschr.* 1902
- Lattes, Leone, *Die Individualität des Blutes*. Berlin: Julius Springer 1925. Praktische Erfahrungen über Blutgruppenbestimmung an Flecken. *Dtsch. Z. gerichtl. med.* 9 (1927)
- Methoden zur Bestimmung der Individualität des Blutes. In *Aberhaldens Handbuch der biol. Arbeitsmethoden*. 1927 Blutgruppendiagnose von Blutflecken. *Ukrain. Zbl. Blutgruppenforsch.* 2, No. 2 (1928)
- Lauer, Alfred, Zur Kenntnis der erblichen Blutstrukturen. *Dtsch. Z. gerichtl. Med.* 11. (1928)
- Lemzowa, O.M. and A.A. Terchowa, Die gruppenspezifische Differenzierung der menschlichen Organe. *Klin. Wschr.* 1929, 5
- Meixner, Karl, Die Blutgruppen in der gerichtlichen Medizin. *Wien. Klin. Wschr.* 1928, No. 4/5
- Müller, M. A., *Das Agglutininanreicherungsverfahren, ein neues Verfahren zur Blutgruppenbestimmung an altem, eingetrocknetem Blute*
- Müller, M. A. and Leo Brunner, Die Isoagglutination in eingetrocknetem Blute und das Agglutininanreicherungsverfahren von M. A. Müller. Diss. Chur. 1927
- Popoff, N. W. Isoagglutination und ihre forensische Anwendung in Russland. *Dtsch. Z. gerichtl. Med.* 9 (1927)
- Zur Frage der individuellen Blutfleckendiagnose mit Hilfe der Isoagglutination. *Ukrain. Zbl. Blutgruppenforsch.* 3, No. 3 (1929)
- Reinheimer, Kritische Übersicht über den gegenwertigen Stand des individuellen Blutnachweis für forensische Zwecke. *Dtsch. Z. gerichtl. Med.* 6 (1926)
- Sauer, A., Zur Kenntnis der menschlichen Blutgruppen. *Naturwiss.* 1930
- Schiff, Fritz, Die Blutgruppen und ihre Anwendung vor Gericht. *Dtsch. Z. gerichtl. Med.* 9 (1927)
- Die Technik der Blutgruppenuntersuchung*. Berlin: Julius Springer
- Serebrjanoff, P. and M. Leitschick, Die Tauglichkeit des Leichenblutes zur Herstellung und Verwendung isohämagglutinierender Standard-seren. *Dtsch. Z. gerichtl. Med.* 12 (1928)
- Strassman, G., Die Bedeutung der Blutgruppen für die gerichtliche Medizin. *Klin. Wschr.* 1924, No. 48
- Thomsen, Oluf and E. Worsaae, Über die Möglichkeit eines Zusammenhanges zwischen den im Serum der O-Gruppe enthaltenen Isoagglutininen Anti-A ( $\alpha$ ) und Anti-B( $\beta$ ). *Z. Rassenhyg.* 2, No. 1 (1929)
- Thomsen, Oluf, E. Worsaae and V. Friedenreich, Die wahrscheinliche Existenz eines neuen, mit den 3 bekannten Blutgruppen (O, A, B) allelomorphen, A' benannten Gens mit den daraus folgenden 2 neuen Gruppen A' und A'B. *Klin. Wschr.* 11, No. 2 (1930)
- Weidemann, M., Über die Konstanz der Blutgruppen nach den Tode. *Eesti Arst* 7, 275-276 (1928)—*Ber. Dtsch. Z. gerichtl. Med.* 13, No. 2 (1929)
- Yosida, Kan-Iti, Über die gruppenspezifischen Unterschiede der Transsudate, Exsudate, Sekrete und Exkrete, Organextrakte und Orgazellen des Menschen und ihre rechtsmedizinischen Anwendung. *Rechtsmed. Inst. Univ. Nagasaki. Z. exper. Med.* 63 (1928)
- Eine einfache Untersuchungsmethode der Blutgruppen aus nichtbluthaltigen Materialien. *Bulteno de la Medicina Fakultato de Nagasaki Japanlando* 2, No. 1

## Investigations on the Medico-legal Usefulness of the Secretion of Blood Group Substances. (Preliminary Report)\*<sup>1</sup>

Dr. Franz Josef Holzer

Institute for Legal Medicine of the University of Innsbruck  
Director: Prof. Dr. Karl Meixner

/234 At first, after the discovery of blood groups, attention was directed only to the blood corpuscles themselves, but by 1910, experiments were begun concerning group substances in the organism besides those in the red blood corpuscles (von Dungern and Hirschfeld, Halpern).

/235 After conducting experiments with semen and saliva, Yamakami along with Shirai published their results in the *Journal of Immunology* 12, 185 (1926). In the same year in the same journal Landsteiner and Levine reported their experiments with semen, after they had worked with this problem for a long time. While these two authors examined semen samples with group-specific immune sera from rabbits, the Japanese workers were testing sperm and cell-free seminal fluid with isoagglutinins. The experiments showed clearly a group-specific inhibition.

There followed reports concerning blood group substances in organ cells (Kritschewsky and Schwarzmann; Witebsky; Witebsky and Okabe; Yosida Kan-Iti), and in leucocytes and in lymphocytes (Thomsen).

In 1924 Schiff demonstrated the presence of group substances also in cell-free serum using group-specific precipitins. Group substances were identified further in saliva, urine, seminal fluid, stomach fluid, amniotic fluid, milk, tears, and so forth (Yamakami, Yosida Kan-Iti, Brahn and Schiff, Schiff, Thomsen, Putkonen, Hirschfeld, Hamburger, Lehre, *et al.*). Group substances were also determined in vaginal secretions (Shirai, Yamakami).

In 1931 Schiff's doctoral dissertation was published by Fischer in Jena with the title, "Concerning Group-specific Substances of the Human Body." In it, Schiff reported the presence of group substances in organs and body fluids, and, of greatest significance, he tested accurately the A substance by means of the sensitive technique of hemolysis inhibition. In these tests Schiff also confirmed the difference between weak A and strong A, in that extracts from organs in the case of weak A were much weaker than such extracts in the

case of strong A (Schiff and Akune; Schiff in *Zentralbl. Bakter.* 98, 91, (1930)). Continuing these studies Schiff, sometimes together with Sasaki, and sometimes with Akune and Weiler, studied the secretion of group substances further, and also analyzed them more exactly. He speculated that the secretion of serological group characteristics O, A, and B was dependent on a simple, Mendelian pair of factors, S and s, without regard to the blood groups, Schiff and Sasaki supported their supposition with observations of 144 twins and of 68 families consisting of 351 persons. In the same study, the authors also noted that non-secretors were more numerous in group O, and that they also found differences in type in infants. Schiff and Sasaki further determined that secretion was dominant over non-secretion.

While Schiff, in the studies of group O, used mostly anti-O agglutinins from normal cattle sera after absorption with AB blood, E. Eisler reported in several studies on the use of heterologous immune agglutinins, which were obtained from goats by immunizing them with Shiga's bacilli (*Bacillus dysenteriae*). These were much more effective according to the reports, and Schiff confirmed this after testing two samples of such serum. Moreover, nothing needs to be absorbed out before the serum can be used.

These important results were impetus enough to test the usefulness of the sera in medico-legal questions, especially since Schiff had already touched on this area.

### My Own Experiments

Our experiments had a multitude of objectives.

1. Could the secretion or non-secretion of group substances in various persons be observed continuously over a rather long period to make possible a judgment concerning the persistence of this characteristic.

2. Could it be tested whether, in the case of secretors, there exist constant relationships between the amount of group substance secreted in the stomach contents and the time elapsed since the last intake of food.

3. Whether secreted group substances resist decay better than those substances do in the blood, and thus make possible diagnosis of blood group in highly decomposed corpses.

Procedure: In order to satisfy practical demands our effort is directed at the identification of all four blood groups. Therefore, from the outset only the agglutinin inhibition

\* Translation of: "Untersuchungen über die gerichtlich-medizinische Wertbarkeit der Ausscheidung von Blutgruppensubstanzen."

in *Deutsche Zeitschrift für die Gesamte Gerichtliche Medizin* 28: 234-248 (1937).

Reprinted with the kind permission of Springer-Verlag, Heidelberg and New York.

procedure could be considered. Despite their higher sensitivity, the complement fixation reaction and the hemolysis inhibition procedure had to be abandoned, since only the A characteristic could be identified in those ways. (Schiff, Hirszfeld).

To meet the requirements of legal medicine, our type of examination must be adapted to testing small quantities and cannot be too difficult to execute. After many attempts, the following procedure has proven itself practical and generally useful.

With a capillary pipette the fluid to be tested is progressively diluted 1:2:4:8:16 on glass plates with concave depressions, such as those we use for serum evaluation. The last well contains a drop of pure, physiological saline solution, equal in volume to the other solutions, to serve as a control. Then, a drop of test serum (agglutinin) is added to each well and mixed thoroughly (beginning at the left and proceeding to the higher dilutions). Finally, the drop of saline solution is mixed thoroughly with a drop of serum. After five minutes, one drop of a 3% blood-corpuscle suspension of the same size is added to each well, and all the samples are again stirred thoroughly in the same fashion.

/237

In numerous comparative experiments, it has turned out that it normally makes little difference whether the addition of the test blood corpuscles takes place immediately after the adding of the serum. One gains some time by this method and also avoids any drying. (The experiment certainly is somewhat more sensitive if one places the dilutions in tubes rather than on plates, and if one allows the mixture of liquid and serum to stand before the addition of the blood corpuscles).

A reading of the test using plates was taken after ten minutes. An observation was made whether, and to what degree of dilution, agglutination failed to take place, i.e. was inhibited. The agglutination in the saline control solution served as a standard of comparison. As testing sera, A, B or even O sera can be used; in the testing of the O substance anti-O sera can also be used, confirming the hypothesis of Schiff, Sasaki, and Eisler. The selection of the sera is not without its effect on the amount of inhibition. If the sera are of very high potency and contain a great quantity of agglutinins, these agglutinins are sometimes not removed in the first dilutions of the material to be tested to a sufficient extent to cause an inhibition effect in the agglutinating of the test blood corpuscles. In the case of weak sera, on the other hand, there is the danger of non-specific inhibition (compare Schiff, *Die gruppenspezifischen Substanzen des menschlichen Körpers*, Jena, 1931). One ought, therefore, to choose sera of a middle strength.

When testing native saliva, its viscous quality makes mixing to homogeneity difficult (often this disturbance is still noticeable in a dilution of 16). In addition the formation of streaks simulates a false agglutination or masks real agglutination. I have, therefore, gone over to the idea that saliva should be heated before carrying out the tests ( $\frac{1}{2}$  hour in boiling hot water) to make it thin enough to flow. One can

do this without any risk, since the group substances resist heat and, as Schiff has shown, the saliva can be kept at a temperature of 126° for two hours or at 150° for one hour without damaging its inhibition effect. The heating of the saliva has still another special purpose which we must mention here. As Schiff and Weiler (*Biochem. Zeitschrift*, 225, 454, 1931) discovered, feces and at times also saliva, have the peculiar property of destroying blood-group substances. By heating for five minutes at 100° this disintegration effect is hindered and the agent destroyed (Schiff and Akune, *Munch. med. Wschr.*, 78, 657, 1931). Recently a repeated attempt was made to characterize more closely the so-called blood-group ferment. Witebsky and later Sievers succeeded in "culturing" the effective principle. While Sievers was unsuccessful in isolating or enriching the bacteria with the enzymatic effect, Schiff reported (*Klin. Wschr.* 1935, 750) that he discovered several strains of gangrene bacilli which were able to destroy the A as well as the B substances in saliva. On these grounds the heating of the saliva is completely justified. In dealing with strongly acidic gastric juice, it has also been recommended that it be neutralized beforehand. One can combat injurious influences due to hypotonicity by means of a corresponding addition of saline solution.

23

Now a few words about anti-O sera. Anti-O sera can be produced by immunizing rabbits with human blood of group O. Although producing such sera is not easy, it has been constantly successful (Landsteiner, Wiener, Schiff). By immunizing with Shiga's bacilli, Eisler has also obtained such sera which have the advantage of not needing to be cleaned up before they are used, and which, in addition, are very effective. Anti-O agglutinins can be more easily extracted from certain cattle sera as Schiff and Sasaki have recommended. By absorption with A<sub>1</sub>B blood corpuscles, the cattle sera lose the agglutinin directed against the foreign type (human blood corpuscles in general), as well as any anti-A or anti-B present. After this treatment, however, suitable cattle sera still possess an agglutinin against O and A<sub>2</sub> blood corpuscles which is, to be sure, often only a weak agglutinin. Such sera are used in the same way as anti-A and anti-B. Among the cattle sera obtained from the slaughter house many are found to be usable, in complete agreement with Schiff's claims.

Experiments to produce an inhibition of anti-M and anti-N sera with saliva or gastric juice have been negative. The antiserum removed for testing was in no way influenced, which also agrees fully with most of the reports to date on the absence of M and N in organs and body fluids.<sup>2</sup>

Our experiments are organized in the following way:

1. Tests concerning secretion in living persons.
2. Tests in corpses.

Saliva was first examined for secretion, then urine, and in some cases seminal fluid, and less often, siphoned gastric juice.

Saliva of different persons was tested, as well as from persons of the same families and from mother-child combi-

nations. Obtaining saliva from adults creates no problems. In order to avoid as much as possible any admixture of blood or epithelial cells, we ask the people to collect the saliva, with their mouths slightly open, without sucking, onto a folded piece of waxed paper and put it into a tube.

/239

In the case of small children and babies, obtaining saliva samples was at first considerably more difficult. After some attempts I tried a simple suction device (Figure 1) with which it was possible to collect an ample quantity of saliva without the least difficulty even in the case of new borns.<sup>†</sup> One puts one tube in the child's mouth and begins to suck lightly on the other tube. The children are immediately pacified, when the small tube is put into their mouths. They stop crying and begin to suck, whereby the saliva secretion is stimulated. The saliva collects in a test tube which is inserted between the baby's tube and that of the researcher. The saliva can be immediately heated in this tube and used for the experiment without being transferred.

As experiments have shown, a small admixture of blood

has no great importance in terms of disturbances (this is true for saliva and for stomach contents.<sup>3</sup>). This was also confirmed in the case of two corpses which had swallowed quantities of blood and even breathed some in. This caused only a comparatively small disturbance.

**Saliva Experiments**

Of 116 persons whose saliva was tested according to the method presented here, 97 were found to be secretors and nineteen to be non-secretors. Table 1 provides a summary. As Table 1 demonstrates, most of the saliva samples were still effective even at a dilution of 1:128 to 1:256. These few tests, as well as the repetition of the test on saliva samples from one and the same person, show that, as a rule, it is possible to distinguish between S and s in the first experiment. It also shows, however, that there are cases in which the weakness of the inhibition effect renders it doubtful whether one is still dealing with a secretor or whether the slight inhibition is due only to admixtures of cells.

240/

We, therefore, took saliva samples for examination from seventeen persons belonging to different groups, repeating the test in some cases as many as sixty times on different days over a lengthy period of time. A summary of these experiments is given in Table 2.

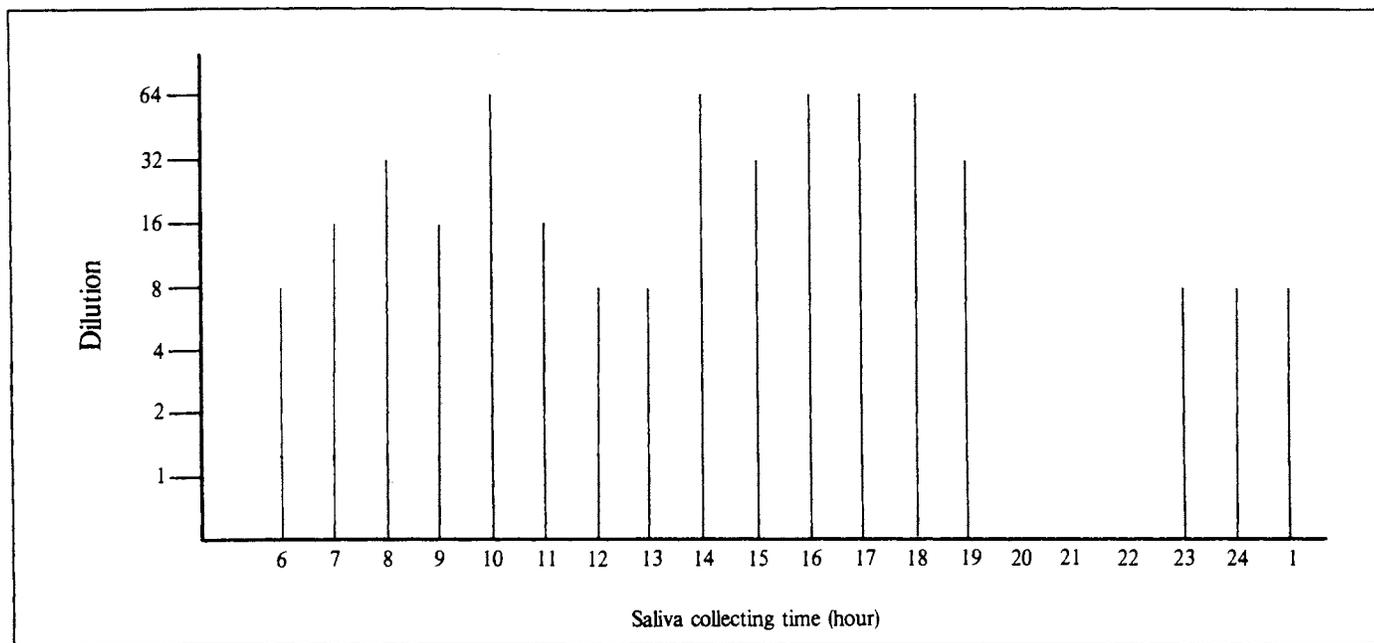
Table 1. Summary of the saliva tests

Blood Group	Secretors S	Dilution														Non-Secretors s
		1	2	4	8	16	32	64	128	256	512	1024	2000	4000	8000	
O	6	-	-	-	2	1	1	-	1	-	-	-	-	-	-	3
A	74	-	2	-	6	9	7	13	15	8	8	4	2	-	-	12
B	12	-	-	-	-	1	1	-	4	4	-	1	1	-	-	1
AB	5	-	-	-	-	2	-	1	-	2	-	-	-	-	-	3

Table 2. Summary of the Repeated Saliva Tests

Blood Group	Secretor S	Dilution															Non-Secretor s
		1	2	4	8	16	32	64	128	256	512	1024	2000	4000	8000		
V.S.	O	16	2	3	-	1	2	1	3	2	2	-	-	-	-	-	1
M.S.	A	35	1	1	2	16	2	3	9	1	-	-	-	-	-	-	-
K.T.	A	26	2	1	4	9	8	2	-	-	-	-	-	-	-	-	-
K.L.	A	23	-	1	1	4	2	5	6	3	1	-	-	-	-	-	3
K.V.	A <sub>2</sub>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	34
Ho.	A <sub>2</sub>	54	4	1	3	12	13	8	6	6	1	-	-	-	-	-	6
Sche.	A <sub>1</sub>	15	1	1	-	1	-	2	1	3	4	-	2	-	-	-	-
Ki.	A <sub>1</sub>	23	-	1	-	1	1	3	5	4	4	4	-	-	-	-	-
Me.	B	19	-	-	2	2	1	1	2	3	3	3	1	-	1	-	-
Fri. E.	A <sub>1</sub> B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	27
Lä.	AB	7	-	-	-	-	-	1	-	2	2	-	1	-	-	-	-
K. Ge.	A <sub>1</sub>	6	-	-	-	-	1	-	-	-	1	-	1	1	1	1	-
Pan.	O	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
V. Bi.	A <sub>2</sub>	5	-	-	-	1	2	1	-	1	-	-	-	-	-	-	-
M. Bi.	A <sub>2</sub> B	5	-	1	-	-	-	3	-	1	-	-	-	-	-	-	-
K. Bi.	B	5	2	-	-	-	-	-	-	2	-	1	-	-	-	-	-
Mö.	O	2	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-

Figure 2.



It is interesting to notice that in this series there are negative samples even among the secretors. On the other hand, it turns out that, in the case of non-secretors, inhibition never takes place despite many repetitions of the test (up to 34) and hourly extractions of saliva. In the case of a secretor from whom saliva was extracted every hour for twenty-four hours, three successive samples were negative, but thereafter, fairly abrupt and clear inhibition effects could be observed (compare Fig. 2). Although the experiments are still small in number, and we must still test further to what extent group enzymes play a role here, the question already can be posed whether there exists another group of humans between the secretors and the non-secretors, a group whose members sometimes secrete and sometimes do not.<sup>4</sup>

Moreover, we have the impression that the intake of food, and the time elapsed since the intake, has some sort of influence, but we do not understand the effect from our quantitative experiments to date. More experiments are required on the relationship of different physiological and pathological conditions to the test results.<sup>5</sup>

Perhaps it would be possible with more sensitive methods to identify inhibition in some samples which were evaluated as negative with the procedure described here.

As we said above, in judging the variations in the content of group substance in saliva, one should also think of a disturbance caused by blood group enzymes, such as those which appear in the intestine. In order to prevent this as much as possible from the outset, it would be expedient to heat the saliva immediately after its extraction.

Our experiments with families agree totally with Schiff and Sasaki, in that secretion was dominant over non-secretion. The designation "Secretion-type" S was very appropriately introduced by Schiff.

In view of the reported findings, we must urgently warn

against the premature use of this fact in paternity cases. A setback due to hastiness could lead to a serious decrease of confidence on the part of the courts in the classical blood groups and in the M and N factors. In 1928 Cuboni says in his summary that secretion was not consistent and that its systematic use in legal medicine seemed to be improbable.

Meanwhile, false determinations could be greatly reduced by means of repeated experiments. Thus, if the dominance of S could be further confirmed, the secretion characteristic could serve to corroborate the improbability of descent demonstrated in other ways. Still this method of testing should not be considered of too great an importance in paternity cases, since the secretion type S predominates to a great extent over the non-secretor so that the possibility of an exclusion is reduced.

The use of secretion of group substances to diagnose the group of human secretions, such as urine, saliva, vomit, semen, from wet or dry stains, is not disputed. Repeatedly, different authors (Schiff, Lattes and others) have confirmed that it is useful in these cases. We have even been able to use successfully the test of saliva stains in legal cases.

In conducting tests with seminal fluid, parallel to the saliva tests, we showed that group substances in fact appear in seminal fluid even where there is a scarcity of spermatozoa. It seems though, that these substances are inferior to those in saliva with regard to the inhibition effect. Group substance was also identified in urine, agreeing completely with what is presently known of secretors, although considerably less than in the respective saliva. In the course of these experiments, A saliva and B saliva were repeatedly tested with anti-O liquid. It was shown in these tests that some A and some B saliva produced a clear inhibition effect, but that AB saliva never does, whether it comes from secretors or non-secretors.

Table 3 (Feb. 29, 1936) presents examples of such reactions in cases of saliva samples of all the groups, and it shows that, in the case of A persons and B persons, a secretion of O never is found without a secretion of A and B.<sup>6</sup> This suggests that the secretion of O together with A and B depends on the presence of an O gene. While inhibition with Anti-O sera could, in the case of A-secretors, be conditioned also by an A<sub>2</sub>, this is not so in the case of B secretors. Possibly, the way is opened here to distinguish homozygous and heterozygous A and B, a distinction which would be of great practical meaning in regard to questions of descent. Now it is well known that A and B blood corpuscles are able also to absorb anti-O sera. According to Schiff (*Zeitschrift Immun.forsch.* **82**, 302, 1934) in experiments with Shiga

bacillus immune serum from goats the A and B secretors can almost always be distinguished from the non-secretors by the inhibition of O-agglutination. The inhibition, however, was often noticeably less than it was in the case of saliva of the O-group. Schiff, therefore, thought that one must ascribe a certain amount of the so-called O factor to the blood corpuscles A<sub>1</sub> (as well as to the other groups). Morzycki (*Zeitschrift Immun.forsch.* **84**, 80, 1935) assumes that the anti-O sera react with such elements which are present in varying amounts in all individuals. Thus, he and Hirszfeld conceive of the so-called O-receptors, first of all, as species receptors and assume that they are present in some individuals only in very small quantities or that they are lacking completely.

Table 3

No.	Boiled Saliva	Group	Anti-	Dilution														NaCl control		
				1	2	3	4	5	6	7	8	9	10	11	12	13	14		15	16
1	V. Sch. Feb. 22, 1936 ...	O	A	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	
			B	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
			O	-	-	-	-	-	-	-	-	++	++	++	++	++	++	++	++	++
2	M. Feb. 22, 1936 .....	A	A	±	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++	
			B	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
			O	-	-	±	±	++	++	++	++	++	++	++	++	++	++	++	++	++
3	K. T. 2/22/36.....	A	A	-	-	±	±	++	++	++	++	++	++	++	++	++	++	++	++	
			B	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
			O	-	-	-	-	±	++	++	++	++	++	++	++	++	++	++	++	++
4	K. I. 2/22/36 .....	A	A	-	-	-	±	+	++	++	++	++	++	++	++	++	++	++	++	
			B	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
			O	-	-	-	-	±	±	++	++	++	++	++	++	++	++	++	++	++
5	K. V. 2/22/36.....	A	A	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	
			B	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
			O	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
6	Kirch .....	A	A	-	-	-	-	-	-	-	±	±	+	++	++	++	++	++	++	
			B	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
			O	-	-	-	-	++	++	++	++	++	++	++	++	++	++	++	++	++
7	Merkl .....	B	A	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	
			B	±	+	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++
			O	-	±	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
8	M.H.....	B	A	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	
			B	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
			O	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
9	K. H. El. ....	AB	A	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	
			B	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
			O	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
10	Lä.....	AB	A	-	-	-	-	±	±	+	++	++	++	++	++	++	++	++	++	
			B	-	-	-	±	+	++	++	++	++	++	++	++	++	++	++	++	
			O	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
11	Ho.....	A	A	-	-	-	-	±	++	++	++	++	++	++	++	++	++	++	++	
			B	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
			O	-	-	±	±	++	++	++	++	++	++	++	++	++	++	++	++	++

O-Serum, 2/13/36: Anti-A 4(8); Anti-B 4(8)  
Anti-O Serum, 2/5/36: Anti-O 2(4)

If, after all, one may express himself only with the greatest reserve and ought not to have too great hopes, it still seems desirable to carry out further tests in the direction which has been taken.

After our experiments concerning the secretion of group substances in the stomach fluid had completely confirmed the reports of earlier authors (Schiff and Akune, Hirszfeld), the experiments which followed were arranged, proceeding from the obvious question regarding the constant relationship between the time elapsed since the last food intake and the amount of secreted group substances in the contents of the stomach.

Shortly after a monitored breakfast, gastric juice samples, taken with a stomach pump, were examined in one experiment together with saliva, which served as a control. But to this day I have only a modest number of these experiment results. In this experiment the inhibition was also small in the case of the secretors, a result that may have been connected with the short duration of the reaction (the stomach was pumped after twenty minutes). Further experiments, and especially self-examinations with the stomach tube, should increase the number of our observations. With one exception, saliva was more effective than gastric juice in the cases tested so far.

A second series of tests was undertaken with twenty-six corpses. Among these were two certain non-secretors and one questionable secretor. The secretors were in a very clear majority here. The stomach contents of these corpses was tested, and, where possible, also the saliva, serum, urine, bile, and the walls of the duodenum, small intestines, and the colon. In order to see how much group substance was contained in the stomach and intestinal walls, the pieces were extracted with a saline solution. The process was uniformly the following: After washing, a piece almost 1 sq. cm. is stamped out (the method of stamping is very easy while at the same time being sufficiently exact). The sections are then placed in small test tubes and mixed with  $\frac{1}{2}$  cc of physiological saline solution. After being shaken they are allowed to stand for a rather long time, usually overnight, but sometimes throughout the day, and are shaken several times during this period. Then the tubes are centrifuged. The agglutinin-inhibition reaction is carried out in the ordinary manner with the supernatant fluid. Usually, it turns out that, despite the rather great dilution produced by adding the saline solution, the extracts still cause a rather strong inhibition effect, often up to a dilution of 1:16. It also turns out that large amounts of group substances are contained in the stomach wall (mucous membrane). It appears, moreover, that the place from which one chooses to take a sample of the stomach membrane is relatively unimportant. While the effective action of the extract from the stomach wall ceased between dilutions of 8 and 32, the stomach contents frequently caused a clear inhibition effect in a dilution of 2000.

There are, thus, considerable differences between the inhibition range of the stomach contents and that of the extracts from the stomach lining. Just as group substances

could be leached from individual sections of the lining, so the stomach of the corpse produced such substances in the liquid with which it was filled. At post-mortem examinations, the stomach contents were collected from the excised stomach. The stomach was then thoroughly rinsed and filled with physiological saline solution corresponding to the amount of stomach contents extracted. Then, at various intervals fluid was extracted through a test puncture. From the test, it turned out that a very considerable quantity of group substance was in fact transferred to the saline solution, and in a short time too. For example, the fluid extracted after six hours had an inhibition effect even in a dilution of 1:16 to 1:32. After 40 hours this had certainly increased somewhat, and after 70 hours an inhibition effect could be observed in dilutions of 1:64 to 1:128. In natural stomach contents which have been left in the stomach, concentrations of group substances grow somewhat stronger from the time of the post-mortem examination, as samples taken by puncture have demonstrated. Experiments in this direction are still continuing. The leaching experiments after death show that, indeed, group substances are still present in large quantities but that the fluid never again reaches the degree of saturation of the original stomach fluid. More experiments on persons who have died shortly after filling their stomachs are now being considered.

In most of our cases we also observed the behavior of the bile. Group substances were also secreted in the bile in rather considerable quantities, though as a rule less than in the stomach mucous membrane. As a control, in testing whether a person is a secretor, the bile is quite important since in dealing with corpses it can be difficult to extract saliva. The stringy quality and viscosity of the bile, however, sometimes create quite a considerable disturbance in the agglutination inhibition, as they do in hemolysis. Here one must, of course, keep hemolysis and agglutination-inhibition separate. Moreover, in the case of bile, nonspecific inhibition appears to take place more easily. With regard to serum and urine, our experiments with corpses have proven of very little value. Even in the case of secretors, one can often fail to recognize them from the urine of the corpse. Also, the pericardial fluid, which we tested at the same time, produced only a weak inhibition. Besides the stomach lining we also tested the duodenal, small intestinal, and the colon walls. Our records here confirm the decrease of demonstrable group substances toward the end of the digestive tract. A noticeable decrease can usually be noticed in the small intestine as well. The colon and rectum were usually found to be free of group substances. There exists the possibility that the secreted substances were reabsorbed. It has been proven, nevertheless, that the intestinal contents destroy group substances by means of an agent whose effective principle, as we already mentioned, is destroyed by cooking (Schiff and Weiler). It is an enzyme which is also active in germ-free filtrates (Schiff and Akune, and cf: Witebsky and Satoh). The finding of a large quantity of inhibiting substance in both the small and large intestines of a newborn, 40 cm long, macerated fetus is

in agreement with the thinking of Witebsky and Satoh (*loc. cit.*) that this inhibition enzyme is first formed in the first months of life. Group substances are already secreted in embryonic life (Schiff, Witebsky and Satoh). Even the secretion of O could be established with our tests on these corpses. As in the experiments with saliva, our observations regarding gastric juice, bile, semen and so forth, argued for a specific inhibition with respect to anti-O serum. We established this with secretors of A and B as well.

The secretion of group substances in the stomach can be used with advantage to secure a positive group diagnosis in the case of badly decomposed corpses.

Let the following case serve as an example. In a highly decomposed corpse of an old man, which had lain in water for more than forty days, the blood had completely hemolysed; the blood corpuscles had completely vanished. The Landsteiner-Lattes test with A blood cells caused an uncertain clumping. The diagnosis with the absorption method produced an incontestable absorption of anti-B. At the same time, to serve as a control, bile, stomach contents, the stomach lining, and urine were tested following the agglutinin-inhibition procedure. In total agreement with the blood test, a definite, specific inhibition reaction, caused by the B substance, was demonstrated in these tests. In the gastric juice the inhibition effect continued up to a dilution of 1:4000.

In view of these results, we think that in all similar cases such testing is valuable as a complement. Obviously, we can evaluate only a positive result, i.e., a clear inhibition, since, with a negative result, we might in fact be dealing with a non-secretor. We should also mention in reference to these cases, that, when possible, throat mucus and saliva should be collected and preserved.

### Summary

In order to test secretion of group substances for medico-legal purposes, different experiments were set up on the agglutinin inhibition procedure.

The tests confirm the findings to date, indicating that the possible to distinguish secretors from non-secretors but that, occasionally, a secretor does not secrete or secretes so little that it is not detected by the test.

The test confirm the findings to date, indicating that the secretion type is a dominant hereditary trait. For use in paternity cases, however, the secretion type can, for the time being, only be employed with the greatest reservations and only after repeated testing.

Experiments on the relationship between the amount of secreted group substance in the stomach and the time elapsed since the last intake of food, in this case the time of death, did not produce any noteworthy results.

The secretion of group substances can serve to corroborate the group diagnosis in highly decomposed corpses.

The inhibition of anti-O serum by saliva from secretors of groups A and B may indicate a way of recognizing the presence of an O gene and thus, of distinguishing homozygous and heterozygous A and B carriers.

### Footnotes

1. Delivered in abstract form at the Conference of Physicians and Researchers in the Natural Sciences in Dresden, September 1936.
2. Schiff was able to produce an anti-M agglutinin by immunizing rabbits with human saliva. He, therefore, concluded that the M characteristic appears as a true antigen in the saliva (Schiff, F., *Über die gruppenspezifischen Substanzen des menschlichen Körpers*, Jena, 1931, p. 42).
3. Compare also the experiments of Matson and Brady (*J. of Immun.* 30, 444, 1936)
4. Almost the same as Hirszfeld's method, according to which he divides the organs into "absolute," "facultative," and "negative" group carriers.
5. In Thomsen's laboratory Fog-Möller (*Z. Immun.forsch.* 84, 359, 1935) tested the secretion of group substances in cases of disease, namely pernicious anemia, and they found here no deviation from the norm.
6. Tests with gastric juice produced similar results (see below).

### Literature

- Brahn, B. and F. Schiff. *Klin. Wschr.*, 1929, 1523 and *Zbl. Bakter. I. Orig.* 95, 43 (1929)
- Cuboni, E. *Boll. Ist Sieroter. Milan* 1, 1928
- v. Dungern and Hirszfeld. *Z. Immun.forsch.* 8, 554 (1910)
- Eisler, E. *Z. Immun.forsch.* 84, 359 (1935)
- Halpern, L. O. *Z. Immun.forsch.* 11, 609 (1911)
- Hamburger, Chr. *Z. Rassenphysiol.* 3, 67 (1930)
- Hirszfeld, L. *Konstitutionsserologie und Blutgruppenforschung*. Berlin: Julius Springer, 1928
- Hirszfeld, L., W. Halber and Z. Laskowsky. *Z. Immun.forsch.* 64, 61 (1929)
- Kritschewsky and Schwarzmann. *Klin. Wschr.*, 1927, 2081 and 1928, 896
- Landsteiner, K. and Ph. Levine. *J. Immunol.* 12, 415 (1926)
- Lattes, L. *The Individuality of Blood*. Oxford, 1932
- Lehrs. Inaug. Diss. Berlin, 1929. Cited by Schiff in *Z. Immun.forsch.* 66, 175 (1930)
- Matson and Brady. *J. of Immun.* 30, 444 (1936)
- Matson, J. *of Immun.* 30, 459 (1936)
- Morzycki, J. *Z. Immun.forsch.* 84, 80 (1935)
- v. Oettingen and Witebsky. *Muench. Med. Wschr.*, 1928, 385
- Putkonen, T. *Acta Path. scand.* (Kobenh.) 64 (1930) and *Acta Soc. Medic. fenn. Duodecim.* 14, 107 (1931)
- Sesaki. *Z. Immun.forsch.* 77, 101 (1932)
- Schiff, F. *Klin. Wschr.* 1924, 679; and 1927, 303; and 1935, 750; and *Zbl. Bakteriol. I. Orig.* 98, 91 (1931); and *Die gruppenspezifischen Substanzen des menschlichen Körpers*. Jena, 1931; and *Z. Immun.forsch.* 82, 46 and 302 (1934)
- Schiff, F. and Akune. *Muench. Med. Wschr.*, 1931, 657
- Schiff and Sesaki. *Klin. Wschr.* 1932, No. 34 and *Z. Immun.forsch.* 77, 129 (1932)
- Schiff, F. and G. Weiler. *Biochem. Z.* 235, 454 and 239, 489 (1931)
- Shirai, S. cited by Yamakami, K. in *J. of Immunol.* 12, 185 (1926)
- Sievers, O. *Z. Immun.forsch.* 85, 163 and 86, 130 (1935)
- Thomsen, O. *Acta path. scand.* (Kobenh.) 7, 250 (1930) and *C.R. Soc. Biol., Paris*, 104, 499 (1930)
- Witebsky, E. *Z. Immun.forsch.* 52, 359 (1927)
- Witebsky, E. and Satoh. *Klin. Wschr.* 1933, No. 24
- Yamakami, K. *J. of Immun.* 12, 185 (1926)
- Yosida-Kan-Iti. *Bult. jurmed. Inst. Nagasaki* (esper) 2, No. 1 and *Z. exper. Med.* 1928, 331

## The Current Status of Blood Group Serology and Its Forensic Importance\*†

F. J. Holzer

Institute for Forensic Medicine of the University of Innsbruck  
(Director: Prof. Dr. F. J. Holzer)

### Classical Blood Groups

416 In the first fifty years since the discovery of blood groups by Karl Landsteiner<sup>1</sup> understanding of and experience with the four classical blood groups has become so well established that their identification now rarely causes difficulties in clinical and forensic medicine.

In the last few years, however, researchers have written much about the subgroups and widened our knowledge of these.

### Subgroups of A

On the technique of differentiating strong from weak A, a series of articles has appeared, written by Thomsen,<sup>2,3</sup> Thomsen, Friedenreich, and Worsaae,<sup>4</sup> Lauer,<sup>5</sup> Lehmann-Facius,<sup>6</sup> Ottensooser and Zurukzoglu,<sup>7</sup> Holzer,<sup>8</sup> Krieger,<sup>9</sup> Wolff and Jonsson,<sup>10</sup> Blinov,<sup>11</sup> Ponsold<sup>12</sup> and others.

Among A types a weaker A<sub>2</sub> was distinguished. Dahr<sup>13,14</sup> tested a seven-month old child with a very slight A-agglutinability and considered a division of the clearly-defined cases of weak A into A<sub>3</sub>, A<sub>4</sub>, and A<sub>5</sub> as still premature; Gammelgaard and Marcussen<sup>15</sup> on the other hand, thought that they had a sound basis for proposing the existence of the additional subgroups A<sub>4</sub>, A<sub>5</sub>, and so forth, because of the presence of clear, quantitative differences and distinct hereditary transmission.

A question which is still not completely clarified is that touched on by Friedenreich<sup>16,17</sup> in 1931, namely that concerning the conversions between the blood groups A<sub>1</sub> and A<sub>2</sub>.

Dahr<sup>18</sup> also admitted the existence of intermediate A forms. Witebsky<sup>19</sup> even claims that a new division (reclassification) into A<sub>1</sub> and A<sub>2</sub> is necessary. Of 100 samples 75 could be identified as A<sub>1</sub>, fifteen as intermediate A, and ten as A<sub>2</sub>. The intermediate group was almost totally separated from the A<sub>2</sub> group.

\* Translation of: "Der gegenwärtige Stand der Blutgruppenserologie und deren forensische Bedeutung."

in *Deutsche Zeitschrift für die Gesamte Gerichtliche Medizin* 42: 416-437 (1953).

Reprinted with the kind permission of Springer Verlag, Heidelberg and New York.

† Delivered in excerpts at the Meeting of the German Society for Forensic Medicine in Munich, Sept. 9, 1952.

Since the communication of Laguna<sup>20</sup> and the case of 41: Haselhorst and Lauer,<sup>21</sup> special attention must be given to the weak A on the part of researchers, particularly the investigators in paternity suits.

Of interest in this connection is Boltz's<sup>22</sup> observation at the Vienna Forensic Medical Institute of a phenotypic latency and late manifestation of blood group A<sub>2</sub>B in the case of a young blood donor, who was at first determined to be a B with a weak  $\alpha$ , and only two years later was diagnosed as an A<sub>2</sub>B with an irregular  $\alpha_1$ . This girl has donated blood fifty-one times, sometimes as an AB, without any complications.

Such observations make comprehensible a certain reluctance in the evaluation of A<sub>1</sub>/A<sub>2</sub> exclusions, a reluctance which one sees in the literature, for example, in the work of Andresen<sup>23</sup> and others.

For subgroups of A, Dahr does not assign the high-probability percentage of 99.8%, i.e., the obviously impossible. The conclusion in assessing exclusions based on the subgroups ought, therefore, not to imply that the subgroup excluded is absolutely impossible, but rather that it is highly unlikely.

The American authors Davidsohn, Levine, and Wiener,<sup>24</sup> write in a recent report concerning the forensic use of blood tests that the subgroups of A, though theoretically of use in cases of disputed paternity, cause problems in practice in distinguishing the subgroups, especially with newborns. Thus, the Committee of the American Medical Association for Forensic Medical Problems claims that tests based on the subgroups of A are not yet to be trusted for forensic medical use.

In contrast to this caution on the part of the American authors is the positive evaluation of the demonstrated capabilities of such tests, expressed by other researchers.

Formaggio (personal communication) found no exceptions.

Wichmann,<sup>25</sup> Mayser,<sup>26</sup> and especially Ponsold<sup>27</sup> emphasize both the reliability and the value as evidence of the hereditary aspect of the subgroups, even in exclusions for paternity cases where the interpretation "clearly impossible" can be made.

Ponsold is a man who has thoroughly dedicated his efforts to the A<sub>1</sub>A<sub>2</sub> problem, especially by using the capillary

method and the absorption identification by means of "exhaustion." Ponsold<sup>28</sup> has shown with the aid of an extremely instructive case, clearly identical with one described by Bohmer and Greiner<sup>29</sup> in 1951, that the scientific question of the value of an elimination based on A subgroups is affected not only by the state of the experiment, but just as much by the selection of the expert and the test which he employs.

### Subgroups of B

/418 In the case of the blood characteristic B there are scarcely any difficulties or mistakes.

P. Moureau reported at the Congress for Blood Transfusion in Paris concerning the appearance of a weak B. Although quantitative differences among the various subgroups of B appear (Mudguti,<sup>30</sup> Honde<sup>31</sup>), they are not so pronounced as between A<sub>1</sub> and A<sub>2</sub>, as Formaggio,<sup>32</sup> a man from the Lattes school, asserted in a recent article.

Basing their arguments principally on the results collected by Matta,<sup>34</sup> Schiff and Boyd<sup>33</sup> believe that the forensic medical use of the subgroups of B still lacks sufficient foundation.

Various authors (Killer,<sup>35</sup> Schiff,<sup>36</sup> Zitzman,<sup>37</sup> Pietrusky,<sup>38</sup> Manz,<sup>39</sup> and Jungmichel<sup>40,41</sup>) have indicated the possibility of indirect exclusions in the case of deceased subjects, and of additional indirect exclusions with living subjects.

In 1943 at the Robert Koch Institute in Berlin, Werner Fischer<sup>42</sup> thoroughly treated the different possibilities in a very careful study.

Fischer considered that the chances were good for successful exclusions when the parties in the dispute were dead. Fischer's statements justify a greater use of indirect blood-group diagnosis in judicial investigations.

The indirect method of determining the heterozygote hereditary type is all the more important since, despite our many-sided efforts, we still do not possess a trustworthy serological method to recognize recessive O in the case of heterozygotes.

In 1938 Dahr<sup>14</sup> set up valuable experiments in this direction; some were confirmed, and some were refuted.

According to Dahr<sup>14</sup> the identification of the heterozygosity of A and B blood is fundamentally possible since the inherited O trait is not completely repressed by the A and B trait inherited at the same time. Boorman, Dodd, and Gilbey<sup>43</sup> speak of a co-dominance of the blood-group gene O with A and B.

It is questionable whether the idea which I expressed in 1937,<sup>44</sup> namely the possibility of recognizing heterozygosity by testing saliva from A and B persons with anti-O agglutinin, can, indeed, be realized. Dahr's co-workers, Manz and Altenberg, recently tried this method.<sup>14</sup> On the basis of his experiments Formaggio<sup>45</sup> considered it to have no prospects, since he observed a secretion of O substance both in A<sub>1</sub>A<sub>1</sub> and A<sub>1</sub>B individuals.

### Secretion in the ABO System

/419 In his latest experiments concerning the secretion of

blood-group substances in 2000 persons tested in Pavia, Formaggio<sup>45</sup> found a statistically good correlation in the division of secretors from non-secretors, which we found and described in the literature.

In the group AB, he found that sometimes A and B were secreted, sometimes only A or only B, and sometimes absolutely no substance.

Consequently, to test for S or s it would not be enough in the case of AB to test for A or B in saliva, although until recently Dahr<sup>14</sup> thought that it was.

Relationships were established between the amount of saliva and the quantity of group substance. If a great quantity of saliva is secreted, the group substance in this saliva is diluted; if the saliva increases rapidly in amount, the secreted group substance can decrease almost to zero, so that such a person could appear as a non-secretor in only a single test.

In complete agreement with Wiener and Kosofsky,<sup>47</sup> Formaggio<sup>46</sup> found no difference between A<sub>1</sub> and A<sub>2</sub> with respect to secretion.

While Wiener advised caution in using secretion in the blood group O because the sera against O are difficult to obtain, Formaggio found the reactions with good anti-Shiga bacilli serum to be trustworthy and clear, even for paternity questions. He presented his conclusions with the aid of the table drawn up from his experiments.

Formaggio<sup>46</sup> has indicated the important potential use of secretion to recognize the especially weak A (namely in the so-called "defective" O).

Even when the A characteristic is so weak that it cannot be demonstrated in the blood corpuscles either by agglutination or by absorption, the testing of the saliva from such secretors showed that A substance was clearly secreted. Thus, especially weak A may still be identified.

### M and N

Iso-antibodies against M and N are uncommonly rare. According to Wiener<sup>48</sup> only seven cases with anti-M in normal human serum were described up to 1946.

In May of this year Wiener<sup>49</sup> described the seventh and eighth cases of a natural anti-M in the sera of male Negro twins, both belonging to the blood group BN<sub>ss</sub> Rh<sub>0</sub>.

Numerous studies concerning the technique of MN identification have appeared.

While Schiff<sup>50</sup> recommended the absorption procedure in doubtful cases, Wiener<sup>48</sup> (p. 226) believes it is better to test with more serum and to repeat the experiment. Moreover, Boltz<sup>51</sup> recently pointed out that the absorption experiment is, in general, less reliable than the agglutination test. One must characterize the absorption experiment as fundamentally more cumbersome, which in unclear cases, ought not to be used again.

Since the discovery of a weak N characteristic by Crome,<sup>52</sup> and its confirmation by Pietrusky,<sup>53,54</sup> there have been other similar observations reported by Friedenreich,<sup>55</sup>

Lauer,<sup>56</sup> Pietrusky,<sup>57</sup> Dombrowsky,<sup>58</sup> and Langenberg.<sup>59</sup> There is currently a wide-ranging discussion of this subject. Dahr<sup>14</sup> (p. 106) has pointed out the difference between the weak N described by Pietrusky and the weak N reported by Friedenreich and Lauer. In their report the weak N displayed approximately  $\frac{1}{4}$  of the agglutinating capability of normal N.

A third allelomorph gene  $N_2$  was assumed.

According to Andresen's<sup>23</sup> report (1947), only eight cases, including the four cases reported by Friedenreich, were observed during a ten-year period at the Forensic-medical Institute in Copenhagen among 20,000 paternity cases.

In 1948 Krah<sup>60,61</sup> described cases of weak N, two of which resembled more closely the cases described by Pietrusky than those described by Friedenreich and Lauer. He indicated that, besides the increase in serum titer, the qualitative character of the sera played an essential role in identifying weak N, since sera of the same titer do not behave in a uniform way. Freshly produced sera are better suited to attach to this weak N receptor than are older, used sera of the same titer.

The absorption test in the normal manner usually fails in the cases first described because the reduction in titer is too small. From his observations Krah reached the conclusion that to recognize the weak N receptor it was necessary to have freshly extracted, high-potency, strongly specific anti-N sera with the greatest possible reactive range.

Since there exist not only weaker N types but also defective types, the terms Nd (defective) and Ns (weak; German: *schwach*) were recommended.

In addition to a weak N, observations concerning a weak M have been published in the last few years. Friedenreich and Lauridsen<sup>62</sup> issued the first report in 1938.

In 1943 Pietrusky<sup>63,64</sup> described in a sample of MN blood a weak M receptor—he called it  $M_2$ —with a clear but nevertheless weaker absorption than that of MN blood. Dahr<sup>65</sup> at that time called attention to the possibility of clarification by quantitative methods and the mosaic-like complex of the human M and N agglutinogens.

In 1943 Pietrusky and Hausbrandt<sup>66</sup> issued a report on a certain type  $M_3$  with the advice that, in view of the difficulties of the MN system, superior expert advice ought to be introduced in all paternity decisions based on the MN system.

Jakobowicz, Bryce, and Simmons<sup>67,68</sup> have observed, in addition, a qualitatively deviant M form.

We ought not to overlook the confirmation of Dahr<sup>69</sup> in 1944 that a considerable quantity of anti-N agglutinin was released from human M-blood corpuscles which had been brought together beforehand with a specific anti-N serum (decanted for testing). Kindler<sup>70</sup> also confirmed this when he continued the experiments at Dahr's institute. He was able to separate complete N antibodies from crude anti-N serum by means of absorption with M blood corpuscles.

In any case we need to devote special attention to the M and N experiments in the future.

The last work I was able to obtain was one by Walter Boltz<sup>51</sup> which recently appeared concerning the deviant forms in the M-N system. It contains an interesting report concerning a weak M in the case of a child and the defendant. Here the blood of both persons was "surprisingly" similar regarding the M characteristic. One could designate the two samples of blood as M(s)N.

In this case the relationships were as follows:

mother	O	N	
child	$A_1$	Ms	N
defendant	$A_1$	Ms	N
witness	$A_1$	N	

One could thereby not only choose the adulterer, but one also had an important indication of the actual paternity as a result of the rare weak-M characteristic in both the child and the defendant.

This work from the Vienna Institute rightly emphasized the following:

False testimony on the part of witnesses in paternity suits is an everyday occurrence, and it does not compare as a source of error to the dwindling error factor resulting from an unrecognized blood-type variant.

Although one must still carefully evaluate a decision made on the basis of the subgroups of M and N, nevertheless, almost all authors and experts are in agreement that the same weight should be given to a decision based on M and N which was reached by experienced investigators, and confirmed by superior expert advice, as to that based on the classical blood groups.

In these cases the judge is sometimes unable to reach a decision, or sometimes, when a judgment based on hereditary biology argues for parentage from simple probability or possibility, it can go against the MN judgment. Such cases, however, should not be held against the method and the scientific evidence.

## The S Characteristic

In 1947, Dr. Walsh and Miss Montgomery<sup>71</sup> discovered in Sydney, Australia, in the serum of an rh negative mother with a dropsical stillborn an agglutinin which did not fit into the systems to date.

In the case of the gene designation S, Race and Sanger,<sup>72</sup> as they confess, overlooked the fact that the letter S was already assigned to the secretion type. They<sup>73</sup> looked into the inheritance of the S characteristic themselves, and spoke of four allelomorphs, MS, Ms, Ns, and NS.

Up to 1950 only seven examples of anti-S agglutinin were discovered.

Experiments conducted by Mourant and Ikin<sup>74</sup> in immunizing rabbits to S were unsuccessful up to that time.

Manz and Orbach<sup>75</sup> by chance possessed the same group constellation as had been present in the case of Walsh and Montgomery in the discovery of the anti-S; they wanted to produce the antibody, anti-S, by means of self-immunizations. Antibodies against Rh showed that the test person was, in general, well suited to build antibodies. The

experiment, however, was not successful in identifying any anti-S, even in traces.

If the routine identification of the S characteristics were made possible by the easier availability of anti-S, then the possibility of making an exclusion in the MN system would be greater. The chances of an exclusion would be even better if the anti-s were available to be used for forensic blood tests, which is not yet the case (Wiener<sup>76</sup>).

Statements concerning blood-group changes have disappeared from the more recent literature.

In view of the enormous number of transfusions which take place today throughout the world, one must think about the possibility of a mistake due to the group characteristics of transfused blood corpuscles in the recipient.

As Schwer-Körner and Kim<sup>77</sup> in Dahr's institute reported in 1948, incompatible blood can cause false determinations regarding MN for a long time—up to fifty-three days.

The experienced researcher, however, will notice that only some of the blood corpuscles were clumped and thus protect himself from an incorrect determination.

## P

The first tests of Landsteiner and his co-workers have already demonstrated that the factor P is developed in varying strengths.

How are these differing reports to be evaluated, when the one is P+++ and the other P+?

In his experiments with his anti-P sera from pigs Jungmichel<sup>78</sup> has divided the P into three groups.

423 Wiener<sup>48</sup> as well as Race and Sanger<sup>72</sup> mention the appearance of the P characteristic in varying strengths.

The determination of the strength of P in the case of both monozygotic and dizygotic twins was made by Dahr<sup>79</sup> as well as by Schmidt and his co-workers, as a result of which it seems likely that the varying strength of the P characteristic is conditioned by heredity.

Henningsen<sup>81</sup> distinguished four classes of P according to the strength of the P gene: strong P, middle P, weak P, and P minus. He discovered, in conducting family studies, that in the case of P+ × P- pairs, the offspring cannot exhibit a stronger P than that of the P-positive parent. This corresponds to several different genes which produce the P antigen of different strength.

These observations of Henningsen should be expanded. If they are confirmed in large series of tests and in family studies, we could expect a further development for paternity cases here as in the case of A<sub>1</sub> and A<sub>2</sub>; the test could demonstrate the improbability of generation, even if all three persons tested are P-positive.

Because of the difficulty of its nature, P is not yet used for forensic medical purposes in America according to the report of the committee<sup>24</sup> although Levine and Wiener have had access from the first to special experience through their experiments.

Krah and Harter<sup>82,83</sup> recently occupied themselves with the difficulties of determining P and of obtaining animal P

anti-serum. They succeeded in obtaining high-potency, anti-P sera from normal pig serum.

Concerning the use of blood groups in criminalistics, there have been in the last few years no fundamental innovations or new methods, as Formaggio<sup>84</sup> admits in a summary report which appeared in 1950.

In general, the old methods are still used and are tried on the new blood-group characteristics.

To better dissolve the agglutinins for the agglutinin identification in dried blood, Faraone<sup>85</sup> recommended warming for thirty minutes to a temperature of 40° to 50° in a hanging drop.

Some very fine and successful results have been obtained from the methods to date. Thus, Moureau<sup>86</sup> was successful in 1948 in achieving an interesting criminalistics group identification on the sweat band of a hat.

Muller and Christiaens<sup>87</sup> were able to convict a thief by identifying N substances in blood stains.

The experiments concerning the identification of Rh in blood stains and secretions are still in the experimental stage.

In the case of Rh identification, bacterial decomposition 424/ of the sera makes its appearance as a disturbance during absorption in the heat. For this reason Formaggio mixes in merthiolate.

## Rh Groups

Since the discovery of the classic blood groups, the discovery of the Rh groups by Landsteiner and Wiener<sup>88</sup> has been the most important development.

Thorough monographs such as the one by Fanconi, Grumbach and his co-workers,<sup>89</sup> those by Formaggio<sup>90</sup> and by Edith L. Potter,<sup>91</sup> and that by Hill and Damashek,<sup>92</sup> as well as those of several others, have appeared which deal chiefly with the clinical and serological problem, as have congresses dedicated especially to the Rh questions (for example, in Turin, in Naples, and in Milan). These demonstrate the importance which the Rh factors have achieved today.

The original anti-Rh is now called anti-Rh<sub>0</sub> or anti-D.

Wiener and Landsteiner<sup>93</sup> had assumed three major genes, R<sub>1</sub>, R<sub>2</sub>, and r. Fisher<sup>94</sup> introduced for the Rh genes the symbols Cc, Dd, and Ee.

Each of these genes can, under certain circumstances, stimulate the corresponding antibody.

Fisher's theory is today generally acknowledged.

Wiener,<sup>95</sup> Landsteiner and Wiener,<sup>96</sup> and Levine and his co-workers<sup>97</sup> have acknowledged since 1941 that different Rh types occur.

In addition to C and c Callender and Race<sup>98</sup> found in 1946 a third allelomorph gene C<sup>w</sup>. Later c<sup>v</sup> and c<sup>u</sup> were described.

In addition to D and d, Stratton<sup>99</sup> described a third gene D<sup>u</sup>, probably identical to Wiener's intermediate gene. According to van Loghem,<sup>101</sup> this gene can have an antigenic effect.

Armytage, Ceppellini, Ikin and Mourant<sup>102</sup> first described

a third allelomorphous antigen E' in addition to E and e. Mr. Schleyer from Bonn will report soon concerning reactions with various E-gene types.

Diamond<sup>103</sup> in 1946 and Hill and Haberman<sup>104,105</sup> in 1948 described the anti-d (anti-Hr<sub>0</sub>).

While at first the anti-Rh sera were obtained by immunizing animals, the sera containing the Rh antibodies we use today come for the most part from humans immunized by transfusion or pregnancy.

On account of repeated stillborn babies due to the anti-Rh, Diamond<sup>106</sup> treated intravenously such anti-Rh sterilized women with varying amounts of Rh-positive blood and was able to produce with very small amounts of blood (0.1cc) an increase in the titer.

Moreover, Wiener (personal communication), Hill, Haberman, and Orozco,<sup>107</sup> Callender and Race,<sup>98</sup> and others were successful with this method.

Wiener and Sonn-Gordon<sup>108</sup> injected Rh-negative donors with 4 cc of a 50% blood-corpusele suspension, repeating the injection after four months. Then days after the second injection they obtained usable anti-Rh sera.

Van Loghem<sup>101</sup> succeeded, after thirteen to seventeen injections, in obtaining anti-C and anti-E in the case of professional donors, especially from those who react to vaccine injections. He was thereby able to confirm Diamond's<sup>109</sup> observations that, in the case of progressive immunization, the agglutinins effective in saline solution change to incomplete antibodies. If agglutinins effective in saline solutions are desired, he recommends that the immunization be interrupted at the right moment.

Maresch<sup>110</sup> and Speiser<sup>111</sup> observed too, how the agglutinins present at the beginning of the immunization were later replaced by univalent antibodies.

### The Methods of Rh Testing

These are based on the antigen identification by means of agglutinins or conglutinins, complete or incomplete antibodies.

In order to be used with all groups, anti-A and anti-B sera, containing anti-Rh, must be purified before use with A<sub>1</sub>B blood of the corresponding Rh genotype, with purified A and B substance, or with saliva of an A<sub>1</sub>B secretor. In doing this, one must keep in mind what Cappell and McFarlane<sup>112</sup> found, i.e., that, after long storage, an unwanted anti-A and anti-B can again appear in absorbed serum. If the sera are strong enough, one can dilute them for use, according to Diamond,<sup>103</sup> most effectively in albumin.

A great number of studies in recent years have dealt with the Rh technique.

For Rh identification the incomplete, conglutinating antibodies are the most important and are predominantly the ones used.

The indirect and the direct Coombs tests serve more than any other method to identify incomplete antibodies.

An enzyme test, the trypsin test, was introduced by Pickles,<sup>113</sup> and Morton and Pickles,<sup>114</sup> a test which proved itself

also in routine experiments according to the assertion of English authors.

In recent years the identification of incomplete antibodies by means of macromolecular substances (polyvinylpyrrolidon, dextran, etc.) has been used.

In 1950 Hummel and Hamburger<sup>115,116</sup> in Germany and Formaggio<sup>117,118</sup> in Italy tried a synthetic colloid (polyvinylpyrrolidon, periston) to identify successfully incomplete antibodies.

Formaggio's method, which has been preserved for us, is the following:

One dilutes the serum with physiological saline solution. One drop of diluted serum and one drop of 2% blood-corpusele suspension are mixed in a tube or in the hollow depression of a slide tray and left to stand for ten to fifteen minutes. Then, one adds a drop of a 12-13% solution of polyvinylpyrrolidon diluted with physiological saline solution. After the mixture is allowed to stand for an hour at 37°, a reading is taken with the naked eye.

When using known, incomplete antibodies, this method, applied in reverse, serves to identify Rh types and other antigens.

The method saves albumin or AB serum or the serum of the required blood-corpusele suspension, as well as the anti-globulin serum.

Hummel and Hamburger,<sup>116</sup> however, emphasize the influence of weather on the sensitive colloid test. This ought not to cause amazement since, in working with colloid on stormy days, changes in the specific gravities—for example, milk coagulating promoted by foul weather—can be observed.

Fisk and McGee<sup>119</sup> report that the same conditions prevail in the gelatin test.

Concerning the gelatin-Rhesus test as a conglutinin test, we have favorable experiments to report. Prokop<sup>102</sup> from Elbel's institute in Bonn emphasized in 1951 both the economy of gelatin solutions and their high-quality, clear results. He warned, however, against dilutions of too great concentration.

According to the experimental results of most authors exclusion of paternity can be determined from the hereditary relationship of individual allelomorphous genes on the basis of C, c, D, E, and e.

The simplest rule runs: the antigens

D	C	E	c	e
Rh <sub>0</sub>	rh'	rh''	hr'	hr''

can only appear in children, if they are present in one of the parents.

Formaggio<sup>121</sup> found repeatedly in his experiments that the Rh-characteristics in newborn babies are already very strongly developed.

Witebsky and Engasser<sup>122,123</sup>, moreover, have shown that, when human immune anti-A and anti-B sera are used, the antigens appear in newborns and in adults at the same strength.

The reliability of the hereditary rules in the Rh system has

best proven itself in wide-ranging family investigations.

Wiener<sup>124</sup> and Levine have used the Rh characteristics for a rather long time in the USA. Also, the most recent report of the committee cites as dependable the test of Rh and even the test of the subtypes.

427 The further use of individual subtypes to ascertain the exact hereditary type is now hindered only by the difficulty in obtaining individual antisera.

The use of only anti-D (anti-Rh<sub>0</sub>) offers a smaller chance of exclusion since according to Race<sup>72</sup> both supposed parents are Rh negative only in about 2.5% of the cases involving whites and in even fewer cases involving colored persons.

Although the establishment of the Rh hereditary type is so desirable and promising, especially for paternity cases, one will have to be satisfied with the testing of Rh-phenotypes for forensic purposes from time to time until anti-Rh subgroup sera are produced in larger quantities. Thus, Ponsold<sup>27</sup> considers the testing of the Rh hereditary type not yet ripe for forensic purposes.

The determination of Rh phenotypes, however, presents a far-reaching differentiation of the blood formula and in many cases an exclusion of paternity.

### Blood Groups Besides the ABO, MN, and Rh Systems

In recent years a few more new blood-corpusele characteristics and antibodies have been described. First, the Lutheran blood groups were discovered by Callender and Race<sup>98</sup> by identifying the corresponding antibody in the serum of a Lutheran patient who had often received transfusions.

Mainwaring and Pickles<sup>125</sup> succeeded in obtaining the anti-Lutheran antibody by means of transfusions of Lu (a+) blood. They distinguished two Lutheran types, a stronger and a weaker, which can be compared to the A<sub>1</sub> and A<sub>2</sub>; they assumed three allelomorphous genes.

The Lutheran groups were introduced into testing processes by Race and his co-workers, and in the future they will perhaps still play a role in paternity suits.

At present, however, the serum is still quite scarce. Second, the Kell blood groups were discovered also in 1946 by Coombs, Mourant and Race<sup>126</sup> by identifying an antibody of incomplete type in the blood of a mother with a child suffering from hemolytic disease. One year later, Wiener and Sonn-Gordon<sup>27</sup> described a second case with an anti-Kell. Moreover, the Kell-characteristic is dominant in hereditary transmission. The genes are K and k, of which 10.17% have been observed as Kell+ and 89.85% as Kell-.

In 1948 Levine<sup>128</sup> discovered in the serum of a Mrs. Cellano an antibody which produced no reaction in only 0.2% of the blood tested. The Cellano characteristic can be conceived of as antagonistic to the Kell characteristic.

428 Third, the Lewis blood groups are of special interest; they were discovered also in 1946 in England by Mourant<sup>129</sup> and were named after the two donors Lewis.

In 1947 Andresen<sup>130</sup> reported that he and Friedenreich discovered antisera which agglutinated 21% of the blood of adults.

Andresen made the interesting observation that L-positive blood is more frequent among children than it is among adults and that adults of type L- can have children of type L+. From this he concluded that, in the case of adults, only LL homozygotes produce the L+ reaction while, in children, Ll heterozygotes also produce the L+ reaction.

Andresen<sup>130</sup> found a second antibody, anti-L<sub>2</sub>. That only 42% of A<sub>1</sub> was agglutinated by anti-L<sub>2</sub> and that many reactions were weak or doubtful Andresen connected with the phenomenon of epistasis<sup>†</sup> concerning which Mr. Prokop from Bonn will offer a closer study in the future.

In 1948 Grubb<sup>132</sup> from Lund made the observation at the Lister Institute in London that practically all Lewis positives were also non-secretors of A, B, or H substance.

The Le (a+) antigen was demonstrated by Grubb and Morgan<sup>133,132</sup> to be present in the saliva of all Le (a+) persons. Moreover, the majority of the Le (a-) persons displayed a weaker anti-Le<sup>a</sup> inhibition effect in saliva. Because in the first years of life the reactions are not so clear as in later years, the use of Lewis blood groups in paternity cases is limited.

Fourth, the Duffy blood groups were described in 1950 by Cutbush, Mollison, and Parkin<sup>134, 135</sup> after discovery of an antibody in the case of a man who, on account of hemophilia, had received numerous transfusions during the previous twenty years.

The antigen was found in 64.9% of the blood samples. Genes: Fy<sup>a</sup> and Fy<sup>b</sup>; genotypes: Fy<sup>a</sup>Fy<sup>a</sup>, Fy<sup>a</sup>Fy<sup>b</sup>, and Fy<sup>b</sup>Fy<sup>b</sup>.

Among the rare blood-group systems which remain to be mentioned are the Levay, the Gr and the Jobbins systems.

Callender and Race<sup>72</sup> discovered the Levay group and Graydon<sup>136</sup> the Gr group in 1946, a year most productive in finding new antibodies. The brother and the father of the blood donor Levay possessed the antigen. Graydon himself thought that the antigen Gr could possibly be identical with the Levay antigen. The rarity of both, however, makes this unlikely.

The Jobbins blood group with an incomplete antibody was 429/ described in 1947 by Gilbey.<sup>137</sup>

In 1951 Orth<sup>138</sup> gave a comprehensive presentation on the new blood-group systems.

Landsteiner, Strutton, and Chase<sup>139</sup> had discovered in 1934 a peculiar factor in the case of Negroes, and that only in persons who exhibit either the N factor or the factors M and N. It seems as though this factor is related to the MN system.

<sup>†</sup> By *epistasis* one means the masking of a hereditary factor by another factor which does not belong to the same allelic order.

If a gene hinders the phenotypical expression of another gene which belongs to a different allelic order, one says that it is epistatic over the other. The gene, which has been hindered in its phenotypical expression, is called hypostatic.

In 1951 Ikin and Mourant<sup>140</sup> also discovered in rabbit immune serum an antibody which reacted in a special way with Negro blood. The rabbit, however, had been pretreated with M blood which argues against assuming that this antigen is identical with the one identified by Landsteiner, Strutton, and Chase, or that it was perhaps a variety of N.

Most recently, strongly individual consanguinity-related cell characteristics have been discovered, and are independent of all systems to date. Here we must place Elbel and Prokop's<sup>141</sup> discovery of the Becker antigen.

Finally, the procedure of Löns deals with the supposition of an individual hereditary gene structure of the body cells and with the existence of antigens closely related to consanguinity.

This short overview ought not to close without reference to the Löns procedure which is still at the stage where it is being tested and evaluated.

The procedure consists of the following. Blood from an especially large number of persons is injected subcutaneously into a goat. In carrying this out, Wassermann blood samples are used. A mixture of the smallest blood samples (0.01cc) from approximately 200 persons is injected twice during a week until the blood from a total of 1000 persons has been used for immunization. In this way serum is obtained which contains antibodies against (?) all possible known and unknown blood characteristics.

This serum is absorbed by the blood corpuscles of the mother and of the possible sire; the corresponding antibodies are bound. Since the child can only have such characteristics which are also present in the parents, the antibodies which could be effective against the child's blood are removed from the goat's serum by the blood of the parents. If the goat's serum, pretreated (absorbed) by the parents' blood, is added to the blood of the child, no agglutination (clumping) should take place. If it does occur, then the supposed father can not have produced the child.

This process is original and includes antigen blood characteristics which are unknown up to the present time.

If the process is confirmed, it means a giant step forward, not only for the exclusion of paternity, but also for its positive-determination capability.

7430 Dahr<sup>142</sup> and Ponsold<sup>143,144</sup> have occupied themselves with a thorough and comprehensive testing of the process. Though there have been arguments advanced against the method up to now, as almost always takes place in the case of a process so complicated and still in a state of development, none have yet brought about its rejection.

It is unnecessary for us to pursue any further the Löns test and the results and follow-up experiments conducted up to now, since Schmidt, Dahr, Sachs, and Ponsold will communicate their personal experiences.

In agreement with Dahr, however, we regret that a process has been taken over by several courts before it has been in fact recognized as sound on the grounds of follow-up testing. We regret that conclusions which are drawn from the results can actually be appealed to as though they had the strength

of the law.

If new processes are dependable, then it is not surprising that they be employed in practice until after general scientific recognition.

If, through follow-up testing, new methods are shown to be unsuitable after they were prematurely publicized, and put to use by the courts, this could easily discredit the use of biological testing methods in legal processes in general.

Even when judges want testing for the entire "alphabet" of the groups and factors, as recently happened, the researcher ought not to leave the sure ground of established methods.

It is here that the forensic physicians as assessors can contribute much to preserving the reputation of blood testing by exercising an essential caution.

I will never forget the words which Karl Landsteiner spoke thirty-five years after his discovery of the blood groups and seven years after his identification of the M, N, and P characteristics: "I am only happy that I have nothing to do with the practical application of blood groups; I could not endure the responsibility."

In the meantime, the use of blood groups has experienced an unexpected growth. The chances are considerably better of excluding a man who has falsely been declared as the father.

Calculations concerning the chances of an exclusion based on blood characteristics have repeatedly been set forth.

In a recent article concerning "The Directions and Perspectives of Blood-group Research for Determining Paternity Based on Forty Years of Application," the old master of serology and the co-creator of the first blood-group hereditary theory, Ludwig Hirszfeld,<sup>145</sup> coined the concept of *complete* and *incomplete* applicability.

Incomplete applicability corresponds to the situation where a dominant characteristic has been established in the case of the child, a characteristic which is lacking in the mother, but must be present in the father.

In the case of complete applicability, the rule has been established that the homozygous DD male (the dominant characteristic is double) cannot be the father of a homozygous-negative child.

Incomplete applicability has as its maximum 8.19% when group frequency is 75%; complete applicability, its maximum as 18.75% when the group frequency is 75%.

Tables, based on the characteristics OAB, MN, Rh (CDE), permit us to predict the probability of exclusion. It is noteworthy that the applicability of the characteristic Rh E was found to be six times greater than that of the characteristic Rh D.

The individuality of the blood, established on grounds of the known blood characteristics, is today well-advanced. Speiser<sup>146</sup> recently published a table which took into consideration the characteristics A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, O, B, M, N, P, Rh types, and secretor with a total of 1,728 combinations.

Race and Sanger<sup>72</sup> (p. 275, table 88) were able to exclude approximately 62% of all men falsely accused of being the

important blood group characteristics following table (table 88) shows the individual exclusion

possibility of excluding a man, falsely being the father.\*

		Through the individual system	Through the combination of systems
1.	O	0.1760	0.1760
2.	MNS	0.2741	0.4019
3.	Rh	0.2520	0.5526
4.	Kell	0.0421	0.5714
5.	Lutheran	0.0333	0.5857
6.	Secretion	0.0258	0.5964
7.	Duffy	0.0496	0.6164

\* According to Race and Sanger ("Blood Groups in Man," Oxford, 72, p. 275, Table 88)

The same book also includes the following extremely revealing table (76) with the phenotypes of the most important blood-group systems and the 29,952 possible combinations.

Table 2. Blood Group Determinations Which Can Be Made in Many Laboratories

Blood Group System	Obtainable sera	Number of Recognizable Phenotypes
A <sub>1</sub> A <sub>2</sub> BO	anti-A-B	6
M N S	anti-M-N-S	6
P	anti-P	2
Rh	anti-C-c-C <sup>+</sup> -D-E-e	26
Lutheran	anti-Lu <sup>a</sup>	2
Kell	anti-K	2
Lewis	anti-Le <sup>a</sup>	2
Duffy	anti-Fy <sup>a</sup>	2
Phenotype combinations		29,952

In these figures D<sup>u</sup>, c<sup>v</sup>, C<sup>n</sup>, A<sub>2</sub>, N<sub>2</sub>, k, and Le<sup>b</sup> are not included.

If all the antibodies mentioned by Race and Sanger<sup>72</sup> were used together, they would produce over one million phenotypes, an amount which is equivalent to a highly-developed individuality of the blood.

These possibilities illuminate at the same time the great progress which blood group serology has experienced in the fifty years of its existence, and they highlight its special importance for forensic medicine.

Andresen in Copenhagen has rendered the field a personal service in collecting and publicizing the titles of numerous new works in this field in his bulletin entitled "Blood-group News."

When we, as the experts, make use of the achievements of blood-group research in proving the truth before the court, we do not want to forget the tremendous pioneering work which has been done; we want to thank all those who have made efforts in the past and are doing so now in the interests of developing this science.

Our greatest thanks, however, transcends the grave to honor in the first place the man who, at the beginning of this century, opened for us the door to this wonderful field of research and to its recognition and its practical world-wide use, a man who recognized its importance for forensic medicine fifty years ago, Karl Landsteiner.

## Literature

- Landsteiner, Karl: *Zbl. Bakter.* 27, 357 (1900).
- Thomsen, Oluf: Der Unterscheid in dem Verhalten der beiden menschlichen A-Blutgruppen (A und A') gegenüber Anti-A-Lysin (in O- und B-Sera). *Munch. med. Wschr.* 1930, 1190.
- Thomsen, Oluf: Über die A<sub>1</sub>- und A<sub>2</sub>-Receptoren in der sog. A-Gruppe. *Acta Soc. Med. fenn. "Duodecim"*, Ser. A 15, 17 (1932).
- Thomsen, O., V. Friedenreich u. E. Worsaae: Die wahrscheinliche Existenz eines neuen, mit den 3 bekannten Blutgruppengenen (O, A, B) allelomorphen A' benannten Gens mit den daraus folgenden 2 neuen Blutgruppen A' und A'B. *Klin. Wschr.* 1930, 67.
- Lauer, A.: Zue Kenntnis der menschlichen Blutgruppen. *Die Naturwiss.* 4, S.86 (1930).
- Lehmann-Faciuss, H.: Die Methode der serologischen Differenzierung der Blutgruppen A<sub>1</sub> und A<sub>2</sub>. *Dtsch. Z. gerichtl. Med.* 19, 58 (1932).
- Ottenssooser, F., u. St. Zurukzoglu: Unterscheidung der Untergruppen A<sub>1</sub> und A<sub>2</sub> durch die Pepsinhemmungsreaktion. *Klin Wschr.* 1932, 1715.
- Holzer, F. J.: Untergruppen in der Blutgruppenforschung. *Klin Wschr.* 1937, 481.
- Krieger, Klaus: Die Technik der A-Untergruppenbestimmung Diss. Heidelberg 1941.
- Wolff u. Jonsson: *Dtsch. Z. gerichtl. Med.* 22 (1933).
- Blinov, N.: Eine vereinfachte Methode zur Differenzierung der Untergruppen A<sub>1</sub> und A<sub>2</sub>. *Klin. Wschr.* 1934, 1025.
- Ponsold, Albert: Absorptionsnachweis der Untergruppen A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub> durch "Erschöpfung" eines Testserums. *Dtsch. Z. gerichtl. Med.* 39, 132 (1948).
- Dahr, Peter: Beitrag zur Blutgruppe A<sub>3</sub>. *Z. Immunforsch.* 102, 13 (1942).
- Dahr, Peter: *Die Technik der Blutgruppen- und Faktorenbestimmung*, 4 u. 5 Aufl. Leipzig: Georg Thieme 1950.
- Gammelgaard, Arne, u. Poul V. Marcussen: Die Blutgruppe A. *Z. Immunforsch.* 102, 259 (1942).
- Friedenreich, V.: Sur les sous-groupes du groupe sérologique. A. De l'interprétation serologique des recepteurs, A<sub>1</sub> et A<sub>2</sub>. *C. r. Soc. Biol. Paris* 106 574 (1931).
- Friedenreich, V.: Sur les sous-groupes du groupe sérologique. Y-a-t-il des transitions entre les sous-groupes A<sub>1</sub> et A<sub>2</sub>? *C. r. Soc. Biol. Paris* 106 578 (1931).
- Dahr, Peter: Welchen Beweiswert hat ein Vaterschaftsausschluss mit den A<sub>1</sub> A<sub>2</sub> Untergruppen? *Dtsch. Z. gerichtl. Med.* 39, 14 (1948/49).
- Witebsky, E.: Interrelationship between the Rh system and the ABO system. *Blood* 3, Suppl. 2, 66 (1948).
- Laguna, St.: *Klin. Wschr.* 1930, 547.
- Haselhorst, G. u. A. Lauer: Über eine Blutgruppenkombination Mutter AB und Kind O. *Z. Konstit.lehre* 15, 205 (1931); 16, 227.
- Boltz, Werner: Phänotypische Latenz und Spätmanifestierung der Blutgruppe A<sub>2</sub>B. *Beitr. gerichtl. Med.* 18, 109 (1949).
- Andresen, P. H.: Reliability of the exclusion of paternity after the MN and ABO systems elucidated by 20,000 mother-child examinations and its significance to the medicolegal conclusion. *Acta path scand.* (Kobenh.) 24, 545 (1947).
- Davidsohn, Israel, Philip Levine and Alexander S. Wiener: Medicolegal application of blood grouping tests. A report of the committee on medicolegal problems. *Amer. Med. Assn.* 1952.

25. Wichmann, Dietrich: Über den Beweiswert eines Vaterschaftsaus- schlusses durch die A-Untergruppen. *Z. Immunforsch* **108**, 516 (1951).
26. Maysen, H.: Neue Schlüsse aus gerichtlichen Blutuntersuchungen eines Sachverständigen in den Jahren 1945–1948. *Dtsch. Z. gerichtl. Med.* **40**, 326 (1951).
27. Ponsold, A.: Blutgruppen. In *Lehrbuch der gerichtlichen Medizin*, S. 366–391. Stuttgart: Georg Thieme 1950.
28. Ponsold, A.: Über den Beweiswert der Blutgruppen A<sub>1</sub> und A<sub>2</sub>. (Im Druck.)
29. Böhmer, K., u. H. Greiner: Über die Sicherheit der geläufigen Untersuchungsmethoden bei der A-Untergruppenbestimmung. *Z. Immunforsch* **108**, 328 (1951).
30. Midguti, *Chiba Igakkai Zassi* **14**, 926 (1936). Zit. nach Schiff u. Boyd, S. 15.
31. Honda: *Chiba Igakkai Zassi* **14**, 851 (1936). Zit. nach Schiff u. Boyd, S. 15.
32. Formaggio, Tiziano: Ricerche sull' Antigene B. *Minerva medicolegale* **72**, Nr 2 (1952).
33. Schiff, F., and W. C. Boyd: *Blood Grouping Technic*. New York: Interscience Publishers 1942.
34. Matta, A.: A critical investigation of the blood groups and their medico-legal application. *Faculty of Medicine of the Egyptian University*, No. 11 S.52–58. 1937.
35. Koller, Siegfried: Statistische Untersuchungen zur Theorie der Blutgruppen und ihrer Anwendung vor Gericht. *Z. Rassenphysiol.* **3**, 121 (1931).
36. Schiff, F.: *Wiss. Woche Frankfurt a. M.* 1934.
37. Zitzmann, *Dtsch. Z. gerichtl. Med.* **27**, 329 (1937).
38. Pietrusky, F.: *Technik der Blutgruppenbestimmung*. Berlin: Springer 1940.
39. Manz: *Dtsch. Recht* **11**, 1176 (1941).
40. Jungmichel, G.: *Die Bedeutung der Blutgruppen und Blutkörperchenmerkmale in der gerichtlichen Praxis*. Berlin 1940.
41. Jungmichel, G.: *Med. Welt* 1942, 907.
42. Fischer, Werner: Über indirekte Blutgruppenbestimmungen, die sich aus ihnen ergebenden Ausschlussmöglichkeiten und die jeweiligen Erfolgsaussichten. *Dtsch. Z. gerichtl. Med.* **37**, 231 (1942).
43. Boorman, Kathleen E., Barbara E. Dodd and B. E. Gibbey. A serum which demonstrates to co-dominance of the bloodgroup gene O with A and B. *Ann. of Eugen.* **14**, 201 (1948).
44. Holzer, F. J.: Untersuchungen über die gerichtlich medizinische Verwertbarkeit der Ausscheidung von Blutgruppen substanzen. *Dtsch. Z. gerichtl. Med.* **28**, 243 (1937).
45. Formaggio, Tiziano: Ricerche sullo sviluppo e l'eliminazione delle proprietà gruppal. III. Diversità di distribuzione del tipo "secretore" nei gruppi sanguigni ABO. *Minerva medico-legale* **72**, 157 (1951/52).
46. Formaggio, Tiziano: L'eliminazione proprietà isoantigene *Minerva medicolegale* **72**, Nr 2 (1952).
47. Wiener, A. S. and L. Kosofsky: Quantitative studies on the group-specific substances in human blood and saliva. II. Group-specific substance A, with special reference to the subgroups. *J. of Immunol.* **42**, 381, (1941).
48. Wiener, A. S.: *Blood Groups and Transfusion*, 3. Aufl. Springfield: Ch.C. Thomas 1946.
49. Wiener, A. S.: Natural Anti-M Agglutinins in the sera of male neogrid twin infants, with a comparison of different methods of treating anemia. *Acta genet. med. et gemellologiae* **1**, 2 (1952).
50. Schiff, F.: *Dtsch. Z. gerichtl. Med.* **18**, 57 (1931).
51. Boltz, Werner: Beitrag zu den abweichenden Formen im M-N-System und ihrer gerichtsarztlichen Bedeutung. *Beitr. gerichtl. Med.* **19**, 27. (1952).
52. Crome, W.: Über Blutgruppenfragen, Mutter M, Kind N. *Dtsch. Z. gerichtl. Med.* **24**, 167, (1935).
53. Pietrusky, F.: *Münch. med. Wschr.* 1936, 1123.
54. Pietrusky, F.: Über eingeeengte Seren und über andere Untersuchungsverfahren zum Nachweis des schwachen N-Rezeptors (N<sub>2</sub>) im Blute. *Dtsch. Z. gerichtl. Med.* **28**, 468 (1937).
55. Friedenreich, V.: Ein erblicher defekter N-Rezeptor, der wahrscheinlich eine bisher unbekannte Blutgruppeneigenschaft innerhalb des MN-Systems darstellt. *Dtsch. Z. gerichtl. Med.* **25**, 358 (1935).
56. Lauer, A.: *Z. Immunforsch.* **99**, 232 (1941).
57. Pietrusky, F.: *Z. Immunforsch.* **105**, 200 (1944).
58. Dombrowsky: *Med. Welt* 1938, 832.
59. Langenberg: *Z. Immun. forsch.* **97**, 48 (1940).
60. Krahe, Ernst: Beobachtungen über den schwachen N-Rezeptor. *Dtsch. Z. gerichtl. Med.* **39**, 213 (1948).
61. Krahe, Ernst: Zum Problem des schwachen N. *Dtsch. Z. gerichtl. Med.* **39**, 222 (1948).
62. Friedenreich, V. and A. Lauridsen: On a variety of human type antigen M and its relation to other M antigens. *Acta path. scand.* (Kobenh.) Suppl. 38, 155 (1938).
63. Pietrusky, F.: Ein schwacher Rezeptor M<sub>2</sub> der Blutgruppeneigenschaft M. *Dtsch. Z. gerichtl. Med.* **37**, 277 (1943).
64. Pietrusky, F.: *Dtsch. Z. gerichtl. Med.* **38**, 299 (1943).
65. Dahr, Peter: Ein schwacher Rezeptor M<sub>2</sub> der Blutgruppeneigenschaft M. Bemerkungen zu der gleichnamigen Arbeit von F. Pietrusky in *Bd. 37*, H. 5 dieser *Z. Dtsch. Z. gerichtl. Med.* **38**, 297.
66. Pietrusky, F. u. F. Hausbrandt: Ein besonderer Typ (M<sub>3</sub>) des Blutgruppenfaktors M. *Dtsch. Z. gerichtl. Med.* **38**, 191, 1943.
67. Jakobowicz, Rachel, Lucy M. Bryce and R. T. Simmons: The occurrence of unusual positive Coombs reactions and M variants in the blood of a mother and her first child. *Med. J. Austral.* **2**, 945 (1949).
68. Jakobowicz, Rachel, Lucy M. Bryce and R. T. Simmons: *Nature* (Lond.) **165**, 158 (1950).
69. Dahr, Peter: *Z. Immunforsch.* **105**, 383 (1945).
70. Kindler, M.: Untersuchungen über die Serologie des Blutkörperchenmerkmals M.Z. *Immunforsch.* **109**, 66 (1951).
71. Walsh, R. J. and Carmel Montgomery: A new human isoagglutinin subdividing the MN blood groups. *Nature* (Lond.) **160**, 504 (1947).
72. Race, R. R. and Ruth Sanger: *Blood groups in man*. Scientific Publications. Oxford: Blackwell 1950. 290 S.
73. Sanger, Ruth and R. R. Race: Subdivisions of the MN blood groups in man. *Nature* (Lond.) **160**, 505 (1947).
74. Mourant, A. E., and Elizabeth W. Ikin: Personal communication. 1949. Zit. bei Race u. Sanger (72).
75. Manz, Rudolf, u. Heinz Orbach: Eine neue agglutinable Blutkörpercheneigenschaft: das Merkmal S. *Dtsch. Z. gerichtl. Med.* **40**, 160 (1950).
76. Wiener, A. S.: Heredity of the M-N-S Blood Types. *Amer. J. Human Genet.* **4**, No. 1 (1952).
77. Schwerkörner, Th. u. B. P. Kim: Bedingt die Transfusion faktorenfremden Blutes eine Fehlermöglichkeit bei gerichtlichen MN-Bestimmungen? (Aus dem Institut von Dahr). *Dtsch. Z. gerichtl. Med.* **39**, 252 (1948).
78. Jungmichel, G.: Der Blutfaktor P. *Dtsch. Z. gerichtl. Med.* **36**, 259 (1942).
79. Dahr, Peter: Serologische Untersuchungen bei 330 Zwillingspaaren. *Z. Rassenphysiol.* **12**, 1 (1941).
80. Schmidt, O., R. Manz u. K. H. Traenckner: Serologische Untersuchungen bei Zwillingen mit besonderer Berücksichtigung der Rh-Untergruppen, sowie des Faktors P, seiner Rezeptorenstärke und der Ausscheidereigenschaft. *Dtsch. Z. gerichtl. Med.* **40**, 197 (1951).
81. Henningsen, K.: On the heredity of blood factor P. *Acta path. scand.* (Kobenh.) **26**, 769 (1949).
82. Krahe, E. u. F. Harter: Über Erfahrungen bei der Gewinnung tierischer P-Antiseren. *Z. Immunforsch* **108**, 307 (1951).
83. Krahe, Ernst: Untersuchungen zur Technik der Bestimmung des Blutgruppenmerkmals P. *Z. Immun. forsch* **109**, 80 (1951).
84. Formaggio, Tiziano: Recenti progressi (1945–1950) nella diagnosi

important blood group characteristics following table (table 88) shows the individual exclusion

of excluding a man, falsely being the father.\*

Blood Groups

	Through the individual system	Through the combination of systems
O	0.1760	0.1760
MNS	0.2741	0.4019
h	0.2520	0.5526
ell	0.0421	0.5714
utheran	0.0333	0.5857
ecretion	0.0258	0.5964
uffy	0.0496	0.6164

\*Sanger ("Blood Groups in Man," Oxford, 72, p. 275, Table 88)

book also includes the following extremely rare (6) with the phenotypes of the most important systems and the 29,952 possible combinations.

#### Blood Group Determinations Which Can Be Made in Many Laboratories

Obtainable sera	Number of Recognizable Phenotypes
anti-A-B	6
anti-M-N-S	6
anti-P	2
anti-C-c-C*-D-E-e	26
anti-Lu <sup>a</sup>	2
anti-K	2
anti-Le <sup>a</sup>	2
anti-Fy <sup>a</sup>	2
Phenotype combinations	29,952

types D<sup>a</sup>, c<sup>v</sup>, C<sup>v</sup>, A<sub>2</sub>, N<sub>2</sub>, k, and Le<sup>b</sup> are not

bodies mentioned by Race and Sanger<sup>72</sup> were they would produce over one million phenotypes which is equivalent to a highly-developed population of the blood.

phenotypes illuminate at the same time the great progress blood group serology has experienced in the past, its existence, and they highlight its special importance in forensic medicine.

Copenhagen has rendered the field a personal contribution by publishing the titles of numerous articles in his field in his bulletin entitled "Blood-group

and the experts, make use of the achievements of forensic search in proving the truth before the court, do not forget the tremendous pioneering work that has been done; we want to thank all those who have contributed to the past and are doing so now in the interests of forensic science.

Our greatest thanks, however, transcends the grave to honor in the first place the man who, at the beginning of this century, opened for us the door to this wonderful field of research and to its recognition and its practical world-wide use, a man who recognized its importance for forensic medicine fifty years ago, Karl Landsteiner.

#### Literature

- Landsteiner, Karl: *Zbl. Bakter.* 27, 357 (1900).
- Thomsen, Oluf: Der Unterscheid in dem Verhalten der beiden menschlichen A-Blutgruppen (A und A') gegenüber Anti-A-Lysin (in O- und B-Sera). *Munch. med. Wschr.* 1930, 1190.
- Thomsen, Oluf: Über die A<sub>1</sub>- und A<sub>2</sub>-Receptoren in der sog. A-Gruppe. *Acta Soc. Med. fenn. "Duodecim"*, Ser. A 15, 17 (1932).
- Thomsen, O., V. Friedenreich u. E. Worsaae: Die wahrscheinliche Existenz eines neuen, mit den 3 bekannten Blutgruppengenen (O, A, B) allelomorphen A' benannten Gens mit den daraus folgenden 2 neuen Blutgruppen A' und A'B. *Klin. Wschr.* 1930, 67.
- Lauer, A.: Zue Kenntnis der menschlichen Blutgruppen. *Die Naturwiss.* 4, S.86 (1930).
- Lehmann-Facijs, H.: Die Methode der serologischen Differenzierung der Blutgruppen A<sub>1</sub> und A<sub>2</sub>. *Dtsch. Z. gerichtl. Med.* 19, 58 (1932).
- Ottenssooser, F., u. St. Zuruzkoglul: Unterscheidung der Untergruppen A<sub>1</sub> und A<sub>2</sub> durch die Pepsinhemmungsreaktion. *Klin Wschr.* 1932, 1715.
- Holzer, F. J.: Untergruppen in der Blutgruppenforschung. *Klin Wschr.* 1937, 481.
- Krieger, Klaus: Die Technik der A-Untergruppenbestimmung. Diss. Heidelberg 1941.
- Wolff u. Jonsson: *Dtsch. Z. gerichtl. Med.* 22 (1933).
- Blinov, N.: Eine vereinfachte Methode zur Differenzierung der Untergruppen A<sub>1</sub> und A<sub>2</sub>. *Klin. Wschr.* 1934, 1025.
- Ponsold, Albert: Absorptionsnachweis der Untergruppen A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub> durch "Erschöpfung" eines Testserums. *Dtsch. Z. gerichtl. Med.* 39, 132 (1948).
- Dahr, Peter: Beitrag zur Blutgruppe A<sub>3</sub>. *Z. Immunforsch.* 102, 13 (1942).
- Dahr, Peter: *Die Technik der Blutgruppen- und Faktorenbestimmung*, 4 u. 5 Aufl. Leipzig: Georg Thieme 1950.
- Gammelgaard, Arne, u. Poul V. Marcussen: Die Blutgruppe A. *Z. Immunforsch.* 102, 259 (1942).
- Friedenreich, V.: Sur les sous-groupes du groupe sérologique. A. De l'interprétation serologique des recepteurs, A<sub>1</sub> et A<sub>2</sub>. *C. r. Soc. Biol. Paris* 106 574 (1931).
- Friedenreich, V.: Sur les sous-groupes du groupe sérologique. Y-a-t-il des transitions entre les sous-groupes A<sub>1</sub> et A<sub>2</sub>? *C.r.Soc. Biol. Paris* 106 578 (1931).
- Dahr, Peter: Welchen Beweiswert hat ein Vaterschaftsausschluss mit den A<sub>1</sub>, A<sub>2</sub> Untergruppen? *Dtsch. Z. gerichtl. Med.* 39, 14 (1948/49).
- Witebsky, E.: Interrelationship between the Rh system and the ABO system. *Blood* 3, Suppl. 2, 66 (1948).
- Laguna, St.: *Klin. Wschr.* 1930, 547.
- Haselhorst, G. u. A. Lauer: Über eine Blutgruppenkombination Mutter AB und Kind O. *Z. Konstit.lehre* 15, 205 (1931); 16, 227.
- Boltz, Werner: Phänotypische Latenz und Spätmanifestierung der Blutgruppe A<sub>2</sub>B. *Beitr. gerichtl. Med.* 18, 109 (1949).
- Andresen, P. H.: Reliability of the exclusion of paternity after the MN and ABO systems elucidated by 20,000 mother-child examinations and its significance to the medicolegal conclusion. *Acta path scand.* (Kobenh.) 24, 545 (1947).
- Davidsohn, Israel, Philip Levine and Alexander S. Wiener: Medicolegal application of blood grouping tests. A report of the committee on medicolegal problems. *Amer. Med. Assn.* 1952.

er Schwan-  
stimulierung  
Bedeutung.  
ous loss of  
s as demon-  
) 158, 880  
he detection  
59. 779 (1947).  
gen über den  
llidontest").  
angue (Mi-  
tti 'azione degli  
51).  
n Rh testing  
737 (1947).  
richtl. Med.  
finerva me-  
subgroups of  
mun. 61, 171  
subgroups of  
mun. 60, 597  
types. Bull.  
ther case of  
capacity for  
: In vivo iso-  
sease. *Lancet*  
tr nsfusionnelle  
jusqu'ici non  
equent occur-  
: notypical as-  
).  
ood group L<sub>2</sub>.  
path. scand.  
secretor char-  
up characters  
r. *Path.* 30, 198  
A new human  
oup. *Heredity*  
*Austral.* 2, 9  
" *Nature* (Lond.)  
ysteme. *Z. Im-*

139. Landsteiner, K., W. R. Stratton and M. W. Chase: *J. of Immun.* **27**, 469 (1934).
140. Ikin, Elizabeth W. and A. E. Mourant: A rare blood group antigen occurring in negroes. *Brit. Med. J.* 1951, 456.
141. Elbel, H. u. O. Prokop: Ein neues erbliches Antigen als Ursache gehäufte Fehlgelburten. *Z. Hyg.* **132**, 120 (1951).
142. Dahr, P. u. M. Kindler: Ist die von Löns angegebene Methode für einen positiven Vaterschaftsnachweis jetzt schon gerichtlich anwendbar? *Z. Immunforsch.* **108**, 427 (1951).
143. Ponsold, A. Der "Positive Vaterschaftsnachweis" nach Löns. (Blutgruppenabteilung des Gerichtsmedizinischen Institutes Münster i Westf.) Sept. 1951.
144. Ponsold, A.: Das Löns-Verfahren im Rahmen der erbbiologischen Begutachtung. *Zbl. Bakter. I. Orig.* **158** (1952).
145. Hirsfeld, L.: Wege und Ausblicke der Blutgruppenforschung für die Feststellung der Vaterschaft. Aus Anlass der 40jährigen Anwendung. *Schweiz. Z. Path. u. Bakter.* **15**, 257 (1952).
146. Speiser, P.: Der derzeitige Stand der Rhesusfrage. *Wien. klin. Wschr.* 1948, Nr 35/36.