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On individual differences in human blood

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Owing to the difficulty of dealing with substances of high molecular weight we are still a long way from having determined the chemical characteristics and the constitution of proteins, which are regarded as the principal constituents of living organisms. Thus it was not the usual chemical methods but the use of serological reagents which led to an important general result in protein chemistry, namely to the knowledge that the proteins in individual animal and plant species differ and are characteristic of each species. The diversity is increased still further by the fact that the different organs contain special proteins, and it therefore appears that in living organisms specific building materials are necessary for each particular form and function, whereas man-made machines, performing a wide variety of operations, can be produced from a limited number of substances.

The problem raised by the discovery of biochemical specificity peculiar to a species - the subject of the investigations which we are about to discuss - was to establish whether the differentiation extends beyond the species and whether the individuals within a species show similar though smaller differences. Since no observations whatever had been made in this direction, I selected the simplest experimental arrangements available and the material which offered the best prospects. Accordingly, my experiment consisted of causing the blood serum and erythrocytes of different human subjects to react with one another.

The result was only to some extent as expected. With many samples there was no perceptible alteration, in other words the result was exactly the same as if the blood cells had been mixed with their own serum, but frequently a phenomenon known as agglutination - in which the serum causes the cells of the alien individual to group into clusters - occurred.

The surprising thing was that agglutination, when it occurred at all, was just as pronounced as the already familiar reactions which take place during the interaction between serum and cells of different animal species, whereas in the other cases there seemed to be no difference between the bloods of different persons. First of all, therefore, it was necessary to consider whether

the physiological differences discovered between individuals were in fact those which were being sought and whether the phenomena, although observed in the case of blood of healthy persons, might not be due to endured illnesses. It soon became clear, however, that the reactions follow a pattern, which is valid for the blood of all humans, and that the peculiarities discovered are just as characteristic of the individual as are the serological features peculiar to an animal species. Basically, in fact, there are four different types of human blood, the so-called blood groups. The number of the groups follows from the fact that the erythrocytes evidently contain substances (iso-agglutinogens) with two different structures, of which both may be absent, or one or both present, in the erythrocytes of a person. This alone would still not explain the reactions; the active substances of the sera, the iso-agglutinins, must also be present in a specific distribution. This is actually the case, since every serum contains those agglutinins which react with the agglutinogens not present in the cells - a remarkable phenomenon, the cause of which is not yet known for certain. This results in certain relationships between the blood groups, which make them very easy to determine and which are shown in the following scheme. The groups are named according to the agglutinogens contained in the cells. (The sign + in the table indicates agglutination.)

Serum of group	*Agglutinins in serum*	*Erythrocytes of group*			
		O	*A*	*B*	*AB*
O	$\alpha\beta$	−	+	+	+
A	β	−	−	+	+
B	α	−	+	−	+
AB	−	−	−	−	−

The question now arises whether iso-agglutination by normal serum is confined to human blood or whether it also occurs in animals. In fact such reactions are found but are distinct in only a small number of species and are hardly ever as regular as in man. Only the highest anthropoid apes - whose blood corpuscles, though scarcely their proteins, differ from those of man - have blood group characteristics, which, in so far as we have yet been able to establish, correspond completely to those of man.

It can be assumed that a comparative examination of a large number of animal species will help to explain how the groups are formed - a phenom-

enon which is not fully understood. One noteworthy result of the examination of animal blood has already been obtained. Very soon after the first observations on iso-agglutination had been made, Ehrlich and Morgenroth described experiments in which, by means of blood-solvent antibodies (isolysins), they demonstrated differences in the blood of goats which arose when the animals were injected with blood of other individuals of the same species. In this case, however, no typical blood groups but, instead, numerous apparently random differences were found - a result which, except possibly for the intensity of the reactions, is roughly what one might have expected. Similar investigations, especially those conducted by Todd on cattle and chickens (Landsteiner and Miller; Todd) indicated almost complete individual specificity.

The apparent contradiction between the observations on man and those on animals has recently been resolved. There were already some pointers in this direction, and I - working in conjunction with Levine - obtained significant results by using special immune sera which had been produced by injecting human blood into rabbits; these results led to the discovery of three new agglutinable factors present in all four groups. Thus, when the breakdown of groups A and AB each into two subgroups (v. Dungern and Hirszfeld; Guthrie *et al.)* - which had recently been subjected to a thorough study at my laboratory and by Thomsen - was taken into account, it was found there were at least 36 different types of human blood. In addition it was shown that weak iso-reactions (Unger, Guthrie et al.; Jones and Glynn; Landsteiner and Levine), which do not follow the group rule and which vary in their specificity, are more common than had previously been assumed - irregular reactions, which can indeed easily be distinguished from the typical ones and which in no way affect the validity of the rule of the four blood groups. These findings justify the assertion that very numerous individual blood differences exist in man, too, and that there are certainly other differences which could not yet be detected. Whether each individual blood really has a character of its own, or how often there is complete correspondence, we cannot yet say.

For the time being, at least, these facts have no importance with regard to the therapeutic application of the blood groups, which will be discussed later, and yet they probably have a close bearing on an important field of surgery, namely the grafting of tissues.

It had long been known that grafts, for instance of skin, were much more successful when the material to be grafted came from the same individual,

and the results were similar where transplantable tumours were transferred to different strains of an animal species - as first described by Jensen. The experience of surgeons was confirmed by investigations on animals, among which the important series of experiments conducted by L. Loeb merit particular mention. Loeb's experiments consisted in the grafting of different tissues taken from an animal's own body, from related and unrelated animals of the same stock, and from members of different varieties and species. The success of the grafts was generally speaking in reciprocal relation to the degree of affinity, and in the light of the observations as a whole it was possible to conclude that the tissues of separate individuals must possess special biochemical characteristics.

The agreement between results obtained by two independent methods is so striking that the immediate assumption is that the differences which give rise, on the one hand, to the individual differences detectable by serum reactions and, on the other, to the individual-specific behaviour of grafted tissues are substantially of the same type. The reason for this assumption is that the group features can be demonstrated in the organ cells as well as in the blood. However, experiments based on this assumption - tissue grafts with blood groups taken into account - gave no clear result. But this is understandable, since the blood groups account for only some of the actually existing serological differences, and even apparently slight deviations can affect the permanent healing of tissues. This removes the doubt raised by these experiments, and the most probable assumption is that the two series of phenomena - the serological difference between individuals and the graft specificity - are basically related and rest on chemical differences of a similar kind. Consequently, the possibility of using the serum reactions for the important work of graft therapy can certainly not be ruled out, but existing knowledge justifies no more than a hope.

To the question of the chemical nature of the individual-specific substances - which I shall now examine - the answer is entirely of a negative character but it is nevertheless not without interest. The praecipitin reactions -mentioned at the beginning of this lecture - which revealed the species difference between proteins gave rise to the view that the substrates of all serological reactions were proteins or substances closely related to them. At first this view was shaken by the study of blood antigens. The solubility of specific substances in organic solvents and in particular the investigation into the heterogenetic sheep's blood antigen (which had been discovered by the Swedish pathologist Forssman in sheep's blood and organs of different

animals), from which a substance specifically binding but not acting directly as an antigen can be separated by extraction with alcohol, led me to the view that the constituents of many cell antigens are not protein-like substances and only as a result of uniting with proteins become antigens, which are appropriately called "complex antigens". This theory was strongly supported by the fact that I was able to restore the antigen action of the specific substance by mixing with protein-containing solutions.

Analogous results were obtained from a study of certain specific substances present in bacteria (Zinsser). Whereas in the case of bacteria the chemical nature of the specifically binding substances (haptens) has been determined for certain - as being colloidal polysaccharides (Avery and Heidelberger) - a conclusive result in the case of the animal cell antigens has not yet been obtamed. It can nevertheless be stated that the biochemical individuality of the animal species rests on the existence of two different classes of species-specific substances (Landsteiner and Van der Scheer; Bordet and Renaux), which show basic differences in the nature of their occurrence.

With regard to the actual subject of this lecture the fact is that group-specific substances too can be extracted from the blood cells by means of alcohol, and in this state normally give rise to the formation of antibodies only in a mixture with antigenic proteins. It can therefore be concluded that the haptens vary within a species, whereas analogous serological differences between proteins are, admittedly, suspected but cannot convincingly be proved. Another peculiarity is the fact that haptens related in their reactions frequently occur in animal species which are very far apart in the zoological system. Thus, the iso-agglutinogen A is related serologically to Forssman's antigen, which is contained in sheep's blood, and therefore immune sera react both with sheep's blood and with human blood of groups A and AB, but not with blood of group O or B (Schiff and Adelsberger). Still more noteworthy is the presence of similar structures in bacteria. This emerges from the fact that lysins from sheep's blood and apparently also agglutinins for blood of group A are present in many antibacterial sera, e.g. immune sera for paratyphus bacilli, and that a dysentery serum (recently described by Eisler), which agglutinates human blood, contains antibodies which influences to a higher degree the one of the two subgroups of group A which is the less susceptible to iso-agglutinin.

According to the result of investigations on artificial complex antigens the genesis of immune iso-antibodies indicating individual differences is probably due to the fact that as a result of combination with other substances

proteins peculiar to the species are enabled to induce the formation of antibodies. If, conversely, haptens identical or closely related with those of the animal are injected in association with foreign proteins, it appears that normally no antibodies arise. An example is provided by Witebsky's experiments, which showed that group-specific immune sera form, following injection of blood of group A, only in rabbits whose organs do not contain substances resembling agglutinogen A. However, experiments conducted by Sachs and Klopstock on the occurrence of Wassermann's reaction in rabbits following injection with foreign serum of mixed alcohol extracts of rabbit organs showed that the rule does not apply universally.

Whereas in this case the antibodies react only with organ extracts, O. Fischer succeeded - in experiments in which foreign serum of mixed extracts of rabbit's blood was injected - in producing auto-antibodies in rabbits which acted upon the intact blood cells but had a haemolytic action only after prior cooling, like the haemolysins which I, together with Donath, found to be the cause of the dissolution of blood in paroxysmal cold haemoglobinuria. This result and the difference between immune sera produced from extracts of erythrocytes of group O and group B, on the one hand, and from intact cells, on the other, indicate that the nature of the combining of the substances contained in the cells also has an influence on the antigen properties.

Following these brief observations on individual differences in blood and individual characteristics of the cell antigens I must now discuss the applications of the group reactions.

The relative frequency of the individual blood groups in various races has been dealt with in a well-nigh endless number of communications since L. and H. Hirschfeld made the noteworthy observation that characteristic differences in this connection are found in different races. Their most important finding was that group A is more frequent than B in northern Europeans, whereas the position is reversed in several Asiatic races. Another striking example is that of the American Indians who, when racially pure, belong almost exclusively to group O (Coca; Snyder), from which it is concluded that in the few cases where groups A and B do occur this is due to mixing of races.

I am not qualified to discuss the results of anthropological investigations on blood groups and the conclusions drawn from them, and in any case various authors differ in their opinions regarding the general principles on which interpretation should be based and regarding individual problems. Never-

theless, the majority view seems to be that the behaviour of the blood groups in conjunction with other anthropological features allows us to draw conclusions regarding the relationship and origin of human races and is of some importance to anthropological research.

One practical application of the group characteristics which immediately suggested itself was the distinguishing between human blood stains for forensic purposes. By means of the praecipitin reactions (Kraus; Bordet; Uhlenhuth) it is not difficult to determine whether a blood stain is of human or animal origin, but forensic medicine knew no way of distinguishing between blood stains from different persons. Since the iso-agglutinins and the corresponding agglutinogens will also keep for a considerable time in a dried condition, the problem can in certain cases be solved, in particular when the bloods in question, e.g. that of the accused and that of the victim, belong to different groups. Reasons for using this method do not of course occur very often and in your country in particular the occasions for using it are few and far between, but nevertheless the test - according to a report by Lattes, who was the first to use it in forensic cases - has proved useful in a number of cases and has been the basis of court verdicts and of the acquittal of accused persons.

To a far greater extent the group reactions have been used in forensic medicine for the purpose of establishing paternity. The possibility of arriving at decisions in such cases rests on the studies of the hereditary transmission of the blood groups; the principal factural results in this field we owe to the work of von Dungern and Hirszfeld. As a result of their research it became established that both agglutinogens A and B are dominant hereditary characteristics and that transmission of these characteristics follows Mendel's laws. The importance of this lies in the fact that in man there is scarcely any other unequivocally identifiable physiological characteristic with such simple hereditary behaviour. The genetic theory that there are two independent pairs of genes, formulated by the above-named authors, had to be abandoned following a statistical investigation by Bernstein. Provided that a population is sufficiently mixed the frequency of the inherited characteristics can be calculated on the basis of a certain genetic hypothesis. Bernstein made this calculation and found that the observed figures were constantly different from the figures calculated on the basis of the theory put forward by von Dungern and Hirszfeld. Complete agreement, on the other hand, was found when the calculation was based on the hypothesis that there are three allelomorphic genes localized at one position in the chro-

mosome. The assumption also leads to certain consequences regarding the children of AB parents, and these consequences - except for a few very isolated cases, which, however, may be explained in accordance with Bernstein's theory - have likewise been proved by experience, as extensive investigations by Thomsen, Schiff, Snyder, Furuhata, and Wiener have shown, and therefore the new theory is now almost universally accepted.

As far as the forensic application is concerned the law of dominance of A and B is decisive. Thus, paternity can be excluded in all those cases where a child belongs to group A or B and where these characteristics are absent in the mother as well as in the alleged father. This test is used fairly frequently in a number of countries - especially in Germany and Austria, though also in Scandinavia. In a survey which appeared last year Schiff reported on some 5,000 forensic investigations in which paternity was excluded, in more than 8% of cases, whilst a calculation of cases in which exclusion would have been possible gives a proportion of approximately 15 to 100. In favour of the method, it can be mentioned that it has also been instrumental in inducing some fathers to recognize their illegitimate children.

It will perhaps be of interest to show how a further development of the paternity diagnosis might be possible. In the light of preliminary results (Landsteiner and Levine) on the transmission of two of the above-mentioned blood characteristics, which are detectable with immune sera and are known by the letters M and N, the most probable assumption is that their presence is due to a pair of genes of which neither is dominant with respect to the other, so that when both are present a mixed type occurs. The existence of three phaenotypes M+N-, M-N+, and M+N+ is then explained by the fact that the third corresponds to the heterozygous form whilst the first and second correspond to the homozygous forms. Accordingly the heterozygous form can be recognized directly as a special phaenotype. The consequences of this hypothesis can be seen from the following schematic representation :

Marriages	*Offspring to be expected*		
	M + N +	*M + N −*	*M − N +*
M + N + × M + N +	50	25	25
M + N + × M − N +	50	0	50
M + N + × M + N −	50	50	0
M + N − × M − N +	100	0	0
M + N − × M + N −	0	100	0
M − N + × M − N +	0	0	100

Our own observations showed some exceptions to these rules, and this prevented us from finally accepting the hypothesis. It is possible, however, that these deviations may have been due to illegitimacy or to imperfections in the experimental method, which is not so simple as determination of the group, and in fact Schiff has found complete agreement with expectation in recently communicated observations on heredity and population statistics. New, unpublished results by Wiener are almost as good.

If this hypothesis should further prove correct the possibility of excluding paternity would be approximately doubled, i.e. a judgment would be possible in roughly one case in three. Even on the basis of data already available, however, assertions can be made with a considerable degree of probability. Use of the subgroups of groups A and B may make possible a further advance (Landsteiner and Levine; Thomsen), if future experience confirms the suspected laws.

More important to practical medicine than the subject with which we have just been dealing is the use of the blood-group reaction in transfusions. It would take too long to go into the details of the interesting history of the transfusion, which goes back for centuries, namely to the time of Harvey's discovery of the circulation of the blood. The possibility of carrying out transfusions had already been debated before this, but the first successful transfusions, prompted by Harvey's great discovery, were performed by Lower on dogs, in 1666 in England, and the next year the first transfusions of animal blood to humans were carried out by Denys in France, and by Lower and King in England. Further efforts were then directed to the inventing of special appliances and led to the experience that there is no need to transfer the blood from vessel to vessel but that even defibrinated blood can be used (Bischoff, 1835). The first transfusion with human blood was probably carried out by Blundell during the first half of the 19th century.

How differently the prospects were assessed can be illustrated by two remarks, which I quote from Snyder. In a history of the Royal Society, Sprat (1607) says: "Hence arose many new experiments, and chiefly that of transfusing blood - that will probably end in extraordinary success." Again in a *History of the Royal Society,* by Thompson (1812), we find the passage : "The expected advantages resulting from this practice have long been known to be visionary." The aim of introducing the method into regular medical practice was not achieved, despite great efforts and lively discussions of the question, and the idea was finally abandoned and that because the operation,

though often very useful, sometimes resulted in serious symptoms and even in the death of the patient.

With regard to the injection of animal blood the reason for the disasters followed from the observations of Landois, who as far back as 1875 discovered the phenomena of agglutination and haemolysis, which frequently occur when blood is brought into contact with serum from an alien species. However, it remained a mystery why the introduction of human blood into the circulation may also be dangerous, since it was regarded as obvious that serum or plasma was an inert medium as far as cells of the same species were concerned, a conviction which may have been strengthened by the fact that such sera were used in histological examinations.

The simple solution to the problem was provided by the discovery of individual differences in blood, and of the blood groups. Animal experiments and more particularly clinical experiments with cases where mistakes were made in group determinations have confirmed this connection and leave no doubt that the transfusion of agglutinable human blood is normally accompanied by harmful consequences. However, the pathogenesis of transfusion shock has not yet been fully explained.

The first blood transfusion in which the agglutinin reaction was taken into account was carried out by Ottenberg, but it was only during the emergencies of the Great War that the method oftransfusion with serological selection of donor was widely adopted - a method which has since remained the normal practice.

It would be out of place here to go into such details as the sources of error in group determination, their control by direct comparison of the blood of the recipient with that of the donor, and the precaution of beginning the transfusion by injecting small quantities of blood. It should only be mentioned that it is not absolutely necessary to use blood of the same group, but that in stead of this, blood of group O for instance (see Ottenberg), the cells of which are not affected by the serum of the recipient, can also be used. In this case, however, care must be taken to exclude donors whose serum has a high agglutinin content, as this can be dangerous, especially to severely anaemic and weakened patients. Use of blood from so-called "universal donors" belonging to group O or of non-agglutinable blood of any alien group can be of great value in emergency cases and for recipients belonging to the rare blood groups.

The most obvious indication for blood transfusion is acute or chronic anaemia, e.g. as a result of wounds or lung haemorrhages, in obstetrical cases

and in those of gastric and duodenal ulcer. The effect, which in cases of haemorrhage often means the saving of a patient's life, is of course primarily due to the replacement of blood, an important factor here being that the transferred erythrocytes retain for several weeks their functional capacity in the circulation. Other important effects are haemostasis due to increased coagulability and presumably also stimulation of blood regeneration in the bone marrow, as has been concluded from changes in the histological blood picture. However, transfusion therapy which used to be widely used for pernicious anaemia has now become almost superfluous as the result of the discovery of liver therapy.

Another wide field of application is shock following severe injury and operations, and it is assumed that in these cases the introduction of blood has a better effect than injection of isotonic solutions, such as the acacia (gum arabic)-containing solution of common salt recommended by Bayliss during the war. In accordance with this indication transfusions are given, often with great success, after major operations - not only for the purpose of replacing blood but also to serve as a stimulant. American surgeons also recommend the treatment before major operations where the patient is in a weakened condition. Good results have also been obtained with haemophilia, thrombopenic purpura and to some extent with agranulocytosis, carbon-monoxide poisoning and burns, whereas with a series of other diseases, e.g. septicaemia, for which transfusion therapy has been tried, the results have been doubtful.

Some figures which I quoted in a report to the Microbiology Congress in Paris provide information on the frequency with which transfusions are given and the relative safety which has been achieved with this method - though it must be remembered that this success is partly due to the great advances in surgical methods. There is a slight variation in the statistics, as some authors in contrast to others still have isolated failures to report. Since these differences are probably connected with the transfusion technique I think I am justified in basing my judgment on the favourable reports, provided that they cover a large number of cases.

The number of transfusions given is surprisingly large, and it may well be that use of this technique has been taken too far. According to statistics which were kindly made available to me by Dr. Corwin of the Academy of Medicine, some 10,000 transfusions were given in New York during 1929. In a recently published communication by Tiber from the Bellevue Hospital in New York, 1,467 transfusions carried out during the three-and-a-half years up to July 1929 are reported. Among these there were two deaths, one

due to incorrect blood-group determination, the other - also probably avoidable - involving a group-A baby in poor condition which was given a transfusion of blood from a so-called "universal donor" of group O. Three fatalities which occurred among the 1,036 cases quoted in a report by Pemberton at the Mayo Clinic were caused by errors in blood-group determination. At Kiel, as reported to me by Dr. Beck, 2,300 transfusions were carried out over a period of about five years without one fatal accident. Mild aftereffects, such as shivering and pyrexia, were felt by 2-3% of the patients. One noteworthy case reported by Beck was that of a patient suffering from pernicious anaemia to whom he gave 87 transfusions within three-and-a-half years, without any serious symptoms.

Good though these results may be, isolated serious and even fatal accidents - which may not be due to technical errors - as well as frequent slight disturbances are still reported as we have already mentioned. It is unlikely that the differences in blood indicated by atypical iso-agglutinins were an important factor in these cases, and if this is so they could easily have been avoided. It has not been established for certain whether, as has been assumed, intense pseudo-agglutination has an injurious effect through the serum of the recipient. Some of the disturbances observed were probably due to allergy to nutritive substances present in the injected blood, whilst others were due to the action of antibodies which formed as a result of earlier transfusions. Another problem which has not yet been investigated sufficiently is whether differences exist between individual proteins, and, if so, whether these may cause antibodies to form.

All in all, the results of blood transfusions are already highly satisfactory, and we have reason to hope that a thorough study of cases with undesirable aftereffects will help us to assess the significance of the suspected causes and perhaps reveal unknown causes, and thus finally virtually eliminate the slight risks which transfusion still involves.

Apart from the solution of this practical problem, the subject with which we have been dealing can also be developed by a study of the biological problem of individual serological differences in general, and in particular by the further improvement of techniques for the finer individual differentiation of human blood as well as by a continuation of the genetic analysis of serological blood differences in humans and animals. As a result of work already done, at least two of the human chromosome pairs - apart from the sexual chromosome - can be regarded as characterized by a specific feature (see also F. Bernstein, in *Z. Inductive Abstammungs-Vererbungslehre,* 57 (1931) 113).