A CRITICAL INVESTIGATION OF THE BLOOD GROUPS AND THEIR MEDICO-LEGAL APPLICATION

T H E S I S

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by

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I am indebted to Professor John Glaister for suggesting the subject of blood grouping as one which would repay further investigation.

C O N T E N T S.

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S E C T I O N I

INDIVIDUALITY OF THE HUMAN BLOOD

The fact that the sera of certain normal persons have the property of agglutinating the red cells of certain other normal persons, led to Landsteiner's (1) discovery of three types of bloods. Shortly after, Decastello and Sturli (2) discovered a fourth type. According to the behaviour of the blood serum and cells, human beings were divided into four groups. Jansky (3) and Moss (4) classified these groups by numbering them I, II, III and IV, of which groups II and III were similar in the two classifications, but I and IV were reversed. In order to exclude the confusion produced by such difference, a new classification was recommended by the Health Committee of the League of Nations, which is the only one used in recent scientific literature.(1) The relation

(1) This classification will be followed throughout this thesis.

between these three systems to that originally designed by Landsteiner, is given in Table No. 1.

TABLE No. 1

CLASSIFICATION OF THE FOUR GROUPS			
LANDSTEINER	JANSKY	MOSS	INTERNATIONAL
C	I	IV	O
A	II	II	A
B	III	III	B
DECASTELLO AND STURLI	IV	I	AB

The reactions obtained when the sera of each of the four blood-groups are mixed with the red cells of persons of each group as was originally discovered by Landsteiner and Decastello and Sturli, are given in Table No. 2.

TABLE No. 2

SERUM OF GROUP	TESTED WITH CELLS OF GROUP			
	O	A	B	AB
O	-	+	+	+
A	-	-	+	+
B	-	+	-	+
AB	-	-	-	-

\+ = cells agglutinated.
\- = cells not agglutinated.

Landsteiner explained these reactions by assuming the existence of two agglutinable substance (agglutinogens) in the red cells and two

corresponding agglutinating substances in the serum. Dungern and Hirszfeld (5) named the agglutinogens A and B, and the agglutinins (a) and (b).(1) In order to ascertain the relation between the agglutinin and agglutinogen present in each blood, Landsteiner formulated the following law.

> "In a given blood containing a given agglutinogen the serum will also contain all the iso-agglutinins which are incapable of acting on the said agglutinogen".

If agglutinogen A is present in the cells of group A and agglutinogen B is present in the cells of group B as was originally designated by Dungern and Hirszfeld, the distribution of the group substances in the different groups will be as shown in Table No. 3.

TABLE No. 3

	DISTRIBUTION OF	
GROUP	AGGLUTINOGEN IN THE RED CELLS	AGGLUTININ IN THE SERUM.
O	Nil. - O	(a) and (b)
A	A	(b)
B	B	(a)
AB	A and B	Nil. (o)(2)

According to Tables Nos. 2 and 3, the blood-group of an individual can be determined by testing the blood cells of this individual against known sera of groups A and B and by testing the serum against known cells

(1) These letters are meant to represent the greek α and β, but owing to the difficulty of printing these letters, they have been substituted by the Roman small letters (a) and (b) which are bracketed.

(2) The small letter (o) will be used to designate the absence of both agglutinins from the serum.

of these two groups. This is shown in Table No. 4.

TABLE No. 4

UNKNOWN SERUM OF BLOOD No.	KNOWN CELLS OF GROUP A	KNOWN CELLS OF GROUP B	UNKNOWN CELLS OF BLOOD No.	KNOWN SERA OF GROUP A	KNOWN SERA OF GROUP B	GROUP OF THE UNKNOWN BLOOD
1	+	+	1	-	-	O
2	-	+	2	-	+	A
3	+	-	3	+	-	B
4	-	-	4	+	+	AB

NATURE OF THE GROUP SPECIFIC SUBSTANCES AND THE REACTIONS WHICH TAKE PLACE BETWEEN THEM

The chemical composition of the agglutinogens was first thought to be of a protein or lipoidal nature. Recently Schiff (6) and others have isolated a group specific substance of a carbo-hydrate nature from the cells of group A. Nothing is yet known about the nature of the iso-agglutinins (a) and (b) except that they are attached to the globulin fraction of the serum. The fact that the agglutinogens A and B are antigenic in nature was shown by Landsteiner (7) and Dungern and Hirszfeld (8). They proved that when cells of groups A or B are used to immunize animals, the anti-serum derived from these contains besides the human species agglutinin, a specific agglutinin against the cells used. In order to obtain a specific immune serum against A or B cells, the species agglutinins must be removed from the serum of the immunized animal, by absorbing it with human cells which do not contain the agglutinogen concerned, for example, cells of group O. Such immune sera are similar in their action to normal

sera of groups B and A respectively, but usually are much stronger, especially if the immunization was carried out over long periods.(1) The immune anti-A and B sera thus prepared are not suitable for carrying out the agglutination test since they usually contain immune antilysins besides the agglutinins, therefore hemolysis of the cells takes place. In order to abolish the action of these lysins the serum is inactivated by heating for half-an-hour at a temperature of 55°C. But when the immune sera are kept in an ice-chest for a long time (4-6 weeks), they lose their hemolysing action and therefore need not be inactivated.

The agglutinogen A was found by Schiff and Adelsberger (9) to be related to Forssman's antigen found in the sheep's red cells. This relation appears from the following observations:-

1) The sera of certain rabbits immunized with sheep's cells can agglutinate the cells of group A.
2) The sera of certain rabbits immunized with A cells can hemolyse sheep's cells.

These reactions do not occur if the anti-sheep sera are absorbed with sheep's cells or if the anti-A sera are absorbed with cells of group A. The fact that the anti-sheep immune serum does not contain anti-human species agglutinins, has led to its frequent use in testing for cells of group A, since no absorption is required, as in the case of sera produced by immunizing animals with human cells of group A. The fact

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(1) For convenience, the writer will use the following terms to differentiate between the immune and natural sera acting on either A or B cells.

a) Normal anti-A (Normal human serum of group B).
b) Immune anti-A (Immune serum against A cells).
c) Normal anti-B (Normal human serum of group A).
d) Immune anti-B (Immune serum against B cells).

that not all immune anti-sheep sera can agglutinate cells of group A suggests that the antigen of group A cells, although related, nevertheless is not identical with Forssman's antigen.

From the above-mentioned facts one may conclude that the reaction which takes place between these agglutinins and agglutinogens is immunological in nature. Like other similar reactions it depends on the union of the antigen (agglutinogen) and its antibody (agglutinin) and in this particular case manifests itself through the agglutination of the cells. Consequently, one would expect that the agglutinin will disappear from the serum when this is mixed with a suitable quantity of cells and after a period of contact is separated and tested. This is confirmed by the following experiment:-

A packed sediment of cells of group B is prepared by taking the blood in citrated saline in order to prevent clotting and the cells separated from the serum by centrifuging. In order to remove all traces of the serum, the cells are washed with saline and re-centrifuged. One part of the cell sediment is mixed with two parts of anti-B serum (normal or immune) in a test tube. The tube is allowed to stand at room temperature for 1-2 hours after which the serum is separated by the centrifuge and tested with cells of group B, when it will be found no longer active.// This method of absorption has been widely employed in investigating the mechanism of blood-grouping and has been the basis on which several theories have been formulated, especially as regards sub-division of the groups. The method involves:-

a) The determination of what agglutinins a particular specimen of red cells can remove from the serum, and comprises also a study of the

behaviour of these agglutinins, when they are again split off by heating the treated red cells at 55°C, so that the agglutinins exist in an active state in the fluid when the cells are removed by the centrifuge.

b) The estimation of the agglutinating action of the absorbed sera in various red cells.

The union between the agglutinins and the agglutinogens takes place over a range of temperature 0°C - 45°C, but agglutination is stronger and occurs more rapidly at 0°C and decreases in proportion to the increase of temperature. The possibility of splitting the agglutinin off the cells used in absorbing the serum proves that the agglutinin has united with the agglutinogen. This is described in the following experiment according to the method used by Landsteiner (10).

Anti-B serum is absorbed for 2 hours at room temperature with one tenth its volume of washed packed cells of group B. The agglutinated cells are separated by centrifuging and then washed three times with cold saline in order to remove the serum thoroughly. A suspension of these cells is made by adding to them an equal volume of saline. The mixture is allowed to stand in a water-bath at 55°C, for five minutes during which the tube is shaken. At this stage the agglutination disappears and if the mixture is centrifuged in a tube with a jacket filled with water at 55°C, it will be found that the separated saline can agglutinate cells of group B.

The specificity of the reactions obtained by these group specific substances can be demonstrated as follows:-

1) Anti-A or B sera whether natural or immune agglutinate only their respective cells.

2) Anti-A serum is exhausted of its agglutinin by absorbing it with a

sufficiency of cells of group A. The same occurs when anti-B serum is absorbed with B cells.

3) Cells of group A are incapable of exhausting anti-B serum and B cells are incapable of exhausting anti-A. serum.

Occasionally it has been noticed that when the serum of group O is absorbed with cells of group A, these will not only absorb the (a) agglutinins but also, to a certain extent, the (b) agglutinin. The same occurs with regard to the agglutinin (a) when B cells are used. This has been explained by Thomsen (11), on the assumption that the compound produced by the union of the agglutinogen A and agglutinin (a) possesses the property of binding the unspecific agglutinin. He claims that cells of group A, although they cannot normally absorb the agglutinin (b) found in group A serum, acquire the ability to do so, if they have been previously used to absorb the agglutinin (a). A more reasonable explanation given by Landsteiner (12) is that the two agglutinins are linked together so that when one is specifically absorbed, the other will be partly bound to it. The writer has found that this phenomenon is only noticeable with weak sera of group O, and therefore he could not confirm Thomsen's statement.

FACTORS AFFECTING THE AGGLUTINATION REACTIONS.

As mentioned, the union between the agglutinins and agglutinogens takes place more readily at low temperatures; the extent of agglutination is more marked at 0°C, than at higher temperatures. The maximum temperature at which agglutination occurs depends on the sensitivity of the cells and the strength of the agglutinin. The maximum temperature at which a weak serum acts is less than that at which a strong serum can still produce

agglutination. The highest temperature at which agglutination still occurs is called by Bialosuknia and Hirszfeld (13) the "thermal amplitude" of the serum. They have found that it is proportional to the titre of the serum i.e. the highest dilution at which the serum can still produce agglutination. The highest thermal amplitude met with is about 45°C. above which temperature no union takes place between the agglutinin and the antibody. According to Bialosoknia and Hirszfeld, the serum contains several fractions of the agglutinin which can be separated by absorbing the serum at various temperatures. For instance, if a serum of group B is absorbed with A cells at 37°C. it can no longer agglutinate cells of group A at this temperature, but it can agglutinate them at lower temperature (0°C. - 25°C.) If the serum is absorbed at 25°C. it can still agglutinate the cells at lower temperature than this. They assume that these fractions absorbed at various temperatures possess different affinities towards the cells. A fraction with high affinity unites with the cells at higher temperature and a fraction of weak affinity can only unite with the cells at a low temperature.

Although a low temperature is more convenient for the production of the reactions, yet it is not suitable for the test of blood-grouping since other agglutinins acting only at low temperature, are found in the human blood. In order to exclude the latter, the test should be performed at least at room temperature (20°-25°C). Naturally the stronger the agglutinin found in the serum, the stronger and quicker will be the reaction. Similarly, the more sensitive the cells towards the agglutinin, the stronger will be the reaction obtained.

On account of the presence of other phenomenae of hemo-

agglutination which may simulate the above-mentioned reactions, certain precautions should be taken when the group of a blood is being determined, otherwise mistakes are liable to occur. A brief description of such phenomenae and the precautions to be taken is given below.

PSEUDO-AGGLUTINATION.

If the serum of individuals suffering from certain infectious diseases is mixed with the cells of other persons of the same group, it will be noticed that the cells are sedimented more quickly than if they have been suspended in a normal serum of the same group. When the cells mixed with the former serum are examined under the microscope, they will be found to be attached together in the form of rouleaux - and that is the reason why the sedimentation was quicker, since these rouleaux are heavier than the separate cells and settle down more rapidly. This phenomenon is thought to be due to an increase in the fibrinogen content of the serum of such persons. When even a normal serum of group AB is used in high concentration, there is a tendency to the formation of such rouleaux, which may unite together and thus form small red clumps visible to the naked eye. The reaction takes place at room temperature and equally well at 37°C. Although these appearances simulate the clumps produced by the action of iso-agglutinins on their corresponding cells, nevertheless it is usually easy to differentiate between them by microscopic examination, since one can readily distinguish the lumps of true agglutination from the chain-like rouleaux in cases of pseudo-agglutination. However, it is not safe to depend on this method because the writer has seen some cases where the clumps were in all respects similar to those of

/12

real agglutination. In order to prevent the occurrence of this phenomenon, Lattes (14)recommended the addition of lecithin to the cell suspension. The writer has found that diluting the serum with 2 volumes of saline and preparing a suspension of not over 5% concentration will be sufficient to exclude the errors which might arise from pseudo-agglutination.

UNSPECIFIC AGGLUTINATION DUE TO CERTAIN BACTERIA.

Hubener (15) and Thomsen (16) and others found that the cells, after being left in the laboratory, may become agglutinable by all sera, even by that of group ABo. This was found to be due to infection with certain micro-organisms. Friedenreich (17) discovered two bacilli (M and J) and Imamura (18) discovered another two (K and S), all of which, as well as others like vibrio cholera, can produce this phenomenon if the cells or the serum become infected with them. Thomsen and Friedenreich are of the opinion that the reactions obtained are due to the presence of latent receptor and agglutinin which are called into action by these bacteria. The reaction takes place at room temperature and usually fails to appear at 37°C. Therefore it was recommended by Sachs and Klopstock (19) to perform the test for group determination at 37°C. in order to prevent the occurrence of this phenomenon. It is true that the iso-agglutination reaction takes place at this temperature, but the reactions at such a temperature are much weaker than at room temperature and may not take place if the serum used is weak or the cells are only slightly sensitive. The addition of formalin was also suggested to inhibit the action of bacteria. According to the writer's own experience in performing the test, it suffices to take precaution to ensure the sterility of the material used.

COLD AND AUTO-AGGLUTINATIONS.

Auto-agglutination was thought to be a form of pseudo-agglutination, but the work of Landsteiner (20) and his assistants has shown that it is due to an agglutinin which can be absorbed by the cells of the same blood. The agglutinin can also be recovered from the cells in the same manner as the iso-agglutinin (a) and (b) previously described. The only difference between the reactions of these and that of the auto-agglutinin is that the latter acts only at low temperatures (0°C - 5°C) and it only acts at room temperature very seldom.

Another similar type of agglutinin is that described by Landsteiner and his assistants, which acts only on certain cells of other groups at low temperatures. These agglutinins, although weakened, yet are not absorbed completely by the individuals own cells and therefore were considered to be specific for certain agglutinable substance in the cells agglutinated by them. However, it was impossible to draw any conclusion with regard to their specificity on account of the variations in the reactions and the difficulty encountered in performing the test at 0°C.

In order to avoid confusion with these reactions the sera to be used in the test should be separated only after leaving the whole blood to stand in an ice-chest, by which method the thermal amplitude of the cold and auto-agglutinin is greatly diminished and therefore if the test is afterwards applied at room temperature (20°- 25°C.) these will not be active.

In order to exclude any doubt about the specificity of the reactions obtained, one may control the reactions given by the sera by testing them with cells of group O and those given with cells by testing

/14

them with serum of group ABo. If there is agglutination in the control specimen, one may then doubt the results and repeat the test with other sera and another sample of fresh cells.

THE DEVELOPMENT OF THE GROUP SUBSTANCES IN THE BLOOD.

The agglutinogens are present in the stroma of the red cells: this can be demonstrated by the inhibiting action of the stroma to the serum, since they absorb the agglutinin in the same manner as the fresh cells. They are also found in most of the tissues and secretions of the body. Their presence here can only be demonstrated by the absorption method. The agglutinogens A and B are developed during early foetal life. Dungern and Hirszfeld (21) found them in a 6 months' foetus and Ohnesorge (22) in a 4 months' foetus, and Kemp (23) in embryos of 2-3 months. The agglutinins are present in the serum and the tissue plasma, milk, tears and other secretions, but not as regularly or so active as in the serum. They only appear in the serum of the child about the third month after birth and in certain cases not before the first year.

The sensitivity of the blood cells varies considerably according to age, and even in individuals of the same age and of the same group. The blood corpuscles of adults are more strongly agglutinated than those of children where there is a possibility of overlooking the agglutinogens, especially in certain cells of group A. Hence it is necessary to use very strong serum when testing children's red cells. Schiff and Hubener (24) found that some cells of group A are agglutinated at a titre of 1:25 while others of the same group are agglutinated up to a titre of 1:800. The difference between the sensitivity of the cells of Group B was not so marked.

/15

The titre of the sera of the same group varies also according to the age and in individuals of the same age. It also varies in the same individual from time to time.

DISTRIBUTION OF THE BLOOD GROUPS IN DIFFERENT POPULATIONS.

L. and H. Hirszfeld (25) were the first to point out that the frequency of the four blood-groups varies in different populations. The study of the distribution of the agglutinogens A and B in different populations revealed that the former is more frequent on the Continent and America than the agglutinogen B, which is more common in Asia. Table No. 5 shows the frequency of the blood groups in some populations, including those tested by the writer.

TABLE No. 5.

NATIONALITY	INVESTIGATOR	FREQUENCY OF GROUP				NUMBER EXAMINED
		O	A	B	AB	
Scots	Alexander	43.6	33.9	16.8	5.7	225
Scots	Writer	49.59	36.59	9.52	4.30	746
Egyptian	Shausha	24.3	32.6	29.2	13.9	417
Egyptian	Writer	26.6	35.8	27.1	10.5	754
German	Schiff	37.8	39.4	16.4	6.4	750
Italian	Mino	35.9	51.1	8.6	4.2	1391
Danish	Johansen	43.0	42.0	12.0	3.0	512
French	Kosovitch	42.1	42.3	11.1	4.5	962
Arab	Altounyan	38.0	34.0	20.0	8.0	1149
U.S.A.	Snyder	45.0	41.0	10.0	4.0	2000
Hindu(North)	L. & H. Hirszfeld	31.3	19.0	41.2	8.5	1000

THE INHERITANCE OF THE GROUP SPECIFIC SUBSTANCES.

The agglutinogens A and B were found to be inherited according to certain Mendelian rules. These will be described later when the theories suggested for their inheritance will be discussed. However it became an established fact that these agglutinogens cannot appear in the blood of a child except when present in the blood of at least one of the parents. With regard to the agglutinin, two different opinions are held by Furuhata and Bernstein respectively. The former is of the opinion that the agglutinins (a and b) are inherited like the agglutinogens. He assumes that they should only appear in the serum when the agglutinogen corresponding to them is absent from the cells. Bernstein assumes that the agglutinins are gradually developed after birth in the form of a universal antibody against the cells of all groups. When agglutinogen A or B is present in the cells, it absorbs in vivo the corresponding agglutinin, once it appears in the serum. These opinions will be discussed later on.

STABILITY OF THE BLOOD GROUPS.

Landsteiner and Richter (26) pointed out that once the blood-group of the individual is developed it remains constant through the whole of life. Few workers have reported that the blood-group was found to change after certain diseases like typhus, malaria, syphilis, carcinoma, and after treatment with certain medicaments or the application of rontgen rays or diathermy. Other rare cases reported were that the blood-group changed after transfusion. Owing to the importance of this question the subject has been investigated by several workers (27), who found that

/17

different treatments and rays had no effect whatever on the blood-group of a person. The blood-group of patients has been tested after blood transfusion and it has been noticed that the donor's blood cells, if of a different group from that of the recipient, could be identified in the mixture of the recipient's blood cells by adding a serum which agglutinated the former but not the latter. This is not a permanent change since the donor's cells disappear from the circulation after about seven weeks.(28). With regard to the serum, it may happen, as found by Landsteiner and his co-workers, than an immune iso-agglutinin to the red cells introduced may develop in the serum of the recipient, especially after repeated transfusions of the same blood. Such agglutinin will no doubt disappear after some time as is the case with other immune agglutinins. Therefore these temporary reactions cannot be interpreted as a definite change of the blood-group. Some workers have watched the blood-groups of several individuals for periods of over 20 years and no change was observed despite the occurrence of severe illness with different diseases during this period. During the last four years the writer had the opportunity of watching the blood-groups of several persons and found them to be constant, although some of them suffered from certain infectious diseases, and were treated with different drugs during this period. Moreover, if there were any relation between certain diseases and blood-groups, one would expect that the frequency of the latter would show an abnormal preponderance in persons suffering from these diseases , which by observation was proved not to be the case.

In no case where changeability of the blood-group has been alleged, have precautions been taken to exclude the unspecific types of agglutination namely:- pseudo-, auto- and cold-agglutination, and also

/18

agglutination caused by infection of the blood in vitrio with certain bacteria. The workers who asserted such changeability relied upon testing the blood cells alone and never controlled their results by testing the serum. Therefore it might have happened that the presence of a weak agglutinogen was overlooked, especially if the serum used in testing the cells was not strong. The importance of controlling the reactions obtained with the cells by examining those obtained with the serum is demonstarted in the following instance:-
Hubner (29) found a blood of a person who seemed to belong to group O according to the negative reactions given by the cells, the serum of which contained only (b) agglutinin. Later when this blood was re-examined, the cells gave weak reactions with normal anti-A serum, and only (b) agglutinin was found in the serum, hence it belonged to group A. Therefore one may explain this by the overlooking of the agglutinogen A which was weak in this particular case. This is supported by the fact that in the first and second examinations the serum showed the presence of agglutinin (b) only, which is characteristic of bloods of Group A. Had the serum not been examined in this case, it could have been considered a change of the blood group.

As the agglutinogens A and B are found in almost all tissues and cells and secretions of the body, it would be necessary, in order to change the blood group of a person, that a complete change-over of the system should take place. The fact that the agglutinogens A and B are regularly inherited according to certain mendelian laws strongly indicates that the blood group, once established in the body, will never change.

DEVIATIONS FROM THE SCHEME OF THE FOUR BLOOD GROUPS.

The classification of the human blood into four groups is based

on the presence of two agglutinogens (A and B) and two agglutinins (a and b), the distribution of which in each group was previously given.(1) According to this classification one may expect certain reactions to take place when samples of blood of different persons representing the four groups are examined by testing each serum against the cells of each blood sample.

These reactions are as follows:-

(1) The serum of group O agglutinates blood cells of group A, B and AB.

(2) The serum of group A agglutinates blood cells of groups B and AB.

(3) The serum of group B agglutinates blood cells of groups A and AB.

(4) The serum of group AB does not agglutinate the blood cells of any group.

According to this scheme, blood cells of group O are not agglutinated by any serum and also the blood cells of any person are neither agglutinated by the persons own serum nor with a serum of a blood of the same group. In addition, one may assume that the agglutinogens A and B are identical in all persons belonging to groups A and B respectively; therefore the cells of different persons in each of these two groups should behave similarly towards their respective agglutinins. Exceptions to the above-mentioned rules, however, were reported. Such exceptions may be divided into two types, namely:-

(1) The absence of expected reactions.

(11) The presence of unexpected reactions.

Each of these will be discussed separately below:-

(1) <u>The absence of expected reactions</u>:

The exceptions of this type may reveal themselves either on the part of the serum or on the part of the cells. With regard to the former, it has been infrequently observed that certain sera of group

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(1) See page 3

/20

O, A or B lack the agglutinin (a) and (b). On this account, Guthrie and his co-workers were led to suggest further classifications. Sera lacking the expected agglutinin seem to be very scarce, since Thomson (30) in 3500 bloods met only 6 cases. The writer examined more than 1000 bloods and did not find a case. This was also the experience of other workers. The absence of the expected agglutinin has never been proved to be a constant character of such sera. This, together with the established facts that the agglutinins are usually absent in newly-born children and that the titre of the agglutinins varies in different persons or even in the same person from time to time, makes the rare absence of the agglutinins in the sera of adults of no grave significance in relation to the validity of the four group scheme. This is supported by the assumption of Bernstein that the agglutinins are not inherited like the agglutinogens, which may be considered as the individualising factor.

The absence of expected reactions on the part of the blood cells reveals itself in respect of certain cells of groups A and AB not being agglutinated with a weak normal anti-A serum - these can only be agglutinated with strong normal anti-A serum, but even with such serum they are not so strongly agglutinated as other specimens of the same group. Therefore two types of cells are found in each of groups A and AB which may be called, for the purpose of differentiation, <u>weak and strong types</u>.

Dungern and Hirszfeld (31) were the first to show the difference between these two types of cells by the absorption method. They absorbed normal anti-A serum with group A cells of the weak type and then found that the serum agglutinated only group A cells of the strong type.

/21

Such unexpected reactions became one of the bases on which subdivision of groups A and AB was made.

(11) The presence of unexpected reactions.

Contrary to expectation, certain reactions were observed, which should not take place according to the scheme of the four blood groups. These reactions were obtained either with the sera or with the cells of certain individuals. The abnormal reactions of the sera are summed up in the following statements.

(1) The sera of certain individuals were found capable of agglutinating their own cells. The cause of such reactions has been previously discussed under the subject of auto-agglutination.(1) This does not interfere with the classification of the four groups, since the auto-agglutinins are only active at low temperature, seldom at room temperature but never at body temperature. A similar agglutinin has been found which acts only at low temperature on the blood cells of other persons of the same group. This occurs only in auto-agglutinating sera and persists when the auto-agglutinin has been removed by letting the serum stand in contact with its own corpuscles at a low temperature. This iso-agglutinin does not conform in its action to the scheme of the blood groups.

(2) The sera of certain individuals of any group were found capable of agglutinating the blood cells of other individuals of the same group at room temperature or even higher. In such cases the reactions were due to the presence of additional agglutinins(2)

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(1) See page 11

(2) The term abnormal agglutinins from now on will be given to agglutinins other than (a) and (B).

in the serum and respective additional agglutinogens in the cells. These agglutinins are characterised by their specificity since they agglutinate constantly the cells of certain persons and not their own cells. Unger (32), Guthrie and his assistants (33), and some others found sera containing these abnormal agglutinins and suggested the addition of new groups. Landsteiner and his co-worker, (34) who investigated this question in detail, came to the conclusion that there are other agglutinins in the sera of certain individuals, which are different from the (a) and (b) agglutinins previously described. These agglutinins are described below, according to the description given by these workers:-

(i) The agglutinin (a_1) is found in the sera of certain persons of groups A and AB: it agglutinates the cells of individuals who belong to the strong type of A and AB, representing the majority.

(ii) The agglutinin (a_2) which is found in the sera of certain persons, usually of group A, agglutinates the cells of persons who belong to the weak type of group A, but as a general rule, does not agglutinate the cells of the weak type of group AB. The sera containing this agglutinin are not so common as those containing (a_1) agglutinin, and are generally active at a lower temperature.

On account of the presence of these two agglutinins, together with the difference between the strong and the weak types of cells belonging to groups A and AB, Landsteiner and his co-workers classified each of these groups into two sub-groups, namely A_1 and A_2, A_1B and A_2B, as will be given in detail in Section 11.

(iii) Apart from the above-mentioned agglutinins, Landsteiner and Levine (35) found other sera containing a residual agglutinin which

/23

they called extra-agglutinin 'l'. This remained in the serum when all the other agglutinins so far mentioned have been removed by absorption. It may occur in sera of the various groups and agglutinates the blood cells of certain persons of every group. The reactions were not similar in all cases, since the blood cells of certain persons were agglutinated moderately and others weakly. They concluded that this agglutinin acts on a special agglutinogen found in the cells which they called P., the presence of which was later proved by the aid of immune and normal animal sera. Still further agglutinins have been found which acted in a different way from those described, as in the case reported by Ottenberg and Johnson (36) in which the serum of a person of group B agglutinated the cells of some other persons of group B and also some of group O. Landsteiner agreed to the unique character of this blood.

Such irregular case did not necessitate further classification since no other similar sera were found. However, it indicates that there are still other agglutinogens in the red cells which may differentiate between individuals of the same group.

Beside the normal reactions observed with the sera, other abnormal reactions were observed regarding the behaviour of the blood cells of certain individuals. Blood cells of group O which were supposed to be non-agglutinable by any serum, were reported by several workers to have been agglutinated with certain sera.

Landsteiner and Levine found that the agglutinin (a_2) which acts on A_2 cells, agglutinates also, and more strongly, all cells of group O. Thomson (37) and Schiff (38) are of the opinion that this agglutinin is specific for O cells and only acts on A_2 cells because of the presence of the O receptor in them. Schiff (39) found that

ox-serum, which agglutinates all human cells, after being absorbed by the blood cells of sub-group A_1B, agglutinates O cells and to a less extent A_2 cells. On the basis of this observation he introduced a new conception, namely, that the cells of group O are characterised by the presence of another agglutinogen and not by the mere absence of agglutinogens A and B.

Hirszfeld (40) contradicts this opinion by assuming that the ox-serum contains only species agglutinins which act on the cells of all human beings to a variable extent.

He assumes that O cells on account of the absence of agglutinogens A and B contain more species receptors than the cells of other groups, and also A_2 cells have more of these receptors than A_1 or A_1B, because of the weakness of their agglutinogen. Therefore when the ox-serum is absorbed with A_1B cells it acts on O and A_2 cells but not on the cells of A_1, B and A_1B. He supports this opinion by his observation that the ox-serum can be completely absorbed by the cells of sub-group A_1B if the absorption is continued for a long time. Thomson is of the same opinion as Hirszfeld, but he uses the term basal receptors instead of species receptors.

Further reactions which indicate the existance of other agglutinogens besides those previpusly mentioned were obtained when immune or normal animal sera were used. The evidence for this is as follows:-

(i) Landsteiner and Levine (41) found that when immune anti-human sera obtained by injecting red cells of any group into rabbits, are absorbed with certain cells of one group, the treated sera will still agglutinate certain other cells of the same group. By this method they purified two different agglutinins acting on two

different agglutinogens which they called M and N. These were equally distributed amongst the individuals of each of the four groups. Three types of blood cells were found according to their content i.e. these new agglutinogens, namely M, N and MN.

(ii) Imamura (42) found that when pig-serum is absorbed with certain cells of any group, it still agglutinates certain other cells of each of the four groups. He named the agglutinogen detected by such sera Q.

(iii) Sugishta (43) found another agglutinogen by the aid of eel's serum which he called E, but this agglutinogen differs from the above-mentioned in that it may be strong in the cells of group O and weak in the cells of the other three groups.

THE MEDICO-LEGAL APPLICATION OF BLOOD GROUPS.

Landsteiner and Richter (44), shortly after the discovery of the blood groups, demonstrated that the group of a dried blood stain can be determined by applying the absorption method. Later Dungern and Hirszfeld (45), after studying the inheritance of the agglutinogens A and B, came to the conclusion that the blood Group of a child is related to that of at least one of its parents. These two facts were later applied in legal medicine. The former is now accepted by several courts of law as evidence for the determination of the origin of blood stains in cases of murder etc. In several cases it has been known that a blood stain found on the belongings of the accused belonged to a group different from his own, but to the same blood group as that of the victim. Such results have led to confession by the accused person, or have been taken as evidence against his statements.

Among the theories laid down as a basis for the inheritance of

/26

the agglutinogens A and B, the Bernstein theory won the confidence of almost all experts on this subject, hence this theory was accepted by some courts of law as evidence in the exclusion of paternity and maternity despite the existence of some observed exceptions, which should not occur if this theory were absolutely correct. These exceptions were findings contrary to Bernstein's assumption, which postulates that an AB mother cannot give birth to an O child. The further complications entailed by the discovery of the further sub-groups based on A_1, A_2 and M and N rendered medico-legal applications difficult and delicate, unless some unity could be introduced into the interpretation of the facts.

Another character which was observed by Schiff (46) is that certain individuals secrete large amounts of agglutinogens A and B in their saliva, while others secrete either small traces or none at all. He called these types secretory and non-secretory, which characters, according to him, are inheritable, and may be used in paternity and maternity cases.

With regard to the P, Q, and E agglutinogens, although they were found to be inheritable by the workers who discovered them, as yet no one else has studied them.

The serious consequences of the application of this knowledge in legal medicine appear from the following considerations.

(1) The results of testing a blood stain might assume importance in certain cases, in relation to the conviction of the accused.

(2) The results of testing the blood groups of a family are practically the only evidence which, in certain cases, can decide whether a child belonged to certain parents and not to others, and can prove

/27

the illegitimacy of a child.

Taking these grave consequences into consideration, the writer has investigated the different problems presented by this subject and has concentrated particularly on the study of the questions which are considered by eminent workers as suitable for medico-legal applications.

This has involved an examination of the bases underlying blood-grouping with special reference to the sub-divisions. The writer believes that his experimental work has led to an orderly and simple interpretation of the facts with reference to the sub-groups which in their present state are highly complicated and confusing. This explanation has a genetic foundation which permits a direct application to the question of paternity and should prove valuable in legal medicine.

S E C T I O N 11

SUB-DIVISION OF GROUPS A, B and AB.

THE EXISTENCE OF TWO TYPES OF THE AGGLUTINOGEN A.

Dungern and Hirszfeld (31) were the first to demonstrate the presence of two types of cells of group A. They absorbed normal anti-A serum with several specimens of group A cells and found that in most cases the serum became inactive towards the cells of all specimens. Occasionally that was not the case since when the cells of certain specimens were used in absorption, the treated serum was rendered inactive towards these cells, but remained active towards the cells of the majority of specimens. This observation was confirmed by Schutze (47), and Coca and Klein (48).

Guthrie and his co-workers (33) found that the sera of certain individuals of group A can agglutinate the cells of other individuals of the same group, which they explained by the presence of additional agglutinins and agglutinogens.

Landsteiner and his assistants (34) studied the observations of Dungern and Hirszfeld and Guthrie and his co-workers, and came to the conclusion that each of groups A and AB include two sub-groups, which they

designated as follows:-

	SUBGROUPS		SUBGROUPS
Group A	A_1, A_2	Group AB	A_1B, A_2B

These workers based this new classification on the following:-

1) The observation of Dungern and Hirszfeld.

Landsteiner and Witt absorbed the normal anti-A serum with half its volume of the blood cells of an individual (X) of group A. The treated serum agglutinated the cells of individual (Y) of the same group, and those of several others but not those of (X) and a few others. On account of this they suspected the presence of an additional agglutinin in the serum and a corresponding additional agglutinogen in the cells of (Y).

This they proved in the following experiment:-

2) The separation of two agglutinins from anti-A serum.

8 c.c. of normal anti-A serum were treated with a small amount (0.25 c.c. of the (X) cells and the mixture was centrifuged after standing for two hours at room temperature. The sediment was washed twice with ice-cold saline and then suspended in 0.7 c.c. saline. The absorbed agglutinin was split off by heating the suspension at 55°C. The fluid obtained was called No. 1.

The 8 c.c. of anti-A serum were re-treated for two hours at room temperature with excess (4.0 c.c.) of (X) cells in order to remove all the agglutinin acting on such cells. The supernatant fluid was re-absorbed with 0.25 c.c. of (Y) cells which were agglutinated with the treated serum as in the previous observation. The absorbed agglutinin was split off in the same manner as before and the fluid obtained was called No. 2. The fluids Nos. 1 and 2 were tested against the cells

/30

of (X) and (Y). The results are given in Table No. 6.

TABLE NO. 6.

Anti A serum absorbed		Split off agglutinin tested with cells of group A			
No. of absorption	with cells	In fluid No.		(X)	(Y)
1st absorption	0.25 c.c. of (X) cells	No. 1.	At room temperature	±	+
2nd absorption	4.0 c.c. of (X) cells	-----			
3rd absorption	0.25 c.c. of (Y) cells	No. 2	At room temperature	-	+
			At 0°C	tr	+

From these results they concluded that there are two different agglutinins in the anti-A serum[1], namely:-

(i) The agglutinin absorbed by the cells of (X) which agglutinated the cells of (X) and (Y) to the same extent and therefore must be the agglutinin (a) which acts on the agglutinogen A present in both the cells of (X) and (Y).

(ii) The agglutinin absorbed with the cells of (Y) which agglutinated these cells at room temperature, but not those of (X), although at 0°C. it gave a weak reaction with the latter. This was considered to be a new agglutinin which they called (a_1). Consequently the cells of (Y) contain, beside the agglutinogen A, an additional agglutinogen which they called A_1; hence the cells of (X) and (Y), although belonging to the same group A, represent two qualitatively different

(1) According to the original classification of the four groups, this serum is supposed to contain agglutinin (a) which agglutinates cells of group A.

types in group A, on account of their respective composition, namely:-

Cells of (X)	Cells of Y
A	AA_1

3) The presence of specific abnormal agglutinins.

Landsteiner, Witt and Levine confirmed the existence of two qualitative types in group A by the discovery of two abnormal agglutinins, each of which is specific for a certain type, namely:-

(i) An agglutinin found in the sera of certain individuals of group A or AB[1], which acts in a similar manner to the (a_1) agglutinin separated from the anti-A serum and therefore similarly designated. This agglutinin, whether obtained from A or AB individuals or prepared by absorbing anti-A serum with the A cells which do not contain the agglutinogen A_1, agglutinated the cells of the majority of individuals of groups A and AB.

(ii) An agglutinin found in the sera of certain other individuals of group A, which agglutinated only the cells of group A which were not agglutinated with the agglutinin (a_1). This was explained by the presence of an additional agglutinogen in these cells corresponding to this agglutinin; they called these A_2[2] and (a_2) respectively. Consequently each of the two types is characterised by a special agglutinogen besides the A which is common to both.

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(1) According to the original classification of the four groups, the sera of group AB contain no agglutinins.

(2) The agglutinin (a_2) was found to agglutinate O blood cells which was explained by Landsteiner and Levine by the presence of related agglutinable factors in the cells of group O and A_2. These factors were not thought to be identical.

They found that the same applies to group AB and therefore each of groups A and AB is classified into two sub-groups, namely:-

Group	Sub-group	Agglutinogens	Agglutinins.
A	A_1	(AA_1)	(ba_2)
	A_2	(AA_2)	(ba_1)
AB	A_1B	(AA_1B)	(a_2)
	A_2B	(AA_2B)	(a_1)

The agglutinin (a_1) was found in the sera of some individuals of the sub groups A_2 and A_2B. The agglutinin (a2) was found in the sera of a few individuals of sub-groups A_1 and A_1B, as shown in the diagram.

Lattes (49), while admitting the existence of these two types, is of the opinion that they differ only in a quantitative manner. He assumes that the A_1 differs from the A_2 in that it is more sensitive and therefore reacts more strongly to the anti-A serum. He supports this opinion by the fact that if the anti-A serum is repeatedly absorbed with A_2 (the weak) cells, it will become completely inactive towards A_1 cells. However, he did not explain the existence of the abnormal agglutinins (a_1) and (a_2)

Thomsen's (37) opinion may be summed up in the following:-

(1) There are two agglutinogens - A_1 and A_2 - both of which react with anti-A serum, but the former reacts more strongly than the latter.

(2) The agglutinin (a_1) found in certain sera of groups A_2 or A_2B is a fraction of the agglutinin (a) and on account of its weakness, it is not bound to the weak cells of A_2 and A_2B and therefore appears in their respective sera. It is similar to the (a_1) prepared by absorbing anti-A serum with A_2 cells, but it is not identical since

/33

the former acts only up to room temperature, while the latter acts at 37°C.

(3) The agglutinin (a_2) is specific for the cells of group O. It acts on the cells of the majority of A_2 individuals because most of these are heterozygotes and thus contain an O receptor. The cells of the few individuals of A_2 which are not agglutinated by this agglutinin are homozygotes. This is supported by the observation that the A_2B cells which do not contain the O receptor, according to Thomsen's theory[1] are not agglutinated by this agglutinin. He explained the inability of the agglutinin (a_2) to act on the cells of the heterozygous individuals of group B and sub-group A_1, by assuming that the agglutinogens A_1 and B are stronger than the A_2, and therefore mask the receptor O. The few cases where A_1 cells were weakly agglutinated were explained by incomplete dominance of the agglutinogen A_1 over O.

Schiff (38) and Sachs (50) differentiated the two types of A cells by observing their absorptive power for immune anti-sheep serum from the rabbit which was found to act on all A cells on account of the similarity of Forssman's antigen, contained in the red cells of the sheep, to the agglutinogen A. They found that the absorptive power of the A_2 cells is much less than that of A_1. Schiff who believes, like Thomsen, that the agglutinin (a_2) is specific for O cells, found that normal ox-serum, after being absorbed with A_1B cells, which are supposed not to contain an O receptor, agglutinates the cells of groups O and A_2.

Hirszfeld (40), who admits the existence of the two types,

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(1) To be discussed under the subject of Heredity. /34

expresses his opinion in the following statement:-

> "The agglutinogens A_2 and A_1 are similar but not identical since they possess unequal avidity for anti-A serum, which contains a series of anti-bodies of decreasing thermal amplitude and affinity."

He contradicts Schiff's opinion with regard to the presence of an O receptor. He assumes that ox-serum contains only species agglutinins which act on all human cells but to a greater extent on A_2 and O cells, since the latter two contain more species receptors than those of A_1, B and AB, on account of the absence of iso-agglutinogens in O cells and the weakness of agglutinogen A_2 in the A_2 cells. His opinion is supported by the fact that if the ox-serum is repeatedly absorbed with A_1B cells, it becomes inactive towards all human cells, even those of group O.

Friedenreich and Zacho (51), Wiener and his co-workers (51) Wolff and Jonsson (53) and others, believe in the existence of two independent agglutinogens A_1 and A_2.

From the above-mentioned statements one may conclude that although most of the workers agreed that there are two agglutinogens - A_1 and A_2 - yet some believe that they are qualitatively different and others believe that they are similar but not identical, both schools of thought having their own reasons.

The discovery of the sub-groups A_1 and A_2 has acquired a special importance owing to the suggestion of its application in paternity cases, on the basis of a theory laid down by Thomson and his assistants. Hence it became desirable to make a systematic study of these sub-groups and their nature.

PERSONAL INVESTIGATIONS (1)

The object of this research was to prove the existence of these sub-groups and then to study the nature of the two agglutinogens A_1 and A_2 and the agglutinins (a_1) and (a_2). In order to study both questions, at one time, the writer had to find sera which contained these abnormal agglutinins. This was attained by testing blood samples from different adult individuals, the number of specimens examined daily varied from 15-25 specimens. 5-10 c.c. of blood were collected in a sterile tube and two drops were put in a small tube containing 2 c.c. of physiological saline containing 1% sodium citrate. The first tube was centrifuged after about 30 minutes, and the serum was separated from the clot and tested with cells of known bloods of groups O, A and B. The blood taken in the small tubes was mixed with the saline and then the tube was centrifuged and the saline was removed. A fresh quantity of citrated saline was added to the cell sediment to make roughly a 5% suspension. The cells thus prepared were tested with known normal anti-A and B sera, of a titre 128-256, which were taken from normal persons and proved to contain only agglutinins (a) and (b) respectively. With the object of detecting any abnormal reaction on the part of the cells, they were also tested with serum of group AB which contained no agglutinins.

The test was made either on slides or in small tubes and at room temperature, which varied in Glasgow from 20-25°C. and in Cairo from 20-35°C.

THE SLIDE METHOD.

The serum was diluted with equal volume of saline before being

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(1) This work was done partly in Glasgow and partly in Cairo.

used, in order to prevent the occurrence of pseudo-agglutination. One drop of the diluted serum was added to one drop of cell suspension and both were mixed by a glass rod; after 3-5 minutes the slide was gently rocked from side to side; after another five minutes the reading was taken by the naked eye and all the preparations were examined by the low power of the microscope.

THE TUBE METHOD.

One drop of the serum, one drop of saline and one drop of the cell suspension were mixed in a small tube which was left to stand at room temperature for one hour. The reading was then taken by the naked eye, and in all the negative preparations one drop was taken on a slide and examined under the low power of the microscope.

After the determination of the blood-group of the specimens collected daily, the sera and blood cells of all specimens of group A were tested against each other and the same was done with bloods of group AB. Occasionally the sera and blood cells of group O and also those of group B, were tested against each other. The object was to detect any abnormal reaction which might take place between the cells and serum of two specimens of the same group.

The material thus examined consisted of 1154 bloods, out of which 400 were examined in Glasgow, in which the frequency of the four groups was as follows:-

Group	O	A	B	AB
Number of bloods	195 (49.0%)	144 (36.0%)	40 (10.0%)	21 (5.0%)

The other 754 bloods were examined in Cairo, in which the frequency of the

/37

four groups was as follows:-

Group	O	A	B	AB
Number of bloods	201 (26.6%)	269 (35.8%)	205 (27.1%)	79 (10.5%)

All the bloods of groups O and B examined in the two series failed to show any abnormal reaction, according to the scheme of the four groups, as described in Section I.[1]

In the first series, four sera of bloods of group A and three sera of bloods of group AB showed abnormal reactions, in that all of them, in a similar manner, agglutinated the majority of group A cells. The fact that these sera did not agglutinate O cells and that the cells of group A which were agglutinated were of the sensitive type, suggested that these sera contain the agglutinin (a_1) described by Landsteiner.[2] This, however, was proved by comparing their action with that of the normal anti-A serum after the latter had been absorbed with the cells which did not react with the abnormal agglutinin. There was no agglutinin found corresponding to the (a_2) described by Landsteiner in the bloods examined in this series.

In the second series 13 sera of group A and 6 of group AB were found to contain the agglutinin (a_1). Other 9 sera of group A and 4 of group AB agglutinated the blood cells of group O, but only two of the former and two of the latter agglutinated A cells which were not agglutinated with the agglutinin (a_1). The fact that these four sera agglutinated both cells of groups O and A indicated that this agglutinin corresponds to the (a_2) found by Landsteiner.

Two sera of group AB showed the presence of active agglutinins.

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(1) See pages 18-25

(2) See pages 22 and 31.

The reactions obtained by them varied from those of (a_1) and (a_2) and therefore will be described later.[1]

THE AGGLUTININ (a_1)

Although the seven sera containing this agglutinin, found in the first series, gave similar reactions, yet two of them were stronger than the other five. The titre of the five weak sera at 20°C varied from 1:4 - 1:8, and that of the strong two was 1:16. The maximum temperature at which the weak five agglutinated the cells was 25°C. The strong sera acted up to 30°C.

The nineteen sera containing the agglutinin (a_1), found in the second series, varied in their titre and their thermal amplitude. Only three were active at a temperature of 30-35°C, and their titre at 25°C. varied from 1:4 - 1:8. The others were only suitable for experiment when the test was made at a room temperature not exceeding 25°C.

As a rule, the agglutinin (a_1) found in the sera of group AB was more active than that found in the sera of group A.

CONSTANCY OF THE REACTIONS OBTAINED WITH THIS AGGLUTININ.

The reactions obtained with this agglutinin were constant, in that the agglutinable cells were always agglutinated when tested with the sera containing this agglutinin, and the non-agglutinable cells were always not agglutinated. The cells of several individuals of both types have been examined on several occasions over a period of three years and always behaved in the same manner.

/39

(1) See Section V.

THE CONSTANT PRESENCE OF THE AGGLUTININS IN THE SERA.

In all cases the serum was re-examined at least once by taking another sample of blood and in all of them the presence of the agglutinin was always demonstrated. Two of the sera of group AB were examined on more than ten occasions over a period of two years and the agglutinin was consistently present.

Sera containing this agglutinin were found to agglutinate 70% - 80% of the cells of individuals of groups A and AB[1] which constitute the sub-groups A_1 and A_1B. The others which were not agglutinated constitute the sub-groups A_2 and A_2B. It is interesting to note that all the bloods in the serum of which the agglutinin (a_1) was present were of the sub-groups A_2 or A_2B.

The reactions obtained with the agglutinin (a_1) were characterized by being weak, since the titre of all the sera containing this agglutinin was not more than 16, while the titre of the normal (a) and (b) agglutinins towards A and B cells respectively was much higher (64-256).

It was also noticed that as a general rule the cells of sub-group A_1B were more weakly agglutinated than the cells of sub-group A_1, and these were not equally agglutinated since some of them were weakly agglutinated like the A_1B cells. Others were more strongly agglutinated. This difference was not noticed amongst the cells of sub-group A_1B.

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(1) The cells of group AB were tested with the sera of group AB containing the agglutinin (a_1). The cells of group A were either tested with these sera or with sera of group A containing the same agglutinin.

/40.

THE RELATION BETWEEN THE SENSITIVITY OF THE CELLS AND THE REACTIONS OBTAINED WITH THE AGGLUTININ (a_1)

The sensitivity of the cells of several persons of each of the sub-groups A_1, A_2, A_1B and A_2B was estimated by testing them with different dilutions of normal anti-A serum. The results given in Table No. 7 represent the reactions obtained with each type.

TABLE No. 7.

CELLS	TITRE WITH AGGLUTININ (a_1)	TESTED WITH THE FOLLOWING DILUTIONS OF NORMAL ANTI-A SERUM						
		1:2	1:4	1:8	1:16	1:32	1:64	1:128
(As)A_2B	-	+	+	tr.	-	-	-	- (1)
(Am)A_2	-	++	+	+	tr.	-	-	-
(Mo)A_1B	1 : 4	++	++	+	+	+	tr.	-
(Sa) A_1	1 : 8	++	++	+	+	+	+	±

This table shows that the cells which are not agglutinated with the agglutinin (a_1) are less sensitive than those which are agglutinated. The cells of A_2B are less sensitive than those of A_2 and the cells of A_1B are less sensitive than those of A_1, also the A_1B cells are less agglutinable with agglutinin (a_1) than are A_1 cells. These observations suggest that the extent of the agglutinability with the agglutinin (a_1)

(1) The following symbols are used throughout the thesis to indicate the quality and extent of reactions.

- \- = negative reaction
- f.tr = traces of agglutination seen by the high power of the microscope.
- tr = traces of agglutination seen by the low power of the microscope.
- ± = agglutination just visible to the naked eye.
- \+ = agglutination easily seen by the naked eye.
- ++ = strong agglutination.
- +++ = still stronger agglutination.

/41

runs parallel with the sensitivity of these cells to agglutination by (a).

THE RELATION BETWEEN THE ABSORPTIVE POWER OF THE CELLS AND THE REACTIONS OBTAINED WITH THE AGGLUTININ (a_1)

Normal anti-A serum was absorbed with the cells of the individuals given in Table No. 7, which represent the four sub-groups. 2 c.c. of the serum were absorbed with ½ c.c. of the washed cell sediment of each individual, for two hours at 30°C. The absorbed serum was tested with the cells of the same four specimens. The results are given in Table No. 8

TABLE No. 8

Normal anti-A Serum absorbed with	Cells	Dilutions of the absorbed serum tested against 1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
(As) A_2B not agglutinated by agglutinin (a_1)	A_2B	-	-	-	-	-	-	-	-
	A_2	tr	-	-	-	-	-	-	-
	A_1B	+	+	+	+	tr.	-	-	-
	A_1	+	+	+	+	+	tr.	-	-
(Am) A_2 not agglutinated by agglutinin (a_1)	A_2B	-	-	-	-	-	-	-	-
	A_2	-	-	-	-	-	-	-	-
	A_1B	+	+	+	±	-	-	-	-
	A_1	+	+	+	+	±	-	-	-
(Mo) A_1B agglutinated by agglutinin (a_1) (1:4)	A_2B	-	-	-	-	-	-	-	-
	A_2	-	-	-	-	-	-	-	-
	A_1B	-	-	-	-	-	-	-	-
	A_1	±	-	-	-	-	-	-	-
(Sa) A_1 agglutinated by agglutinin (a_1) (1:8)	A_2B	-	-	-	-	-	-	-	-
	A_2	-	-	-	-	-	-	-	-
	A_1B	-	-	-	-	-	-	-	-
	A_1	-	-	-	-	-	-	-	-

These results show that the cells which are not agglutinated by the

agglutinin (a_1) possess weaker absorptive power than those which are agglutinated. It is also noticed that the extent of the agglutinability with the (a_1) is proportional to the absorptive power of the cells.

THE RELATION BETWEEN THE AGGLUTININ (a_1) AND THE AGGLUTININ PRESENT IN ANTI-A SERUM

The fact that the cells of sub-group A_2B and A_2 which are not agglutinated by the agglutinin (a_1) are less sensitive and possess a weaker absorptive power towards anti-A serum, than the cells of A_1B and A_1, which are agglutinated by the agglutinin (a_1), suggests a certain relation between this agglutinin and the agglutinin found in the anti-A serum.

This, however, was proved by the following experiment:-

4 c.c. of normal anti-A serum were absorbed for two hours at 30°C with 2 c.c. of washed cell sediment of (As) who belonged to sub-group A_2B. The absorbed serum was compared with the agglutinin (a_1) which was found in the serum of the same individual (As), by testing both against the cells of sub-groups A_1B and A_1.

Another 4 c.c. of anti-A serum were absorbed for two hours at a temperature of 30°C with 2 c.c. of washed cell sediment of (At) who belonged to sub-group A_2. The absorbed serum was compared with the agglutinin (a_1) present in the serum of the same individual, by testing both with the cells of sub-groups A_1B and A_1.

The results are given in Table No. 9.

/43

TABLE No. 9.

SERUM	TESTED WITH CELLS		IN THE FOLLOWING DILUTIONS				
			1:2	1:4	1:8	1:16	1:32
Anti-A serum absorbed with cells (As) A_2B	A_1B	(Mo)	+	+	tr	-	-
	A_1	(Sa)	+	+	+	±	-
Agglutinin (a_1) present in the serum of (As)	A_1B	(Mo)	+	±	-	-	-
	A_1	(Sa)	+	+	±	-	-
Anti-A serum absorbed with cells (At) A_2	A_1B	(Mo)	+	±	?	-	-
	A_1	(Sa)	+	+	±	?	-
Agglutinin (a_1) present in the serum of (At)	A_1B	(Mo)	±	-	-	-	-
	A_1	(Sa)	+	±	-	-	-

The results included in this table show the following:-

(1) The normal anti-A serum absorbed with cells of sub-group A_2B (As) behaves similarly to the agglutinin (a_1) present in the serum of the same person.

(2) The normal anti-A serum absorbed with cells of sub-group A_2 (At) behaves similarly to the agglutinin (a_1) found in the serum of the same person.

(3) The agglutinin (a_1) found in the serum of sub-group A_2B is stronger than that found in the serum of sub-group A_2, likewise the anti-A serum absorbed with cells of A_2B is stronger than that absorbed with cells of A_2.

One may conclude, therefore, that the normal anti-A serum contains a similar agglutinin to the (a_1) present in the sera of certain individuals

/44

of sub-groups A_2 and A_2B.[1]

ARE THE PREPARED AND NATURAL (a_1) AGGLUTININS IDENTICAL?

The writer has examined more than 500 specimens of cells of groups A and AB with both the natural and prepared agglutinin (a_1) and found that they give similar reactions, provided the normal anti-A serum has beem absorbed properly with sufficient A_2 cells to render it inactive towards all cells of this sub-group. As a general rule the prepared agglutinin (a_1) gave stronger reactions than the natural. The difference between the reactions was more marked when the room temperature was above 25°C. Thomsen believes that these two agglutinins, although similar, are not identical, because he found that the prepared agglutinin has a higher thermal amplitude (37°C) than the natural agglutinin (25°C.). The writer has previously mentioned that some of the sera containing the natural agglutinin were active up to 30°C.

In order to study this question, the following experiment was made:-

4 c.c. of normal anti-A sera were absorbed for two hours at room temperature with 2 c.c. of washed cell sediment of sub-group A_2, and the absorbed serum was tested with cells of sub-group A_1 at different temperatures.

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(1) In order to differentiate between these two similar agglutinins, the following terms will be used:-

(i) natural (a_1) = the agglutinin normally present in the sera of A_2 and A_2B.

(ii) prepared (a_1) = the agglutinin prepared by absorbing anti-A serum with the cells of A_2 and A_2B.

/45

another 2 c.c. of the normal anti-A serum were absorbed for 24 hours at room temperature with 2 c.c. of washed cell sediment of the same cells A_2 and the absorbed serum was tested with cells of sub-group A_1 at different temperatures. In order to compare the reactions of these two absorbed portions of anti-A serum with the agglutinin (a_1), the latter was tested under similar conditions with the same A_1 cells. The results are given in Table No. 10.

TABLE No. 10.

SERUM	ABSORBED WITH	TIME OF ABSORPTION	TESTED WITH CELLS A_1 and A_1B AT 25°C.	AT 30°C.
Normal anti-A	½ volume of A_2 cells	2 hours	A_1B +	tr
			A_1 ++	±
Normal anti-A	equal vol. of A_2 cells	24 hours	A_1B tr	-
			A_1 ±	-
A_2B (a_1)	----------	--------	A_1B ±	-
			A_1 +	-?

These results show that the agglutinin prepared by the absorption of anti-A serum for 2 hours with half its volume of A_2 cells is stronger than the natural agglutinin (a_1) and of a higher thermal amplitude, but when the absorption is made with an equal volume of cells and continued for a long time, the prepared agglutinin is weaker and of a less thermal amplitude than the natural.(1) Therefore one may conclude that both the

(1) This observation supports Hirszfeld's opinion in that the serum contains several fractions of the agglutinin which possess various thermal amplitudes.

natural and prepared agglutinin are identical but the thermal amplitude of the prepared agglutinin differs according to the time of the absorption and the volume of cells used.

THE SPECIFICITY OF THE AGGLUTININ (a_1).

The fact that agglutinin (a_1) whether natural or prepared, constantly agglutinates certain cells and not others, suggests that it acts in a specific manner.

Landsteiner and his assistants who proved the specificity of this agglutinin by the absorption method,(1) concluded that this agglutinin acts on an additional agglutinogen A_1 which is present beside the agglutinogen A in the cells of sub-groups A_1B and A_1. If we assume according to these workers, that the cells of sub-groups A_2B and A_2 do not contain the agglutinogen A_1, one should expect that such cells will not react with the agglutinin (a_1). This, however, was contradicted by the following observations:-

(1) The agglutination of cells of A_2 and A_2B at low temperature.

Both sera containing the natural and prepared agglutinin (a_1) were tested with the cells of sub-groups A_2 and A_2B at 0°C, and agglutination was produced in all cases.

In order to study the relation between the reactions of A_2 and A_2B obtained at low temperature, and those of A_1 and A_1B at room temperature, the following experiment was performed:-

The serum of A_2B containing the agglutinin (a_1) was absorbed at 0°C

(1) See page 29.

with ½ its volume of cells of groups O, B, A_2 and A_1. The absorbed portions of the serum as well as the unabsorbed serum were tested with these cells and also with those of A_2B and A_1B. The results are shown in Table No. 11.

TABLE No. 11

SERUM OF A_2B (a$_1$) (1)		TESTED AT 0°C WITH CELLS OF					
		O	B	A_2	A_1	A_2B (1)	A_1B
Before absorption		±	±	+	++	+	++
After absorption at 0°C.	With O cells	-	tr.	+	++	+	++
	" B cells	-	-	+	++	+	++
	" A_2 cells	-	tr.	-	+	-	±
	" A_1 cells	tr.	tr.	-	-	-	-

These results show the following:-

(i) Agglutination of A_2 and A_2B cells still occurred when the serum was treated with cells of groups O or B, but did not occur when the serum was absorbed with A_2 or A_1 cells. This indicates that the agglutinable factor in A_2 cells is different from that in O or B cells, but similar to that in A_1 cells.

(ii) Agglutination of A_1 and A_1B cells was not affected when the serum was treated with the cells of groups O or B, but was diminished after treating the serum with A_2 cells. This also indicates that the agglutinogens in A_1 and A_2 cells are similar and the former reacts more strongly towards the agglutinin (a_1) than the latter.

(1) The A_2B serum and A_2B cells used in this experiment belong to the same individual.

(2) The ability of the cells of A_2 and A_2B to absorb completely the agglutinin (a_1).

If the cells of sub-groups A_2 and A_2B lacked the agglutinogen (A_1) which reacts with the agglutinin (a_1), one would expect that such cells would not absorb this agglutinin. What actually happens contradicts this assumption, since when a serum containing the agglutinin (a_1) whether natural or prepared, is repeatedly absorbed with A_2 or A_2B cells, it becomes inactive towards A_1 cells.[1] This is shown by the following experiment:-

The serum of sub-group A_2B which contains the natural agglutinin (a_1) was absorbed on three successive occasions with cells of sub-group A_2 by using each time a quantity of cell sediment equal to half the volume of the serum absorbed.

The serum containing the prepared agglutinin (a_1) (the normal anti-A serum absorbed with A_2 cells) was similarly absorbed on three successive occasions with cells of A_2.

Both sera were also absorbed in a similar manner with the cells of sub-group A_2B.

The absorptions were made, in all cases, at room temperature and the period was three hours.

The sera thus absorbed were titrated with cells of sub-group A_1.

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(1) This was also proved by several workers including Landsteiner (34).

/49

The results obtained are given in Table No. 12.

TABLE No. 12.

SERUM CONTAINING	ABSORBED WITH CELLS	TITRE OF SERUM WITH A_1 CELLS 1:1	1:2	1:4	1:8	1:16
The Natural (a_1) (A_2B) (1)	Before absorption	+	+	+	tr.	-
	1st absorption with (A_2B (1)	+	+	+	-	-
	1st absorption with (A_2	+	+	tr.	-	-
	2nd absorption with (A_2B	+	tr.	-	-	-
	2nd absorption with (A_2	+	-	-	-	-
	3rd absorption with (A_2B	tr.	-	-	-	-
	3rd absorption with (A_2	-	-	-	-	-
The Prepared (a_1)	Before absorption	+	+	+	+	±
	1st absorption with (A_2B	+	+	+	tr.	-
	1st absorption with (A_2	+	+	+	-	-
	2nd absorption with (A_2B	+	+	tr.	-	-
	2nd absorption with (A_2	+	±	-	-	-
	3rd absorption with (A_2B	±	-	-	-	-
	3rd absorption with (A_2	-	-	-	-	-

When these two sera were similarly absorbed with A_1B and A_1 cells, they became inactive after one absorption.

(1) The A_2B cells and A_2B serum used in this experiment belong to the same individual. The fact that this agglutinin is completely absorbed with the serum's own cells is confirmed by letting the whole blood stand for two days at 0°C, after which time, if the serum is separated while the blood is cold, it will be found to be inactive towards A_1 cells at room temperature.

/50.

These results confirm those mentioned in observation No. 1 (page 47) in that the cells of A_2B and A_2 react with the agglutinin (a_1) whether natural or prepared, but to a much less extent than those of A_1B and A_1. Therefore one may conclude that the cells of the four sub-groups possess one kind of agglutinogen, which is more sensitive in cells of sub-groups A_1B_1 and A_1 than in those of A_2B and A_2. One may assume that this variation is due to the cells of the former two sub-groups containing a greater quantity of the agglutinogen than the cells of the latter two. This is proved later.(1)

Consequently one may suppose that the agglutinin (a_1) reacts with the same receptors of these red cells as it is actually an agglutinin (a) but of a weaker affinity, therefore it agglutinates only the more sensitive cells of sub-groups A_1B and A_1.

This is contrary to the opinion of Landsteiner who stated that he could separate two different agglutinins (a) and (a_1) from anti-A serum,(2) the former of which agglutinated the cells of A_2 and A_1 to the same extent, while the latter agglutinated only cells of A_1. The only possible explanation of such results seems to be the existence of qualitative differences but if we assume that the serum contains several fractions of the agglutinin, as Hirszfeld has indicated,(3) one may easily explain them on a quantitative basis. According to Hirszfeld's assumption, anti-A serum contains several fractions of agglutinin (a) which possess different

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(1) See Section III.

(2) See Page 30.

(3) See page 34 - also page 10.

/51.

affinities and different thermal amplitudes. When such serum is mixed with blood cells which contain agglutinogen A, it is to be expected that this agglutinogen will unite first with the fraction of the agglutinin (a) which possess the highest affinity, hence if the serum is separated at this stage it will contain only the agglutinin fraction of low affinity. If we separate the fraction absorbed with the cells by heating them to 55°C as Landsteiner has done, and compare its action with the fraction left in the absorbed serum, by testing both with the sensitive cells of group A persons, we will find that the former is stronger than the latter. When both are tested with the weak cells of a group A person, it is to be expected that these will be agglutinated with the agglutinin fraction separated from the cells, and not agglutinated by the agglutinin fraction left in the serum, since on account of the weakness of the agglutinogen in these cells, it can react only with the agglutinin fraction of high affinity.

Therefore one may explain the agglutinins (a) and (a_1) separated by Landsteiner by assuming that the former is the strong fraction of the agglutinin (a) and the latter is the weak fraction of the agglutinin (a). Naturally the stronger the affinity of the agglutinin, the less marked will be the difference between the reactions obtained with cells possessing different quantities of the agglutinogen. This explains why the agglutinin separated by Landsteiner agglutinated the A_1 and A_2 cells to the same extent. This is supported by the fact that with immune anti-A serum, where the affinity can be raised by active immunization, the difference between A_2 and A_1 cells is less marked than with normal anti-A serum.

The assumption that the two agglutinins (a) and (a_1) are identical as regards the agglutinogen (receptor) with which they combine

but of different affinities and thermal amplitudes is supported by the behaviour of some normal anti-A sera.

Coca and Klein, Wiener and the writer noticed that when certain normal anti-A sera were absorbed with A_2 cells, they lost the power to agglutinate A_1 cells. This might be explained by the absence of the agglutinin (a_1) in these sera. Hence one would expect that such anti-A sera should contain only the agglutinin (a) which, according to Landsteiner, must agglutinate cells of A_2 and A_1 to the same extent. This, however, did not agree with what actually happened when one of these sera was titrated with the cells of the four sub-groups as shown in Table No. 13.

TABLE No. 13

SERUM	TESTED WITH CELLS	REACTIONS OBTAINED WITH THE SERUM DILUTED 1:2	1:4	1:8	1:16	1:32	1:64
Normal anti-A which did not contain (a_1)	A_1	+	+	+	±	tr	-
	A_1B	+	+	+	tr	-	-
	A_2	+	±	-	-	-	-
	A_2B	±	-	-	-	-	-

When the reactions obtained with such sera are compared with those obtained with a strong normal anti-A and those obtained with the serum containing agglutinin (a_1), one observes that there are three types of sera, namely:-

(1) Strong normal anti-A serum which agglutinates the A_1, A_1B cells at higher titres than A_2 and A2B cells, and which contains agglutinin (a_1).

/53

(2) Weak normal anti-A serum which agglutinates A_1 and A_1B cells at higher titres than A_2 and A_2B cells, but the titres in this case are less than those in the previous one, and the serum does not contain agglutinin (a_1).

(3) Serum containing agglutinin (a_1) which agglutinates A_1 and A_1B cells but at lower titre than the previous two sera, and does not agglutinate A_2 and A_2B cells.

The existence of the second type is contrary to Landsteiner's view, and, as already mentioned, can be explained on the assumption of the presence of one agglutinin with different fractions which vary in their action according to their affinity.

The nature of the difference between the sub-groups A_1 and A_2 was studied by immunizing rabbits and goats with the cells of each type(1). If the cells of sub-groups A_1 contain two agglutinogens A_1 and A and if the cells of A_2 contain agglutinogen A as well as A_2, but not A_1, one would expect the following, according to Landsteiner's view:-

(1) The immune serum prepared by injecting A_2 cells will produce agglutinin (a) which acts similarly on the cells of both sub-groups A_1 and A_2, and it should not contain agglutinin (a_1).

(2) The immune serum prepared by injecting A_1 cells will contain both agglutinins (a) and (a_1): the former can be absorbed with cells of A2 but not the latter.

What occurred was contrary to these expectations. The immune serum prepared with A_2 agglutinated the cells of A1 at a higher titre than A2 and when it was absorbed with a suitable quantity of A_2

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(1) This is described in detail in Section III.

/54

cells, a similar agglutinin to the (a_1) remained in the serum.

The immune serum prepared with A_1 cells behaved in a similar manner and when it was repeatedly absorbed with A_2 cells it became inactive towards A_1 cells. Therefore one may conclude that Landsteiner's assumption of two different agglutinins (a and a_1) is not a suitable explanation of the above-mentioned results which, however, can be explained on the assumption of quantitative difference, as previously described.

There remains the explanation of the existence of the agglutinin (a_1) in normal sera of groups A and AB. This, however, can be supplied on the basis of Bernstein's hypothesis regarding the development of agglutinins in the body. He assumes that a universal agglutinin is gradually developed in the serum of each individual against the cells of all groups. When an agglutinogen, for example, A, is present in the cells, it will fix *in vivo* the agglutinin (a) and therefore will leave agglutinin (b) in the serum. If the agglutinogen A is weaker than usual, as in cells A_2, these will absorb only the strong fraction of agglutinin (a) and therefore will leave the agglutinin (b) and the weaker fraction of agglutinin (a). The latter shows itself in the form of what we call (a_1). This opinion is supported by the fact that such agglutinin is only present in the sera of the sub-groups A_2 and A_2B, where the agglutinogen A is weaker than in A_1B and A_1. It is stronger in A_2B than in A_2 serum because of the agglutinogen being still weaker in the former red cells than in A_2.

Therefore the assumption of quantitative difference explains, and in a more logical way, all the points on whcih Landsteiner and his assistants have based the qualitative sub-division of these groups.

Moreover, serological data have been brought forward which are not compatible with Landsteiner's hypothesis.

Naturally then the question arises as to what regulates the development of the agglutinogen A so as to render it strong in the cells of one person and weak in those of others. An explanation of this will be found in Section III.

THE AGGLUTININ (a_2)

Out of the 269 bloods of group A and 79 of group AB examined in Cairo, 9 sera of sub-group A_1 and 4 sera of sub-group A_1B agglutinated all blood cells of group O at 30°C, but only one of them was active at 37°C. Four sera out of the thirteen agglutinated A_2 cells at 30°C, but all agglutinated these cells at 20°C. The reactions obtained with O cells were stronger than those obtained with A_2, hence they behaved like the sera containing the agglutinin (a_2) described by Landsteiner and his assistants.

The reactions were not as strong as those obtained with the agglutinins (a) and (b) and their respective cells. Those of O cells were similar in degree to the reactions obtained with (a_1) and A_1 cells, the titre of the serum against O cells at 30°C being not more than 1:8 and in most cases 1:4. But those of A_2 were weaker. The cells of few specimens of sub-groups A_1 and A_2B were agglutinated but still more weakly.

CONSTANCY OF THE REACTIONS.

Several cells were examined with more than one serum and the same reactions were obtained on each occasion, some cells were examined

/56

occasionally over a period of one year and the reactions were found to be constant.

THE CONSTANT PRESENCE OF THE AGGLUTININ IN THESE SERA:

The second sample of each of the 13 sera was re-examined and in one instance the serum was tested several times over a period of 6 months and the agglutinin was always found.

SPECIFICITY OF THIS AGGLUTININ:

The fact that the normal anti-A serum did not agglutinate cells of group O indicated that the agglutinin (a_2) is different from the agglutinin (a).

Landsteiner and his assistants assumed that this agglutinin acts on A_2 cells on account of the presence of an extra agglutinogen which they called A_2. They explained its action on O cells by assuming that the O and A_2 cells contain related but not identical substances. Thomsen and Schiff are of the opinion that it is specific for O cells and only acts on the cells of the heterozygous individuals of A_2.

In order to study this question the following experiment was made:-

3 c.c. of a serum containing agglutinin (a_2) were absorbed for ½ an hour at 30°C with ½ c.c. of washed cell sediment of A_2. Another 3 c.c. were absorbed in a similar manner with O cells. The absorbed serum was titrated with both cells and the results are given in Table No. 14.

/57

TABLE No. 14

SERUM CONTAINING (a_2)	TESTED WITH CELLS	TITRE OF THE ABSORBED SERUM 1:1	1:2	1:4	1:8	1:16	1:32
Before absorption	A_2	+	+	tr	?	-	-
	O	+	+	+	tr	?	-
Absorbed with A_2 cells	A_2	-	-	-	-	-	-
	O	+	?	-	-	-	-
Absorbed with O cells	A_2	-	-	-	-	-	-
	O	-	-	-	-	-	-

These results show that the A_2 cells are less agglutinable and also possess a weaker absorptive power towards this agglutinin than O cells. This may be explained either on a qualitative or a quantitative basis. The latter explanation proved to be the more reasonable since when the absorption with A_2 cells was continued over a longer period or if larger quantities of these cells were used for a similar period, the treated serum failed to agglutinate O cells also. This experiment suggested that this agglutinin is specific for an agglutinogen found in O cells and A_2 cells but in larger quantities in the former than in the latter.

This was confirmed by splitting the agglutinin off from the cells of A_2 and O used in absorbing the serum in the previous experiment. The sediment of cells after treatment with serum, was washed twice with ice-cold saline and then re-suspended in 0.5 c.c. of saline. The tubes were heated for 5 minutes at a temperature of 50°C and the fluid separated

/58

by centrifuging the tubes while surrounded by water at this temperature, in bigger tubes. The fluids were tested with A_2 and O cells at 30°C. The agglutinin separated from O cells agglutinated O and A_2 cells: the former were more strongly agglutinated than the latter. The agglutinin separated from A_2 cells agglutinated O cells weakly but not A_2. When the latter were tested at a lower temperature (20°C) they were agglutinated.

One would expect the contrary if this agglutinin was specific for A_2 cells, as was thought by Landsteiner. Therefore it seems that this agglutinin is specific for O cells and that it acts on A_2 cells because they contain an O agglutinogen.

Thomsen, who believes that the agglutinogen in A_2 cells is independent of that in A_1 cells, assumes that the agglutinin (a_2) acts on the majority of the cells of A_2 individuals because these are heterozygous. He supports this opinion from his own observation that the cells of a few individuals of sub-group A_2 and those of all A_2B individuals are not agglutinated by this agglutinin, because they do not contain an O agglutinogen.

In order to study this question the writer tested the cells of 204 specimens of group A and 24 specimens of group AB with both (a_1) and (a_2) agglutinins. The results were as follows:-

			Reactions with (a_1)	Reactions with (a_2)
Group A				
	163	A_1	+	-
	3	A_1	+	+
	38	A_2	-	+
Group AB				
	18	A_1B	+	-
(1)	2	A_2B	-	+
	4	A_2B	-	-

/59

(1) On account of the negative reactions with (a_1) these are classified under the type A_2B.

These observations are contrary to Thomsen's view, since all the 38 individuals of A_2 were heterozygotes and also the agglutinogen O seemed to be present in the cells of certain individuals of sub-group A_2B. This was strongly indicated by the fact that these cells could absorb the agglutinin (a_2).

According to these observations, the cells of only 3 out of 166 individuals of sub-group A_1 were agglutinated by (a_2). This number is very small compared to the expected frequency of heterozygotes in this sub-group. The same was observed by Landsteiner, Thomsen, Friedenreich and Zacho. This, however, may be explained by assuming that on account of the greater development of the agglutinogen A in sub-group A_1 [(1)], the O agglutinogen will be less developed in this sub-group than in sub-group A_2 and therefore cannot be demonstrated with the weak anti-O serum. This is supported by the fact that the reactions obtained with these three individuals were weaker than those obtained with A_2 cells. When several specimens of blood of sub-group A_1 were tested at a low temperature with this agglutinin - at which temperature the agglutination reactions are stronger than at room temperature - the majority were agglutinated to variable extents. The fact that some specimens were not agglutinated suggested that the reactions were specific. This was proved by the absorption method, since the agglutinin was absorbed in a specific manner only by the cells which were aggltuinated. The same was noticed with cells of individuals of group B, some of which could absorb the agglutinin (a_2), while others could not.

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(1) See page 21.

/60

This did not occur when the agglutinin (a_1) was absorbed with cells of group B: in this case the treated serum was as active toward A cells as the untreated serum. The behaviour of B cells towards the agglutinin (a_2) will be described in detail under the subject of Sub-division of this Group.

The independence of the agglutinin (a_2) from the auto-agglutinins was proved by treating the serum of A_1B (1) which contained a strong (a_2) with its own cells, after which it was found not to have been absorbed. The same happened when the whole blood was allowed to stand in an ice-box for 24 hours.

Therefore the agglutinin (a_2) differs from (a_1) since it was proved that all sera containing the (a_1) could be rendered inactive if they were repeatedly absorbed with their own cells at 0°C.

From the above-mentioned observations one may assume that agglutinin (a_2) is specific for an agglutinogen mainly present in group O cells and present to a less extent in A_2 cells and in a still smaller quantity in cells of A_1 and some A_2B cells. This, however, has been proved by the aid of immune sera, as will be shown in Section III.

THE EXISTENCE OF TWO TYPES OF THE AGGLUTINOGEN B.

The writer examined about 200 bloods of group B by testing the serum and cells of each blood with known test cells and sera respectively. Occasionally when there were several specimens of this group in the daily batches examined, the sera and cells of each of these specimens were tested against each other. There was no exception to

(1) The cells of this blood were proved later to contain no O substance.

the scheme of the four groups. This agreed so far with the results obtained by other workers in that the agglutinogen B is, unlike A, identical in the cells of all blood. The presence of agglutinogen O in certain cells of group B could be demonstrated by the absorption method as mentioned before.(1)

At that time the only available serum containing the agglutinin (a_2) was of sub-group A_1 and it was impossible to separate the (a_2) agglutinin from (b), since the former was absorbed by the few specimens of group B cells used for this purpose. Therefore the effect of (a_2) on B cells could not be thoroughly studied. During that time the writer was preparing immune sera for the agglutinogens O, A and B and when an immune anti-O serum (from rabbits or goats) was purified(2) the cells of a number of specimens of group B were tested. The cells of one specimen were strongly agglutinated, those of another were not agglutinated at all, and the rest gave weak reactions. The bloods of these individuals, all medical students, were taken and the sera and cells of each were examined against those of the others.(3) The sera of two specimens showed abnormal reactions as given in Table No. 15 which also shows their effect on cells of Group O.

TABLE No. 15.

GROUP B SERUM OF	BLOOD CELLS OF GROUP B										
	Ad.	Ba.	Hif.	Has.	Ga.	Y.S.	Ho.	Sh.	M.I.H.	Isk.	O Cells
Y.S.	tr.	±	±	±	±	-	tr.	tr.	+	+	-
M.I.H.	-?	-	-	-	-	±	-	-?	-	-	+

(1) See page 59.

(2) See Section III

(3) Such sera are very rare.

/62

The fact that the serum of M.I.H. agglutinated the cells of group O and of only a few specimens of group B, indicated that the agglutinin present in this serum is similar to the agglutinin (a_2) previously described. The serum of (Y.S.) did not agglutinate the cells which were agglutinated by the former serum, but agglutinated all the others. This may be explained by an agglutinin which acts on the majority of B cells just as the agglutinin (a_1) acts on A_1 cells. This was confirmed by titration and absorption methods.

The cells of 20 specimens of group B (including those given in Table No. 15), 12 of sub-groups A_1B and 6 of sub-group A_2B were titrated with anti-B sera.

This revealed that the cells of these two groups vary in their sensitivity towards anti-B sera. The difference was not so marked as in group A, but still it agrees with the results obtained with the above-mentioned sera of Y.S. and M.I.H.

The reactions obtained with B specimens were of three degrees, one was highly agglutinated, one was comparatively weak and the third was intermediate.

The specimens of the sub-group A_1B were either weakly or moderately agglutinated and the same was the case with the specimens of the sub-group A_2B.

The results representing the varying degrees of agglutination are given in Table No. 16. The first three specimens are given in Table No. 15.

/63

TABLE No. 16

BLOOD CELLS OF GROUP		ANTI-B SERUM 1:4	1:8	1:16	1:32	1:64	1:128	1:256
M.I.H.	(B)	++	+	+	+	±	tr.	-
Ad.	(B)	++	+	+	+	±	-	-
Y.S.	(B)	++	+	±	±	[illegible]	-	-
Ri.	(A_1B)	++	+	+	±	f.tr.	-	-
Su.	(A_1B)	++	+	±	tr.	-	-	-
A.S.	(A_2B)	++	+	+	±	tr.	-	-
Mo.	(A_2B)	++	+	±	tr.	-	-	-

On comparing the reactions given with the first three specimens with the reactions of these specimens with the sera of Y.S. and M.I.H. shown in Table No. 15, the following points are noticed:-

1) The cells agglutinated with the serum of M.I.H. are less sensitive towards the anti-B serum than those which were not agglutinated. This strongly indicates that the agglutinin of this serum, which also agglutinated O cells, is similar to (a_2) and therefore may as well be called (b_2). Both of these should be called anti-O agglutinin.

2) The cells which were agglutinated with Y.S. serum are more sensitive towards anti-B serum than those which were not agglutinated. It is interesting to note this parallelism. This, together with the fact that the cells of Y.S., whose serum gave these reactions,

/64

were of the weakly sensitive type of B cells, strongly indicates that we are dealing with an agglutinin similar in its nature to the agglutinin (a_1) regarding its effect on A_1 cells. Therefore one may call it (b_1) and the cells which were agglutinated with its serum B_1, and those which were not agglutinated B_2.

With regard to the presence of these types in group AB cells, they were not tested with these two sera which contained also agglutinin (a). But the two types B_1 and B_2 could be easily differentiated in each of sub-groups A_1B and A_2B, according to their sensitivity towards normal anti-B serum; therefore four sub-groups are present in group AB, namely:-

A_1B_1 (Ri).

A_1B_2 (Su).

A_2B_1 (A.S.)

A_2B_2 (Mo.)

This was further confirmed by the absorption method. Normal anti-B serum was absorbed for 2 hrs. at room temperature with a ½ of its volume of the washed cell sediment of M.I.H. (B_1), Y.S. (B_2), Ri. (A_1B_1), Su (A_1B_2), A.S. (A_2B_1) and Mo. (A_2B_2). The absorbed sera tested with these cells and the results obtained are given in Table No. 17.

/65

TABLE No. 17.

ANTI-B SERUM ABSORBED WITH CELLS		TESTED WITH CELLS					
		M.I.H.(B_1)	Y.S.(B_2)	Ri.(A_1B_1)	Su.(A_1B_2)	A.S(A_2B_1)	Mo.(A_2B_2)
M.I.H.	(B_1)	-	-	-	-	-	-
Y.S.	(B_2)	±	-	f.tr.	-	tr.	-
Ri.	(A_1B_1)	-	-	-	-	-	-
Su.	(A_1B_2)	±	f.tr.	tr.	-	tr.	-
A.S.	(A_2B_1)	-	-	-	-	-	-
Mo.	(A_2B_2)	±	-	f.tr.	-	tr.	+

These results show that when anti-B serum is absorbed with A_1B_2, A_2B_2 or B_2, the serum can still agglutinate the B_1, A_2B_1 and A_1B_1 cells. On the other hand absorption with B_1, and A_2B_1, and A_1B_1 completely removes the agglutinating action of the serum for B_1, A_2B_1 and A_1B_1 cells. This is similar to what happens with anti-A serum when absorbed with A_1 and A_2 cells; the only difference is that with anti-B serum, the absorption with B_2 cells weakens the action of the serum more than in the instance when anti-A serum is absorbed with A_2 cells.

Several other sera of group B were tested but only one more was found to contain agglutinin (b_2) (anti-O), but none was found to contain the agglutinin (b_1).

THE SPECIFICITY OF AGGLUTININS (b_2) and (b_1)

The agglutinin (b_2).

The serum of M.I.H. which contains the agglutinin (b_2) was

/66

titrated with the cells of group O and sub-group B_2 at 35°C and it was found that the titre with the first was 1:4 and with the second 1:2. Cells of both these groups absorbed the agglutinin completely. Certain cells of sub-group B_1, namely (Ad) and (Hif) the reaction of which are given in Table No. 15, could absorb this agglutinin although they were not agglutinated with it. The cells of M.I.H. and Isk. of the same sub-group could not absorb the agglutinin even after repeated absorption. Therefore one may conclude that the agglutinin (b_2) is specific for agglutinogen O, which is also present in all B_2 and some B_1 cells, but to a lesser extent in the latter since its presence could only be proved by absorption and not by agglutination.

The agglutinin (b_1).

The serum of Y.S. containing the agglutinin (b_1) was absorbed with the cells of sub-groups B_2 and B_1, and it was found that the B_1 cells absorbed the agglutinin in a single absorption and the B_2 cells weakened its action at the first absorption but could only remove it completely after a second absorption. The same result was obtained with the agglutinin (b_1) prepared from anti-B serum. This is similar to what has been noticed with the agglutinin (a_1) when it was absorbed with A_2 cells, the only difference being that the B_2 cells can more readily absorb the agglutinin (b_1) than the A_2 cells do with the agglutinin (a_1). This, together with the weakness and rarity of the agglutinin (b_1) may account for the fact that other workers have not considered the possibility of the sub-division of group B. This, however, can be accounted for by assuming that the B agglutinogen is stronger than the A and therefore reacts more strongly towards the anti-B serum even if present in small

/67

quantities as in B_2 cells than the A with its corresponding antibody. Consequently one may assume that the difference between the B_1 and B_2 cells is based on the presence of a larger quantity of the agglutinogen B in the former than in the latter, as is the case with the sub-groups A_1 and A_2.

The cells of 50 bloods of group B were tested with the agglutinins (b_1) and (b_2) found in the sera of Y.S. and M.I.H. respectively. The cells of 44 were agglutinated by (b_1) only and the cells of 6 bloods were agglutinated by (b_2) only. The reactions obtained with (b_2) were always equal and distinct but those obtained by (b_1) were sometimes strong and sometimes weak.

The blood cells of 30 bloods of group AB were tested with the serum of sub-group A_1B_1 which contains (b_2) or (a_2) agglutinin and also with the agglutinin (b_1) prepared by absorbing the anti-B serum with cells of A_1B_2 (Su.); the results were as follows:-
11 belonged to sub-group A_1B_1, 13 to sub-group A_1B_2, 3 to sub-group A_2B_1 and 3 to sub-group A_2B_2.
All the specimens were tested with anti-O serum (a_2 or b_2) which was found in a serum of sub-group A_1B_1 and only the specimens which belonged to sub-group A_2B_2 were weakly agglutinated; all the others were negative.

Recently the publication of N. Masaki (54) has come into the hands of the writer, in which he mentions that by titrating anti-B serum against different B cells, two types of these were found, one being agglutinated up to a titre 1:60 and the other up to a titre 1:160, the second type was more common than the first. He also tested the absorbing power of 13 blood cells of group B and found that in three cases the serum was absorbed completely by 1/8th of its volume, in five cases by 1/4, in three

cases by 1/2, and in two cases by an equal volume of cells. His results seem to confirm those obtained by the writer which were explained by the existence of sub-groups in group B. He did not find the abnormal agglutinins.

The discovery of these types in group B strongly supports the assumption that the sub-division of the groups A, B and AB rests on a quantitative basis, since it would have been strange to find that only the agglutinogen A could be present in different quantities. It has also yielded further knowledge with regard to anti-O serum (a_2 or b_2) and the presence of an agglutinogen in O cells which is also present in A_2 and B_2 cells, as well as in the cells of certain individuals of sub-groups A_1 and B_1, and group AB.

The pattern according to which the distribution of the three agglutinogens A, B and O in the cells of the four groups is regulated and the explanation of these sub-groups are considered in Section III.

S E C T I O N III

THE RELATION BETWEEN THE AGGLUTINOGENS O, A AND B IN THE FOUR BLOOD GROUPS

The study of the abnormal agglutinin (a_1)[1] confirmed the existence of the sub-groups A_1, A_2, A_1B and A_2B already described by Landsteiner and his assistants. It has also revealed that the agglutinogens A_1 and A_2 are of the same quality and only differ quantitatively. The study of the agglutinin (a_2) showed that it is specific for an agglutinogen present in the cells of group O and therefore confirmed the opinion of Thomsen and Schiff. The action of this agglutinin on the cells of all A_2 specimens examined by the writer was

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(1) See Section II.

/70.

explained by the assumption that all such specimens contain agglutinogen O but in a lesser quantity than that found in O cells. This is contrary to the view of Thomsen, that this agglutinin acts only on the heterozygous persons of sub-group A_2.(1) The fact revealed from family observations that several A_1 parents could give birth to O children indicates that these individuals must be heterozygotes. Amongst the 166 specimens of A_1 examined with the agglutinin (a_2) only 3 specimens were weakly agglutinated. This number was less than expected. However the presence of the agglutinogen O in other specimens of A_1 was proved by the ability of such cells to absorb the agglutinin (a_2). The same was proved with some specimens of group B. The inability of the (a_2) agglutinin to agglutinate such cells was explained by its weakness. This was also indicated by its low titre against O cells.

At this stage the writer decided to prepare an anti-O serum by absorbing ox serum with the cells of sub-group A_1B, according to the observations of Schiff (38) and Greenfield (55). Several ox sera which gave stronger reaction with O cells than with those of the other groups, were treated in this manner, but only in a few cases was a treated serum obtained which agglutinated weakly the cells of groups O and A_2 but did not agglutinate A_1 or B. The reactions were not stronger than those given by the agglutinin (a_2).

The results of Hooker and Anderson (56) regarding the preparation of immune anti-O from rabbits encouraged the writer to attempt the preparation of immune sera, in order to study the nature of the different agglutinogens found in the sub-groups known at that time. By this method, most of the animals immunized produced a specific serum

/71

(1) See Section IV.

for O cells, which also acted strongly on A_2 cells. The ability of the immune anti-O serum to agglutinate strongly the cells of a certain individual (Y.S.) and not those of another individual (M.I.H.) both of whom belonged to group B, led to the discovery of the sub-division of group B in the same manner as group A,[1] and further sub-division of sub-groups A_1B and A_2B, hence nine different types of bloods could be differentiated namely:-

$$O,\ A_1,\ A_2,\ B_1,\ B_2,\ A_1B_1,\ A_1B,\ A_2B_1,\ A_2B_2.$$

The study of the immune anti-O serum revealed the existence of six additional types as described below.

IMMUNIZATION EXPERIMENTS

The object of these experiments was to prepare immune sera against the agglutinogens O, A, B, M and N, and also to study the nature of the agglutinogens present in sub-groups A_1 and A_2.

Five goats and twelve rabbits were immunized with the cells of groups O, A_2, A_1 and B respectively. The bloods of the five goats, but not those of the rabbits, were tested before immunization. The goat cells were tested with six human sera, of groups A, B and O. The results are given in Table No. 18 which also shows the reactions given with A and B cells.

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(1) See Section II.

TABLE No. 18

CELLS OF GOAT	TESTED WITH SERUM OF GROUP					
	A (T.S)	B (T.S)	O (82)	O (92)	O (184)	O (188)
1	tr.	±	-	±	-	±
2	tr.	±	-	±	tr.	+
3	tr.	tr.	tr.	tr.	tr.	+
4	tr.	tr.	-	-	-	+
5	-	tr.	-	tr.	-	+
Human A	-	++	++	++	++	++
Human B	++	-	++	++	++	++

The reactions obtained with the goat cells were different from those obtained with A and B cells which indicates that the former do not contain agglutinogens A or B, therefore, the animals appeared suitable for the production of immune sera against A and B cells.

The sera of the five goats were found to agglutinate all human cells. They were absorbed by different cells in order to ascertain whether they contained agglutinins (a) and (b) or whether the reactions were due to species agglutinins.

Table No. 19 shows the results obtained by testing the absorbed sera with different blood cells.

TABLE No. 19

SERUM OF GOAT No.	ABSORBED WITH O CELLS AND TESTED WITH			ABSORBED WITH A_1 CELLS AND TESTED WITH			ABSORBED WITH B AND TESTED WITH		
	O	A_1	B	O	A_1	B	O	A_1	B
1	-	±	±	±	-	-	tr.	±	-
2	-	tr.	tr.	tr.	-	-	-	tr.	-
3	-	tr.	tr.	-	-	tr.	tr.	±	-
4	-	+	tr.	-	-	-	-	±	-
5	-	tr.	±	-	-	±	tr.	tr.	-

The results show that the reactions were either negative or weak, which made it difficult to tell if such sera contained specific agglutinins for the agglutinogens O, A and B. However, these reactions were taken into consideration in choosing animals suitable for immunization by the cells of each group, e.g. Goat No. 5 was immunized with B blood cells because its serum showed a tendency to agglutinate these cells to a greater extent.

Six types of blood cells were used by the writer in the immunization experiments, namely, OM, OMN, A_2M, A_1N, BN and A_2MN (1) the last of which reacted only with the sera containing strong (a_2) agglutinins. The animals immunized with each type of the above-mentioned cells are shown in Table 20.

TABLE No. 20

RABBITS Nos.	GOATS Nos.	IMMUNIZED WITH CELLS OF TYPE
1 and 2	1	OM
3 and 4	2	OMN
5 and 7	3	A_2M
8 and 9	4	A_1N
10 and 11	5	BN
12 and 13	-	A_2MN

(1) At the time of immunization, the sub-group B_1 and B_2 were not known to the writer. /74

METHODS OF IMMUNIZATION:

Various methods used by different workers have given satisfactory results (57). Here are three examples which form the basis of the different methods:-

1. Four or more intraperitoneal injections with an interval of 4 to 7 days between each injection.
2. Four or more intravenous injections with an interval of 2 to 4 days.
3. Daily intravenous injections for the first week, followed by an interval of one week, after which another course is given. The first injection is given subcutaneously or intraperitoneally, and the others intravenously. A third course may be given if found necessary.

In all these methods, the cells were washed twice with saline and then made into a 30% suspension in saline before injection.

The method applied by the writer was as follows:-
The blood was taken in small jars containing glass beads and, after being defibrinated, was centrifuged. The blood cells were washed with saline, re-suspensed in fresh saline, and then filtered through a small piece of cotton-wool to remove clots. The cells were then washed again, and a 30% suspension in saline made. Every precaution was taken to keep the blood sterile, by the sterilisation of all the vessels used in the experiments, together with the instruments and saline used in the preparation. The cells of the first five types, namely:-
OM, OMN, A_2M, A_1N and BN were obtained from two or three donors for each type; while the cells of the last type (A_2MN) were taken from one donor. All the bloods were used on the same day as they were taken.

/75

The average weight of the rabbits was about 2 kilos, and that of the goats 8-10 kilos.

Six injections were given to each animal, the first of which was intraperitoneal in the instance of the rabbits and intramuscular in the case of the goats; the other five injections were given intravenously in all the animals. 2 c.c. of 30% suspension of the cells were given to each rabbit, and 10 c.c. were given to each goat. The interval between each injection was 2 days, so the course was completed in 11 days. The reactions observed were very slight in all animals with the exception of Goat No. 2, immunized with Group O blood cells, in which there was a severe rigor accompanied by hemo-globinuria after the fifth and sixth injections, which disappeared after a few hours. All the animals survived the full course of injections.

Three days after the last injection, the blood was taken and its serum titrated against all human cells, when it was found that all of them had attained a high titre strength. On the seventh day another sample was taken, and the titre was found to be still higher in most of them. Two days after this, the rabbits were finally bled by cutting the carotids, and between 30 to 40 c.cs. of blood were obtained from each rabbit, while about 100 c.c. of blood was taken from the jugular vein of each goat without any serious after-effect.

The blood which was put into centrifuge tubes was allowed to stand in the ice-chest over night and next morning the tubes were centrifuged, after which the clear serum was placed in sterile tubes which were corked, sealed, and kept at a temperature below 0°C.

PURIFICATION AND PROPERTIES OF THE SERA OBTAINED BY IMMUNIZING ANIMALS WITH O BLOOD CELLS

The sera of goats 1 and 2 and rabbits 1, 2, 3 and 4, immunized with O cells were titrated with cells of groups O, A_2, A_1, B and A_1B. The results obtained, which are given in Table No. 21, show that each serum agglutinated all the cells to the same extent. The titre of the six sera varied from 320-5120.

TABLE No. 21

SERUM No.	OM	ON	OMN	A_1M	A_1N	A_2M	A_2N	BM	BMN	A_1BMN
G.1	640	640	640	320	640	640	640	320	320	320
G.2	320	320	320	320	320	320	320	320	320	320
R.1	1280	1280	1280	1280	1280	1280	1280	1280	1280	1280
R.2	1280	1280	1280	1280	1280	1280	1280	1280	1280	1280
R.3	5120	5120	5120	5120	5120	5120	5120	2650	5120	2650
R.4	2650	1280	2650	2650	1280	2650	1280	2650	2650	2650

ABSORPTION TESTS.

The sera were inactivated by placing them in a water bath at 55°C. for half an hour to prevent hemolysis. This, however, was not necessary in all cases, since, when the serum had been kept in the ice-chest for 4-6 weeks, on being diluted, it did not hemolyse the blood cells.

The sera of rabbits were diluted from five to ten times

according to their titre. The serum of goat 1 was diluted 1:3 and that of goat 2 was used undiluted. Cells of sub-group A_1BMN (Ri), the serum of which contained a comparatively strong normal anti-O agglutinin (a_2 and b_2), were used for absorbing the above-mentioned sera.

The object in selecting these cells, was to absorb the species agglutinins together with any specific agglutinin for A, B, M and N agglutinogens, but not that for O agglutinogen.

According to the presence of the normal anti-O agglutinin in the serum of this blood, one would expect to find no O agglutinogen in these cells, and, therefore, they were suitable for preparing anti-O agglutinin.

The absorption was performed by adding to a small quantity of serum half its volume of washed cell sediment. After two hours standing at room temperature, the serum was separated and tested with a specimen of the same cells as had been used in absorption: when a serum was found still to give a reaction with these, it was re-absorbed by a further quantity of fresh cells. In some cases, the absorption had to be repeated three times before the serum was rendered negative towards these cells.

The absorbed sera were then tested, and the results given in Table No. 22 show that the sera of goats 1 and 2, and rabbits 1 and 3 contain an agglutinin, which acts strongly on O and A_2 blood cells, and gives weak reactions with both A_1 and B cells, but not with A_1B cells, used in the experiment. The fact that it agglutinated OM, ON and OMN strongly, but not A_1BMN and A_1BM differentiated this agglutinin from anti-M and N agglutinins.

/78

TABLE No. 22

ACTION OF PURIFIED IMMUNE ANTI-O SERA

SERUM No. ABSORBED WITH A_1BMN	TESTED WITH CELLS OF TYPES										
	OM	ON	OMN	A_2M	A_2N	A_1M	A_1N	BM	BMN	A_1BMN	A_1BM
G. 1	++	++	++	++	++	tr.	tr.	±	±	-	-
G.2	++	++	++	++	++	tr.	tr.	±	±	-	-
R.1	+++	+++	+++	++	++	±	±	±	±	-	-
R.2	-	-	-	-	-	-	-	-	-	-	-
R.3	+++	+++	+++	++	++	±	±	±	±	-	-
R.4	±	±	±	tr.	tr.	-	-	-	-	-	-

The writer thought at first that the reactions with A_1 and B cells, were due to species agglutinins, and so reabsrobed the serum with a fresh quantity of A_1BMN cells, and let the tubes stand for 24 hours, but the reactions were still obtained and at the same strength as before.
The similarity of the reactions, obtained with these different sera, may be explained on the basis of one of the following two assumptions.

1. That the sera of these animals contain one similar agglutinin, which acts on a corresponding agglutinogen, present to a variable extent in the cells which give positive reactions, and that, therefore the serum acts strongly on the cells containing large quantities of this agglutinogen and weakly on those which contain small quantities.

2. That the serum in each animal contains more than one agglutinin and the cells contain different agglutinogens.

In order to elucidate this point, the following experiment was made:-

The serum of goat 1 was absorbed with each of the following cells:- OM, ON, A_2M, A_2N, A_1M, A_1N, BM and BMN, by using a quantity of cells equal to half the volume of the serum. The absorbed sera were tested with the same cells. The cells of O and A_2 were found to absorb the agglutinin completely, but A_1 and B cells only weakened the action of the serum to some extent, that is cells of types A_1 and B were not agglutinated by sera treated with either of these blood cells, while those of O and A_2 were moderately agglutinated, as shown in Table No. 23.

TABLE No. 23

SERUM OF GOAT 1	ABSORBED WITH CELLS	TESTED WITH CELLS								
		OM	ON	OMN	A_2M	A_2N	A_1M	A_1N	BM	BMN
Undiluted	Unabsorbed	++	++	++	++	++	tr.	tr.	±	±
	OM	-	-	-	-	-	-	-	-	-
	ON	-	-	-	-	-	-	-	-	-
	A_2M	-	-	-	-	-	-	-	-	-
	A_2N	-	-	-	-	-	-	-	-	-
	A_1M	++	++	++	+	+	-	-	-	-
	A_1N	++	++	++	+	+	-	-	-	-
	BM	++	++	++	+	+	-	-	-	-
	BMN	++	++	++	+	+	-	-	-	-

The goat serum was diluted 1:8 and then was absorbed with cells of A_1, B, and A_1BMN (Ri). By this procedure the former two cells were /80

found to absorb the agglutinin acting on A_2 and O which did not occur with A_1BMN (Ri.) cells.[1] The results are given in Tabel No. 24.

TABLE No. 24

SERUM OF GOAT 1	ABSORBED WITH CELLS	TESTED WITH CELLS								
		OM	ON	OMN	A_2M	A_2N	A_1M	A_1N	BM	BMN
DILUTED 1:8	Unabsorbed	+	+	+	±	±	-	-	tr.	tr.
	A_1M	-	-	-	-	-	-	-	-	-
	A_1N	-	-	-	-	-	-	-	-	-
	BM	-	-	-	-	-	-	-	-	-
	BMN	-	-	-	-	-	-	-	-	-
	A_1BMN (Ri).	+	+	+	±	±	-	-	tr.	tr.

These two experiments demonstrated the following four points:-

1. This anti-O serum is different from anti-M and N sera, because while it could be absorbed completely with the cells of the types OM, ON, OMN, A_2M and A_2N, it was not affected by A_1BMN (Ri.) cells and only to a slight extent by A_1M, A_1N, BM and BMN.

   One might assume that M and N are developed in group O to a greater extent than in A_1 and B blood cells, but this was not found to be the case as will be described under the study of M and N types.[2]

2. The strong agglutination of O and A_2 cells and the weak reaction with A_1 and B cells are due to the presence of a corresponding agglutinogen in all these cells.

   Thus it would appear that:-

   (i) O and A_2 blood cells absorbed the whole agglutinin, so that the

(1) This result depends on the particular A_1BMN used.

(2) Section V.

treated serum no longer agglutinated any of the cells which were previously agglutinated.

(ii) Treatment with A_1 and B cells weakened the action of the undiluted serum on A_2 cells but not on O cells (see Table 6), and rendered the diluted serum negative towards both O and A_2 cells.

3. The difference in the reactions with, and in the absorptive power of O and A_2 cells, on the one hand, and A_1 and B cells on the other, led to the conclusion that the agglutinogen common to the four types of cells is present to a greater extent in the first two than in the second two. The fact that the absorption of the serum with A_1 or B cells weakens the reactions with A_2 more than with O, proves that O blood cells contain more of this agglutinogen than A_2 does. This was confirmed later by absorbing the serum with repeated small doses of A_2 cells, until the reaction of these cells became negative, at which point the O blood cells were still weakly agglutinated.

4. A_1BMN (Ri) cells could not absorb the agglutinin from the diluted serum, and it has been previously shown that this was the case also with the undiluted serum even after repeated absorptions. The results indicate that this agglutinin is not an anti-human species agglutinin.

When this serum was tested with further specimens of blood, it was found to give a strong reaction with the cells of one individual (Y.S) of group B, and a negative reaction with the cells of another individual (M.I.H.) of the same group. The study of the bloods of these two individuals revealed the existence of the other sub-groups

in groups B and AB. Thus nine blood types were obtained, namely:-

O, A_1, A_2, B_1, B_2, A_1B_1, A_1B_2, A_2B_1 and A_2B_2 (1)

STUDY OF THE ACTION OF THE ANTI-O SERUM ON CELLS OF DIFFERENT TYPES.

Sera of rabbit 3 and goat 1 were absorbed with the cells of A_1BMN (Ri.) which, after the discovery of the sub-groups in group B, proved to be A_1B_1. The absorbed sera were used in testing the cells of 140 bloods (2) of different sub-groups, most of which have been already examined. The sub-groups of the specimens of groups A, B and AB were determined according to the technique described in Section II. The two sera behaved exactly alike with all the cells tested.

Four distinct types of reactions were obtained with the various specimens tested, namely: strong, moderate, weak or negative.

The results obtained with the sera of goat 1 and rabbit 3 are given in Table No. 25 according to the different reactions obtained with the different types.

TABLE No. 25

ACTION OF PURIFIED IMMUNE ANTI-O SERUM

GROUP	O	A				B				AB			
SUB-GROUP		A_2	A_1			B_2	B_1			A_1B_1	A_1B_2	A_2B_1	A_2B_2
REACTION	St.	St.	Mod.	We.	Neg.	St.	Mod.	We.	Neg.	Neg.	We.	We.	Mod.
NUMBER OF SPECIMENS EXAMINED	50	12	12	7	5	6	12	5	4	9	12	3	3
			24				21						
	50		36				27				27		
TOTAL	140												

St. = strong Mod. = moderate We. = weak Mod. = moderate

(1) See Section II.

(2) These bloods were taken by selection, hence the distribution of the groups and sub-groups in them does not represent the actual frequency in Egypt.

/83

The results given in this table show that:-

1. All the blood cells of groups O, A_2 and B_2 are strongly agglutinated.
2. Some A_1 and B_1 cells and all A_2B_2 cells are moderately agglutinated.
3. Some A_1 and B_1 cells and all A_1B_2 and A_2B_1 cells are weakly agglutinated.
4. Some A_1 and B_1 cells and all A_1B_1 cells are negative.

The reactions so far obtained confirmed the existence of the already-known sub-groups and revealed the presence of three further types in each of sub-groups A_1 and B_1. The existence of these types was confirmed by titrating the serum against the cells of the specimens which were agglutinated. The reactions with each type are given in Table No. 26.

TABLE No. 26

CELLS OF TYPE	REACTION WITH UNDILUTED SERUM	REACTION WITH DILUTED SERUM 1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
O	very strong	+++	++	++	++	+	+	±	-
A_2	strong	++	++	++	+	±	tr?	-	-
B_2	strong	++	++	+	+	±	-	-	-
A_1	moderate	+	+	±	tr.	-	-	-	-
B_1	moderate	+	+	tr.	-?	-	-	-	-
A_2B_2	moderate	+	+	±	-?	-	-	-	-
A_1	weak	tr.	f.tr	-	-	-	-	-	-
B_1	weak	tr.	-?	-	-	-	-	-	-
A_1B_2	weak	tr.	-?	-	-	-	-	-	-
A_2B_1	weak	tr.	f.tr	-	-	-	-	-	-

? means in some specimens of cells positive and in others negative.

/84

EFFECT OF ABSORPTION WITH THE CELLS GIVING MODERATE, WEAK OR NEGATIVE REACTIONS.

Previously it was shown that O and A_2 cells can completely absorb the agglutinin present in this immune anti-O serum (see Table No. 23. In order to study the effect of the cells of other sub-groups, the serum was absorbed with the cells of sub-group B_2 as well as with the cells of the three types of A_1, B_1 and all the types of group AB, which were agglutinated moderately, weakly, or were negative respectively. The serum was absorbed with half its volume of blood cells for two hours and the absorbed sera were tested with all cells. Table No. 27 shows the results obtained with the types of A_1 and AB. The B_2 cells absorbed the agglutinin completely and the three types of B_1 behaved similarly to those of A_1, therefore have not been inserted in the Table.

TABLE No. 27

SERUM TESTED WITH	O	A_2	A_1			B_2	B_1			A_1B	A_1B_2	A_2B_1	A_2B_2
			Mod.	We.	Neg.		Mod.	We.	Neg.	Neg.	We.	We.	Mod.
Before absorption	+++	++	+	tr.	-	++	+	tr.	-	-	tr.	tr.	+
Absorbed with													
A_1 moderately agg.	+	±	-	-	-	±	-	-	-	-	-	-	-
A_1 weakly "	++	+	-	-	-	+	-	-	-	-	-	-	-
A_1 not "	+++	++	±	tr.	-	++	±	tr.	-	-	tr.	tr.	±
A_1B_1 negative	+++	++	±	tr.	-	++	±	tr.	-	-	tr.	tr.	±
A_1B_2 weakly "	++	+	tr?	-	-	+	-	-	-	-	-	-	f.tr.
A_2B_1 weakly "	++	+	-	-	-	+	-	-	-	-	-	-	-
A_2B_2 moderately "	+	±	-	-	-	±	-	-	-	-	-	-	-

These results show that the cells which were not agglutinated by this

serum has no effect whatever on the absorbed serum. Those which were moderately agglutinated had weakened the action of the serum to a more marked extent than those which were weakly agglutinated.

In order to test the effect of repeated absorption, each portion of the serum was re-absorbed twice with ¼ its volume of fresh blood cells of the same specimen as was used in the first absorption. The serum was titrated after each absorption with the cells of group O and sub-groups A_2 and B_2.

It was found that the cells which were not agglutinated with this serum had no effect even after repeated absorptions: those which were moderately agglutinated absorbed the agglutinin completely after the second absorption, and those which were weakly agglutinated absorbed it only after the third absorption.

The results obtained with the two types A_2B_1 and A_2B_2, which represent the cells which are moderately or weakly agglutinated are given in Table No. 28.

TABLE No. 28

No. OF ABSORPTION OF SERUM OF G. 1.	PROPORTION AND TYPE OF CELLS USED		TESTED WITH CELLS	TITRE OF ABSORBED SERUM 1:1	1:2	1:4	1:8	1:16	1:32	1:64
1st absorption	1:2	A_2B_1	O	++	++	+	+	±	tr.	-
			A_2	+	+	±	tr.	-	-	-
			B_2	+	+	±	f.tr.	-	-	-
		A_2B_2	O	++	+	+	±	tr.	-	-
			A_2	+	±	tr.	-	-	-	-
			B_2	+	±	tr.	-	-	-	-
2nd absorption	1:4	A_2B_1	O	+	±	tr.	-	-	-	-
			A_2	tr.	-	-	-	-	-	-
			B_2	tr.	-	-	-	-	-	-
		A_2B_2	O	-	-	-	-	-	-	-
			A_2	-	-	-	-	-	-	-
			B_2	-	-	-	-	-	-	-
3rd absorption	1:4	A_2B_1	O	-	-	-	-	-	-	-
			A_2	-	-	-	-	-	-	-
			B_2	-	-	-	-	-	-	-

These observations were finally confirmed by diluting the serum 1:8 at which dilution it was still capable of agglutinating O, A_2 and B_2 cells, and then by absorbing it with all the cells which gave either negative or positive reactions with the undiluted serum. It was found that the diluted serum was completely absorbed with the cells of all the types which gave positive reaction, whether weak or strong. Those which gave negative reactions namely some A_1, B_1 and all A_1B_1 cells were incapable of absorbing the agglutinin from the diluted serum even after repeating the absorption twice. A_1B_1 cells were used in absorbing the serum at 0°C and the serum still remained active.

From the above-mentioned results one may conclude that all the specimens which were agglutinated by this serum possess a similar agglutinogen which reacts with a corresponding agglutinin found in this serum. The fact that some of these specimens reacted in various degrees indicates strongly that this agglutinogen is present in various quantities in them.

DEFINITION OF THIS AGGLUTININ-AGGLUTINOGEN PAIR.

Taking into consideration the nature and strength of the reactions, the following conclusions may be drawn regarding this agglutinogen.

(i) It is absent in the cells of all specimens of sub-group A_1B_1 and in certain specimens of A_1 and B_1.

(ii) It is present in small quantities in all the specimens of sub-groups A_1B_2, A_2B_1 and in certain specimens of A_1 and B_1.

(iii) It is present in moderate quantities in all the specimens of sub-group A_2B_2 and in certain specimens of A_1 and B_1.

(iv) It is present in large quantities in all specimens of sub-groups A_2 and B_2.

/87

(v) It is present in still larger quantities in the cells of group O.

On this account one may be justified in calling this agglutinogen O, in the sense of the letter O, but not in a negative sense. This nomenclature is adopted for the purpose of avoiding confusion due to the use of a new designation. The reason for changing the sense of the designation is that the presence of an agglutinogen characterising the cells of group O is definitely proved. Accordingly, the serum which acts on these cells in this particular case, is called immune anti-O.

THE RELATION BETWEEN THE THREE AGGLUTINOGENS O, A and B.

In Section II the writer has previously shown that there exists only one kind of A and B agglutinogens respectively. He has indicated that the difference in behaviour between A_1 and B_1, on the one hand, and A_2 and B_2, on the other, towards anti-A and B sera respectively, is simply due to the fact that A or B agglutinogen is more developed in the former two sub-groups than in the latter two, and is not due to the presence of an additional agglutinogen named A_1 by Landsteiner in the case of group A, and similarly B_1 in the case of group B.

In this Section the writer has shown that agglutinogen O exists in the cells of many sub-groups in various quantities.

The question now arises as to whether agglutinogen O does not play a part with A and B agglutinogens in the classification of human bloods into 4 groups. A positive answer to this question may be found in the study of the theories of heredity which will be discussed later. From the sero-logical point of view it may suffice, for the time being, to say that the immune serum produced by O cells acts in a specific manner on all cells of group O, in exactly the same way as immune anti-A and B

act on their own cells. If this agglutinogen is, like M or N, independent of the A and B agglutinogens, we should expect it to classify each of the four groups in a similar manner, according to its absence or presence. What actually has been demonstrated is that this agglutinogen has differentiated four sub-groups in groups A, B and AB but not in group O.

THE RELATION BETWEEN THE QUANTITY OF EACH OF THE THREE AGGLUTINOGENS PRESENT IN THE DIFFERENT SUB-GROUPS

It has been observed that each of the three agglutinogens may be present in variable quantities in different sub-groups. The relation between the extent of these three agglutinogens in a given sub-group may be studied by estimating the quantity of each agglutinogen in that sub-group.

A rough estimation of these quantities may be obtained either by testing the power of absorption of the different cells or by titrating them against the different sera. As the latter method is quicker and just as safe, it was selected by the writer.

He titrated several O, A, B and AB cells with anti-O, A and B sera. The results were not conclusive in all the bloods tested, but anyone who has had any experience with titration experiments will realise how difficult it is to obtain the expected results in all cases, or even similar results with the same material.

The writer submits, in Table No. 29, the results which represent the majority of the cells tested in each type of blood. The same sera were used in testing all the different cells and the titrations were all carried out under the same conditions.

TABLE No. 29

CELLS NAME	CELLS GROUP	HIGHEST TITRE AT WHICH THE CELLS ARE AGGLUTINATED ANTI-O SERUM	ANTI-A SERUM		ANTI-B SERUM
Mo.	O	128 (tr.)	-		-
N.D.	A_1	-	128 (±)	256 (tr.)	-
M.G.	A_1	-	128 (±)	256 (-)	-
Sa.	A_1	2 (tr.)	64 (+)	128 (f.tr.)	-
Wod.	A_1	4 (tr.)	64 (+)	128 (tr.)	-
Am.	A_1	16 (tr.)	64 (tr.)	128 (-)	-
Da.	A_1	16 (f.tr.)	64 (f.tr)	128 (-)	-
Amer.	A_2	32 ± 64 f.tr	32 (tr.)		-
Hil.	A_2	32 ± 64 -	32 (tr.)		-
Isk.	B_1	-	-		128 (+)
Okas.	B_1	2 (tr.)	-		128 (tr.)
At.	B_1	16 (f.tr.)	-		64 (±)
Y.S.	B_2	32 (±)	-		32 (tr.)
Mo.	A_1B_1	-	64 (f.tr.)		64 (tr.)
Sw.	A_1B_2	2 (tr.)	64 (tr.)		32 (f.tr.)
Ab.	A_2B_1	4 (tr.)	8 (±)	16 (-)	64 (±)
Moch.	A_2B_2	16 (tr.)	16 (tr.)		32 (tr.)

The variation in quantity is more marked by the titration method with O and A agglutinogens than with B agglutinogens which was explained by assuming that B has a stronger affinity than the other two and so it gives stronger reactions even when developed in small quantities.(1) The

(1) See Section II.

/90

agglutinogen O seems to be weaker than A because it reacts with difficulty when it is present in small quantities, therefore the anti-O serum is more suitable to differentiate these types from each other.

The results given in this table show a constant relationship between the quantity of each of the three agglutinogens present in the different sub-groups A, B and AB. To make this more clear, one may classify the different sub-groups according to the behaviour of the agglutinogen in each sub-group, as shown in Table No. 30.

TABLE No. 30.

		AGGLUTINOGEN		
GROUP	TYPE	O	A	B
A	1	-------	very strong	-------
	2	weak	strong	
	3	moderate	moderate	
	4	strong	weak	
B	5	-------	-------	very strong
	6	weak		strong
	7	moderate		moderate
	8	strong		weak
AB	9	-------	moderate	moderate
	10	weak	moderate	weak
	11	weak	weak	moderate
	12	moderate	weak	weak

The writer did not meet with a case where AB blood was negative with anti-O and the A weak and B strong, or the A strong and the B weak. If such types of blood exist, they must be very rare.

THE EXPLANATION OF THESE RESULTS.

According to Bernstein's theory there are three allelomorphic genes, O, A and B, each of which may be transmitted from each parent to the child in the form of one unit, therefore the child's genotype will be composed of 2 units, which may be similar or different. The genotypes obtained according to this theory are:- OO, AO, AA, BO, BB and AB. Thus there are two types in each of groups A and B and one type in group AB, and therefore such a theory does not explain the existence of the sub-groups O, A_1, A_2, B_1, B_2, A_1B_1, A_1B_2, A_2B_1 and A_2B_2, which have been described in Section II. This, however, was explained by the modification of this theory by Thomsen who assumed the presence of two genes A_1 and A_2 and later B_1 and B_2.

The writer has already shown in Section II that the genes A_1 and A_2 are actually one, but differ quantitatively, the same was also shown with B_1 and B_2.

Despite this, let us assume that Thomsen's assumption is correct, and see how far this theory can explain the above-mentioned results.

If we consider that A_1 and similarly B_1 are independent genes from A_2 and B_2 respectively, we will obtain the following genotypes:-

Gr. O (OO	Group A	Group B	Group AB
	A_2O	B_2O	A_2B_2
	A_2A_2	B_2B_2	
	A_1A_2	B_1B_2	A_2B_1
	A_1O	B_1O	A_1B_2
	A_1A_1	B_1B_1	A_1B_1

If, as Thomsen says, A_1 masks the agglutinogen O to a greater extent than A_2 does, and naturally the same applies to B_1 and B_2, the cells

/92

of either group A or B will give two kinds of positive reactions with anti-O serum, namely a strong reaction with A_2 and B_2 cells and either a moderate or a weak reaction with A_1 and B_1, but not a strong reaction with some A_1 and B_1 specimens and a weak reaction with others. Therefore this does not explain the three different positive reactions already mentioned. One may assume that some A_1 cells are more sensitive than others and so react more with anti-O. If this be true, we should expect that the A_1 cells which react more strongly with anti-O will react more strongly with anti-A and those which react weakly with anti-O will also react weakly with anti-A: but this is just the contrary to the results reported in Table No. 29, where one may see that the cells which are weakly agglutinated with anti-O are more strongly agglutinated with anti-A than those which react moderately with anti-O. The same holds with B_1. Hence it appears that there are three different types in the sub-groups called A_1 and B_1, namely:-

1) Cells reacting moderately with anti-A and anti-O.
2) Cells reacting strongly with anti-A and weakly with anti-O, and
3) Cells reacting more strongly with anti-A, but not with anti-O.

One might also try to explain the different reactions of these three types with anti-A by assuming that the agglutinogen A_1 reacts to a variable extent in the different genotypes A_1A_2, A_1O, A_1A_1. The answer to this is that it only explains the different reactions with anti-A but does not explain the different reactions with anti-O. Even according to this assumption A_1O alone of the three should be agglutinable by anti-O - A_1A_2 and A_1A_1 being unagglutinable by this anti-serum.

Another important point with regard to these results is that according to Bernstein's and Thomsen's theories, persons of group AB should not contain the agglutinogen O: but the writer has definitely

proved its existence in this group in two forms, either in small quantities as in both sub-groups A_1B_2 and A_2B_1 or in moderate quantities as in sub-group A_2B_2.

The facts prove that each of the three agglutinogens, O, A and B take part in classifying the human bloods into four groups, and especially that the three agglutinogens are present together in the cells of certain individuals of group AB (A_1B_2, A_2B_1 and A_2B_2). This explains why it is impossible for the above-mentioned theories to account for the results obtained by the writer. The reason is simply because these theories are based on the assumption that the genotype of the individual is composed of two genes and therefore the three agglutinogens will never meet together in one blood.

In order to explain the presence of the three agglutinogens in such individuals of group AB, one must assume that the genotype contains three units representing O, A and B. As the genotype of a child can never be composed of three units, since it receives an equal number from each parent, then the next possible genotypic formula will be the one containing four units, two of which are transmitted from each parent.

If a large number of sets of counters marked respectively A, B, O, are placed in a bag and sets of 4 are picked out, it is clear that the following combinations will be obtained.

1	OOOO	4	AAOO	7	ABOO	10	AAAO
						11	BBBO
2	AAAA	5	BBOO	8	AABO	12	AOOO
						13	BOOO
3	BBBB	6	AABB	9	ABBO	14	ABBB
						15	AAAB

To find out to what extent this hypothesis will explain the data obtained by the writer, the formulae obtained according to this

/94

hypothesis are compared with the results given in Tables Nos. 29 and 30. One may see that the first 13 of these formulae represent the presence of the three agglutinogens in the 13 sub-groups already obtained. This is shown in Table No. 31.

TABLE No. 31

FORMULAE OBTAINED ACCORDING TO THE HYPOTHESIS		SUB-GROUP OBTAINED BY THE WRITER GIVEN IN TABLES Nos.29 & 30 DEVELOPMENT OF AGGLUTINOGEN		
		O	A	B
Group O	OOOO	very strong	-	-
Group A	AAAA	-	very strong	-
	AAAO	weak	strong	-
	AAOO	moderate	moderate	-
	AOOO	strong	weak	-
Group B	BBBB	-	-	very strong
	BBBO	weak	-	strong
	BBOO	moderate	-	moderate
	BOOO	strong	-	weak
Group AB	AABB	-	moderate	moderate
	AABO	weak	Moderate	weak
	ABBO	weak	weak	moderate
	ABOO	moderate	weak	weak
	AAAB	These two types	strong	weak
	ABBB	were not found.	weak	strong

With regard to the last two formulae, it may be supposed either they are very scarce and so have not been found or they may not even exist on account of something which we cannot yet explain.

However, their non-discovery is not an obstacle to the application of this hypothesis, since it is not a fact incompatible with the hypothesis. The hypothesis will be discussed later in detail under

the subject of Heredity of the Blood Groups.

WHAT DOES THIS HYPOTHESIS EXPLAIN?

This hypothesis, although novel, seems to explain all the difficulties which could not be readily explained by other theories, namely:-

1. The existence of sub-groups can be explained without need of adding new agglutinogens namely A_1, A_2, B_1 and B_2, and A_1B_1, A_1B_2, A_2B_1 and A_2B_2 as already described in Section II.
2. The presence of agglutinogen O in various sub-groups, especially in certain ones of group AB.
3. The rare occurrence of natural anti-O serum (agglutinins a_2 and b_2) which may now be explained by the fact that this agglutinin exists in an active form in the sera of the sub-groups which do not contain an O agglutinogen and which are actually very rare. The reason why the writer found a larger number of these natural agglutinins than workers on the Continent or in America, is due to the fact that the frequency of group O is much higher in these countries than in Egypt, hence in these countries one would expect to find a smaller number of homozygote A or B persons, as well as AB persons lacking the agglutinogen O.

The reason why the normal anti-O serum failed to agglutinate the A_1, B_1 and AB cells which contain the agglutinogen O, is explained by the presence of smaller quantities of this agglutinogen in such cells than in A_2 and B_2 cells. Accordingly the cells of the former three sub-groups did not react on account of the weakness of the serum.

/96

4. The occurrence of (a_1) and (b_1) agglutinins in the sera of A_2 and B_2 bloods respectively, or their purification from anti-A and anti-B sera by absorption with A_2 and B_2 cells (as described in Section II).

5. The difference in the strength of reactions obtained with different cells of sub-groups A_1 and B_1 which might be explained by the presence of three types in each of sub-groups A_1 and B_1. (See Table No. 29).

6. The weak cells of the type A_2 which were found by Friedenreich[1] who called them A_3 and indicated the presence of a third quality in Group A. As he says, the A_3 reacts weaker than A_2 with anti-A serum but anti-O serum agglutinates A_2 and A_3 to a practically equal degree. Thus it may be assumed that both are of the type (AOOO) but for some reason or other the sensitivity is lowered; on account of having a very small quantity of A compared to that of O, one would expect that the weakness would be more marked with anti-A than with anti-O, which difference may altogether fail to be detected by ordinary technique. Therefore it seems unnecessary to assume that there is a third quality called A_3.

7. The fact that the writer, in addition to other workers, could not prepare anti-O serum by absorbing ox-serum with A_1B cells by which method Schiff and others succeeded, since it may be assumed that the A_1B cells used by the writer or the other workers were of the type A_1B_2 (AABO) and therefore they could absorb the anti-O.
Hirszfeld found that all ox-sera could be rendered inactive towards

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(1) See the proceedings of the IInd Congress of the International Society of Microbiology. July 1936.

/97

O cells after being repeatedly absorbed with A_1B cells, which may be explained on the same basis as above.

8. Moureaux (58), after a detailed study, came to the conclusion that it is impossible to differentiate the heterozygote from the homozygote individuals of groups A and B, since they behaved similarly towards anti-O serum prepared from ox-serum. He has chosen his homozygote persons from the following family.

FAMILY	SUB-GROUP	GENOTYPE
Father	A_2B	A_2B
Mother	A_1B	A_1B
Child 1.	A_1	A_1A_2
Child 2.	B	BB

According to the new classification an A_2B parent may be AOBO and the other parent may be AABO. Therefore the A_1 child may be AAOO and the B child BBOO or even BOOO. From that one may see that Moureaux was not dealing with pure types of A and B, as he thought, but with heterozygotes which contain agglutinogen O.

ADVANTAGES DERIVED FROM THE USE OF IMMUNE ANTI-O SERUM.

At first the blood group of a person was determined by testing the cells with anti-A and B sera, and the serum was tested with A and B cells. Later, after the discovery of (a_1) and (a_2) and also (b_1) and (b_2), their application was suggested to differentiate the sub-groups from each other. In practice, however, this was difficult owing to their weak reactions and their scarcity. With regard to (a_1) it could be prepared by absorbing anti-A serum with A_2 cells which gives usually a strong (a_1) agglutinin and so in this case the difficulty was overcome.

/98

With respect to agglutinin (a_2), some workers namely Schiff and Thomsen and his co-workers obtained a similar anti-serum by absorbing ox-serum with A_1B cells but other workers could not prepare it, and so the difficulty still remained.

Now, it would appear that it is possible to prepare a much stronger agglutinin by immunizing animals with O blood cells and absorbing the serum with any cells which do not contain the O agglutinogen. By the aid of such a serum we can identify the blood cells of group O by positive reactions and not by merely negative reactions with anti-A and B sera. We can also detect the presence of the agglutinogen O in the blood cells of other groups, which was thought to be impossible before. By means of the reactions obtained with the anti-O, A and B sera, the four blood groups have been classified into 13, if not 15 types.

THE ACTUAL NUMBER OF THE BLOOD GROUPS.

Landsteiner and Decastello and Sturli classified human bloods into four groups according to the presence or absence of the agglutinogens A or B or both together. Having now a third agglutinogen O, the proper classification will be seven and not four groups, as shown in Table No. 32.

TABLE No. 32

NUMBER	GROUP	FORMULA		AGGLUTINOGENS PRESENT
1	O	OOOO		Only O
2	A	AAAA		Only A
3	B	BBBB		Only B
4	AO	AAAO AAOO AOOO		A and O
5	BO	BBBO BBOO BOOO		B and O
6	AB	AABB AAAB ABBB	Theoretical	A and B
7	ABO	AABO ABBO ABOO		A, B and O

/99

However, for the time being, it is sufficient to differentiate these types according to the formula of each as has been suggested before.

THE NATURE OF AGGLUTINOGEN O.

Although the writer considers that the nature of agglutinogen O has not any bearing on the results obtained, still he would like to say something in regard to the two opinions expressed by Professor Thomsen and Professor Sachs on the occasion of his reading a paper at the IInd Congress of the International Society of Microbiology held in London on the 25th July 1936. While they appreciate the importance and possibilities of the results obtained by the writer, Sachs was of the opinion that the so-called O agglutinogen was in reality species in character and not similar to A or B. Such species agglutinogen, he believed, was present to the greatest extent in group O cells and to a variable extent in the cells of other groups. Thomsen said that these results could be explained by assuming that the agglutinogen O was a basal substance present in all cells.

In groups A, B and AB, the agglutinogens A and B "cover" this basal substance to a variable extent and therefore it usually gives weaker reactions with the anti-O serum in these groups than in group O.

The writer does not see any difference between these two opinions, which are really derived from the Hirszfeld hypothesis, previously discussed, namely that all the cells of all groups contain human species agglutinogen. After the development of A and B agglutinogens this human species agglutinogen will be displaced or suppressed by them, and therefore, will remain more prominent in the cells of group O.

/100

IS O REALLY A SPECIES AGGLUTINOGEN?

The answer to this question may be found in the following paragraphs:-

1. According to Hirszfeld's hypothesis any immune anti-human serum will agglutinate cells of group O at a higher titre than the cells of the other groups especially those of group AB. The writer has titrated 6 anti-human sera produced by O cells with cells of all groups and found that AB blood cells were agglutinated nearly to the same extent as O blood cells (See Table 21). An anti-human serum which was produced by B cells agglutinated A cells at a much higher titre than O cells. Another serum produced by A cells agglutinated B cells at a higher titre than O cells, and it is interesting to note that these two sera which were prepared from goats did not contain anti-M or N agglutinins, therefore one might feel satisfied that one is dealing with a purely species agglutinin. This would indicate that the species agglutinogen is not related to the agglutinogen O.
2. According to Hirszfeld's hypothesis any immune anti-human serum is suitable for preparing anti-O. That was not the case in the immunization experiments made by the writer. He could only prepare anti-O serum from animals immunized with O blood cells or A_2 cells. Two out of the 6 injected with O cells which did not produce anti-O, produced very strong anti-species agglutinins. The development of anti-O serum by injecting A_2 cells is explained by the fact that the A_2 contains large quantities of the O agglutinogen.

/101

3. According to Hirszfeld's hypothesis, such agglutinogen is found in all blood cells but the writer has shown that it is absent from the cells of sub-group A_1B_1 and from the homozygous types of groups A and B.
4. According to Hirszfeld's hypothesis, it should be possible to absorb anti-O serum by all cells, even with those of group A_1B_1. The writer has proved that certain types of cells namely AABB, AAAA, BBBB, cannot absorb this serum even when diluted or when the absorption is repeated.

From these considerations it is clear that O is not merely a species agglutinogen. Further, there are reasons for thinking that, on the contrary, it is a specific agglutinogen for O cells. These are:-

1. The presence of natural anti-O serum (a_2 and b_2) which, although it gives weak reactions, nevertheless agglutinates in a specific manner O blood cells and which is not absorbed except by cells containing this agglutinogen.
2. The constant relation between the presence of the three agglutinogens and the fact that the inheritance of the agglutinogens A and B is governed by that of O, as will be shown in Section IV.

PREPARATION OF IMMUNE ANTI-A AND B SERA

The writer immunized six rabbits and two goats with blood cells of group A and two rabbits and one goat with blood cells of group B, as shown in Table No.20. The object was to find out if these cells contain additional agglutinogens to A and B.

/102

Rabbits 5 and 7 and goat 3 were immunized with A_2M blood all of which gave a strong anti-A immune serum, also an anti-O serum was produced by rabbit 5 and goat 3. This anti-O serum was as strong as that prepared from the other goats and rabbits immunized with O cells.

Rabbits 8 and 9 and goat 4 were immunized with A_1N cells and all of these gave a powerful anti-A serum but not an anti-O serum.

Rabbits 12 and 13 which were immunized with A_2MN cells gave a moderate anti-A serum and a strong anti-O serum.

Rabbits 10 and 11 and goat 5 were immunized with BN blood cells; all of these gave a very strong anti-B serum, but not an anti-O serum.

The production of an anti-O serum in the animals immunized with A_2 cells and not from the other animals is a definite proof that these cells contain a large quantity of agglutinogen O. It also proves that the anti-O serum is not related to the species agglutinins which were highly developed in all the sera of the above-mentioned animals.

PREPARATION OF IMMUNE ANTI-A SERUM.

Anti-serum of rabbits 5, 7, 8, 9, 12 and 13 and goats 3 and 4 were absorbed with BMN and OMN blood cells in the same way as described for anti-O serum. The sera absorbed were tested with OM, ON, A_2M, A_2N, A_1M, A_1N, BMN, ABM, ABN cells and the results obtained are given in Table No. 33 which shows that all the animals have produced a specific agglutinin for A blood cells.

These absorbed sera will be referred to as "purified" anti-A sera.

/103

TABLE No. 33

SERUM OF	OM	ON	A_2M	A_2N	A_1M	A_1N	BMN	ABM	ABN
R.5	-	-	+	+	++	++	-	+	+
R.7	-	-	+	+	++	++	-	+	+
R.8	-	-	++	++	+++	+++	-	++	++
R.9	-	-	+	+	++	++	-	+	+
R.12	-	-	±	±	++	++	-	±	±
R.13	-	-	±	±	++	++	-	±	±
G.3	-	-	+	+	++	++	-	+	+
G.4	-	-	++	++	+++	+++	-	++	++

The titre of these sera before and after absorption is given in Table 34.

TABLE No. 34.

SERUM No.	TITRE OF UNDILUTED SERUM BEFORE ABSORPTION A_1	DILUTED BEFORE ABSORPTION	TITRE OF THE DILUTED SERUM AFTER ABSORPTION X DILUTION A_2	A_1
R.5	2560	1:5	320	1280
R.7	2560	1:5	160	640
R.8	5120	1:8	1024	4096
R.9	2560	1:5	160	640
R.12	1280	1:4	64	256
R.13	2560	1:5	80	320
G.3	1280	1:4	128	512
G.4	5120	1:8	1024	4096

The titre is multiplied by the dilution factor in order to make it /104

comparable with the titre of the serum before absorption.
This table shows that all the animals have produced more or less powerful anti-A serum which, after being absorbed, agglutinated A_2 and A_1 cells at different titres. The difference of titre at which these two types of cells are agglutinated is not so marked with the strong sera as with the weak sera.

When all these purified anti-A sera were absorbed with A_2 blood cells for a short time they could agglutinate A_1 but not A_2 cells. When the absorption was repeated twice, or in some cases three times, the sera became inactive towards A_1 cells, even when the titre was as high as 512. This is a strong indication that the two kinds of A_2 and A_1 cells are of a similar nature but contain different quantities of agglutinogen A, as was shown with the natural serum.

All the purified sera were absorbed with A_1 cells and in no case could the treated serum agglutinate A_2 cells, even when the serum had been produced by A_2 cells, which is contrary to what is expected if the A_2 cells contain besides the A agglutinogen another agglutinogen which Landsteiner has called A_2.

PREPARATION OF IMMUNE ANTI-B SERUM

The sera of rabbits 10 and 11 and goat 5 were absorbed with A_1MN and OMN cells in the same way as was described with anti-O serum, and the absorbed sera were tested with cells of OM, ON, A_2M, A_1M, A_1N, BM, BN, A_1BM and A_1BN and the results are given in Table No. 35.

/105

Table No.35.

Absorbed serum of	Tested with cells								
	OM	ON	A_2M	A_1M	A_1N	BM	BN	A_1BM	A_1BN
R.10	-	-	-	-	-	+++	+++	++	++
R.11	-	-	-	-	-	+++	+++	++	++
G.5	-	-	-	-	-	+++	+++	++	++

Later these sera were titrated with cells B_1 and B_2 before and after absorption with a mixture of A_1MN and OMN cells and all were found to be strong, as is shown in Table No.36.

Table No.36.

Serum No.	Titre of undiluted serum before absorption	Diluted before absorption	Titre of the diluted serum after absorption x dilution B_2	B_1
R.10	5120	1:8	2048	4096
R.11	5120	1:8	512	2048
G.5	2560	1:5	640	1280

The relation between the titres at which the two kinds of cells were agglutinated was constant in the three sera.

When B_2 cells were used in absorbing the prepared sera, they remained only weakly active towards B_1, and the same happened with normal anti-B sera from which the writer failed to prepare any strong (b_1) agglutinin. B_1 cells were constantly capable of absorbing the whole agglutinins from the serum, which could not, thereafter, agglutinate B_2 cells. This supported the opinion that B_1 and B_2 differ in their reactions simply because of the different quantity of agglutinogen B present in each.

Evidence was obtained that goats are more suitable than rabbits for the preparation of immune anti-O, A and B sera, since no anti-M and N sera

/106

were produced in goats, as was the case with rabbits. The presence of anti-M and anti-N sera make the purification of anti-O, A and B sera somewhat difficult, owing to the large quantity of cells needed for absorption.

The goats were bled a month after the first bleeding and it was found that the species agglutinins became much weaker and the specific agglutinins also became weaker, but not to the same extent.

A third bleeding was made after another month, and only in two goats could specific agglutinins be demonstrated.

S E C T I O N IV.

HEREDITY OF THE BLOOD GROUPS AND SUB-GROUPS

The possibility of the inheritance of the blood groups was originally suggested by Ottenberg and Epstein (59). This was confirmed by Dungern and Hirszfeld (60) who, after testing the blood of 72 families, came to the conclusion that the groups were inherited according to certain Mendelian laws. This observation was followed by an extensive study by several workers who examined the blood groups of about 9000 families including about 20,000 children (61).

Four theories were advanced by different workers in order to explain the basis on which the qualities characterising the four blood groups were inherited, namely:-

1. The Dungern and Hirszfeld Theory (62), according to which the heredity of agglutinogens A and B depends upon two pairs of independent allelomorphic genes, namely A and (a) which represent the presence or absence of agglutinogen A, and B and (b) which represent the presence or absence of agglutinogen B.

/108

2. The Bernstein Theory (63) according to which the inheritance of A and B agglutinogens depends on three allelomorphic genes, R, A and B, of which A and B are dominant and R is recessive.
3. The Furuhata Theory (64) according to which the bloodgroups are inherited by means of two pairs of allelomorphic genes, A and (a), B and (b), of which A and B are domnant over (a) and (b) - A and B representing the agglutinogens and (a) and (b) representing the agglutinins. The difference between this theory and that of Dungern and Hirszfeld is that the four genes are transmitted in three completely linked pairs namely Ab, aB and (ab).
4. The Bauer Theory (65) according to which there are two pairs of genes A and (a), B and (b), which are not independent like those of Dungern and Hirszfeld, and not completely linked like those of Furohata. Each pair is present in the same chromosome, but they are far apart from each other, and hence crossing over may take place between them.

DUNGERN AND HIRSZFELD THEORY.

According to this theory A is inherited independently from B. The former is inherited by the pair Aa, and the latter by the pair Bb. Considering A alone, one obtains three different genotypes representing two phenotypes, namely:- AA, Aa, which represent the phenotype reacting with anti-A serum, and (aa) which represents the phenotype which does not react with anti-A serum. Similarly three genotypes are obtained with B, namely BB, Bb and (bb). When the genotypes of A and B are crossed together the genotypes representing each group can be obtained - these are given in Table No.37.

/109

Table No.37.

PHENOTYPES	GENOTYPES	
	HOMOZYGOTE	HETEROZYGOTE
O	a a b b	Aa
A	A A b b	Aabb
B	a a B B	aaBb
AB	A A B B	Monohybrids AaBB AABb; Dihybrid AaBb

According to this theory the agglutinogens A and B can only appear in the blood of a child when present at least in one of the parents. Table No.38 shows the children possible and impossible to be obtained from each mating.

Table No.38.

PARENTS	CHILDREN	
	POSSIBLE	IMPOSSIBLE
O X O	O	A, B, AB
O X A	O, A	B, AB
O X B	O, B	A, AB
A X A	O, A	B, AB
B X B	O, B	A, AB
A X B	O, A, B, AB	----------
O X AB	O, A, B, AB	----------
A X AB	O, A, B, AB	----------
B X AB	O, A, B, AB	----------
AB X AB	O, A, B, AB	----------

Some exceptions to this rule were found but they were few and could be explained either by mistakes in the technique or by illegitimacy. In 1925 Bernstein (63) tested the accuracy of this theory from the statistical point of view and found that the frequency of the different blood

/110

groups expected according to this theory did not agree with what was observed in different populations.

The expected percentage of group AB in the English and Greek populations are 6.5 and 15.5 respectively, while the actual percentages are 3.0 and 4.0.

According to the theory of Dungern and Hirszfeld, the following equation should hold: O X AB = A X B. The theory was tested against different populations and it was found that O X AB was much lower than A X B. For example, among the population of Glasgow, personal investigation has shown that the frequency of the blood groups was as follows:-

O	A	B	AB
49.6%	36.6%	9.5%	4.3%

From that, value of O X AB = 0.496 x 0.043 = 0.021528

and A X AB = 0.366 x 0.095 = 0.03577

which shows a marked inequality.

BERNSTEIN'S THEORY.

When Bernstein found that the Dungern and Hirszfeld theory conflicted with the statistical point of view, he proposed another theory, based on the existence of three allelomorphic genes, A, B and R. According to this theory A and B which represent the agglutinogens A and B are dominant and R, which represents their absence, in group O, is recessive. Therefore we have four phenotypes and six genotypes, as given in Table No.39.

Table No.39.

PHENOTYPE	GENOTYPE: HOMOZYGOTE	GENOTYPE: HETEROZYGOTE
O	RR	--
A	AA	AR
B	BB	BR
AB	--	AB

/111

According to the Bernstein theory there are two rules governing the heredity of the three genes, namely:-

(1) As A and B agglutinogens are dominant, they only appear in the blood of a child when present in at least one of the parents, and therefore, this theory agrees with the Dungern and Hirszfeld theory concerning this point.

(2) An AB parent has only one genotypic formula, namely AB, so that one of these genes will be transmitted from this parent to the child which will contain either A or B, and consequently cannot belong to Group O, even if the other parent belongs to this group, which is possible according to the first theory. The children, possible and impossible, to result from each mating, according to this theory, are given in T Table No.40.

Table No.40.

PARENTS	CHILDREN	
	POSSIBLE	IMPOSSIBLE
O X O	O	A, B, AB
O X A	O, A	B, AB
O X B	O, B	A, AB
A X A	O, A	A, AB
B X B	O, B	A, AB
A X B	O, A, B, AB	----------
O X AB	A, B	O, AB
A X AB	A, B, AB	O
B X AB	A, B, AB	O
AB X AB	A, B, AB	O

/112

When this theory was tested by comparing the expected frequency of the blood groups with their observed frequency, they agreed in a satisfactory manner. If r, p and q represent the frequency of the genes R, A and B respectively, in a homogeneous population, then according to Bernstein, values of r+ p+ q = 1. The values of r, p and q were calculated according to the theory (66) and it was found that:-

$$r = \sqrt{O}$$
$$p = 1 - \sqrt{\text{group O + B}}$$
$$q = 1 - \sqrt{\text{group O + A}}$$

From the frequency of the blood groups in Glasgow already mentioned, the values of r + p + q were found to be 1.007 which is really not different from what was expected and such was the case with other populations tested by Lattes, Wiener and Snyder. Therefore the Bernstein theory is suitable from the statistical point of view to explain the frequency of the blood groups in different populations. From the point of view of heredity there were many exceptions to the Bernstein theory, according to which, when one of the parents or both of them belong to group AB, no O children could be obtained. Several O children were found in such families but that was explained by the supporters of this theory as due to faulty technique, and they pointed to the fact that fewer exceptions were recorded following the advancement of this theory than before it was known. According to Wiener the percentage of the exceptions against this theory before and after its application are as follows:-

MATING	BEFORE 1926	AFTER 1927
O X AB	26.26	3.61
A X AB	3.77	0.87
B X AB	11.11	0.83
AB X AB	---	---

He explained such decrease by improvement in the technique, and by the

/113

fact that when an exception to the theory was found, the blood was re-examined. The few exceptions encountered after 1927 were explained by illegitimacy when the exception was against the father, but there were some cases in which the exception was against the mother, which could not be explained by illegitimacy. These as Wiener states, can be explained by the occurrence of a recessive mutation (from A to R or from B to R) or according to Levine, as a result of non-disjunction, both of which explanations are theoretical. Schiff suggested testing this theory by the study of the blood groups of mothers and their children, and after a large number had been examined by him, Wiener and Thomsen, no exceptions were found. Therefore the Bernstein Theory was considered by all the leading workers to be reliable as a basis for the inheritance of the blood groups.

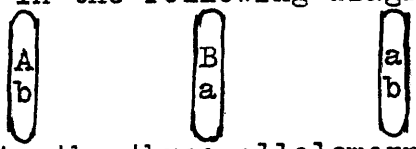
With regard to its medico-legal application, it became accepted as evidence in paternity suits by several courts of law on the Continent. Some experts, as Landsteiner (67), Marx (68) and Hirszfeld (69), and others, although they do not doubt the validity of this theory, recommend that in paternity cases where the child is of group O and the father of group AB, or vice versa, the court should be informed that at least one such exception against the mother was recorded by Haselhorst and Lauer (70), and to express the opinion that such exceptions when found against the father are to be considered in all probability, but not in certainty, as evidence of illegitimacy. In Germany and in other countries, this theory seems to be accepted without any reservation.

FURUHATA'S THEORY.

According to Furuhata's Theory, there are three completely linked pairs, namely Ab, Ba, and (ab), each of which is situated in one

/114

chromosome, as shown in the following diagram.



If we compare these to the three allelomorphs of Bernstein which may be represented in the following diagram,

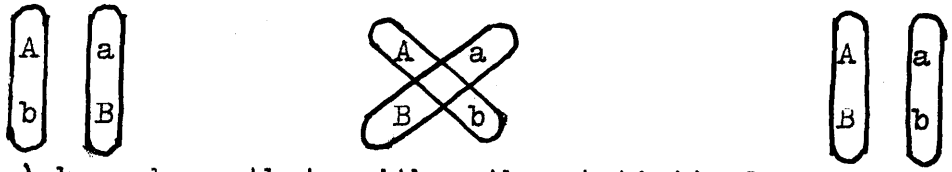
A B R

one will expect to obtain the same results from both theories. However, these differ in that, according to Furuhata, the two agglutinins are equally inherited like the agglutinogens. Bernstein assumes that the agglutinogens alone are inherited and that both agglutinins are developed in all sera of all groups. When the blood cells contain A or B agglutinogens, these will absorb (a) or (b) agglutinins, respectively, in vivo. The fact that the agglutinogens are developed early in foetal life and the agglutinins later, after birth, is more in favour of the Bernstein Theory. Moreover, Furuhata's theory does not explain the presence of (a_1), (a_2), (b_1), and (b_2) agglutinins. The writer has shown in Section II that the Bernstein Theory can explain their presence and therefore this is to be preferred to that of Furuhata.

BAUER'S THEORY.

Kirihara and Haku (71) and Bauer, in order to explain the presence of O children resulting from AB parents suggested a theory midway between that of Dungern and Hirszfeld and that of Furuhata. According to their theory, there are two pairs of genes Ab and Ba, which are partially linked, that is, present in one chromosome, but far enough apart from each other to allow crossing over to take place. This only affects the results in group AB parents so that in some cases O children may be obtained, as shown in the following diagram:-

/115



Snyder (71a) has shown that neither the statistical observations nor the family histories support this theory.

THE INHERITANCE OF SUB-GROUPS.

Landsteiner and Levine (72) studied the inheritance of sub-groups A_1 and A_2, and found that the qualities characterising them are inherited. Thomsen, Friedenreich and Worsaae (73) confirmed this point and formulated a theory on the basis of the Bernstein Theory to explain the inheritance of sub-groups A_1 and A_2. They assumed the presence of four allelomorphic genes, namely, R, A_2, A_1 and B, of which R is recessive and the other three dominant, but when A_1 is present, beside A_2, the former is dominant. If this be applied to the sub-groups of Group B, there will be found five genes, namely:- R, A_2, A_1, B_2 and B_1.

The genotypes and phenotypes obtainid according to Thomsen's Theory are given in Table No.41.

Table No.41.

GROUP	PHENOTYPES	GENOTYPES
O	O	RR
A A_1	A_1	A_1A_1, A_1A_2, A_1R
A A_2	A_2	A_2A_2, A_2R
B	B	BR, BB
AB A_1B	A_1B	A_1B
AB A_2B	A_2B	A_2B

This theory does not interfere with the original rules of Bernstein but suggests to Thomsen and other workers the application of additional rules.

/116

(1) Agglutinogen A_1 can only appear in the blood of the child when present in at least one of the parents.

(2) An A_1B parent cannot give birth to a child of sub-group A_2, and vice versa.

(3) An A_1B parent cannot give birth to a child of sub-group A_2B unless the other parent supplies the gene A_2, therefore the children, possible and impossible, to be obtained from each mating are given in Table No.42.

Table No.42.

PARENTS	CHILDREN	
	POSSIBLE	IMPOSSIBLE
1. O X O	O	A_2, A_1, B, A_1B, A_2B
2. O X A_1	A_1 or A_2 and A_1, or O and A_1	B, A_1B, A_2B
3. O X A_2	O, A	A_1, B, A_1B, A_2B
4. O X B	O, B	A_2, A_1, A_1B, A_2B
5. O X A_1B	A_1, B	O, A_2, A_1B, A_2B
6. O X A_2B	A_2, B	O, A_1, A_1B, A_2B
7. A_2 X A_1	O, A_2, A_1	B, A_1B, A_2B
8. A_2 X A_2	O, A_2	A_1, B, A_1B, A_2B
9. A_1 X A_1	A_1, or O and A, or A_2 and A_1	B, A_1B, A_2B
10. A_2 X B	O, A_2, B, A_2B	A_1, A_1B
11. A_1 X B	O, A_1, B, A_1B, A_2, A_2B	----------
12. B X B	O, B	A_2, A_1, A_1B, A_2B
13. A_2B x A_2	A_2, B, A_2B	O, A_1, A_1B
14. A_2B X A	A_2, A_1, B, A_1B, A_2B	O
15. A_2B X B	A_2, B, A_2B	O, A_1, A_1B
16. A_2B X A_2B	A_2, B, A_2B	O, A_1, A_1B

/117

Table No.42 (cont.)

PARENTS	CHILDREN POSSIBLE	IMPOSSIBLE
17. A_2B X A_1B	A_1, B, A_1B, A_2B	O, A_2
18. A_1B X A_2	A_1, B, A_2B	O, A_2, A_1B
19. A_1B X A_1	A_1, B, A_1B, A_2B	O, A_2
20. A_1B X B	A_1, B, A_1B	O, A_2, A_2B
21. A_1B X A_1B	A_1, B, A_1B	O, A_2, A_2B

Further exclusions can be made if the number of children are three or more. This is shown in Table No.43.

Table No.43.

MATING	POSSIBLE CHILDREN EITHER	OR
A_1 X O) A_1 X A)	A_1 and O	: A_1 and A_2
A_1 X B	A_1 & O alone or with B	: A_1 & A_2 alone or with B
A_1 X A_1B	A_1, A_1B and A_2B	: A_1, A_1B and B
A_1 X A_2B	A_1, A_2, B and A_1B	: A_1, A_2, A_2B and A_1B

Landsteiner and Levine in 69 families found three exceptions to the first rule, namely, when one of the parents was A_2 and the other was not A_1, three children were found of sub-group A_1. Thomsen and Friedenreich and Worsaae reported one family A_2 X O with two children A_1, which was explained by them by the fact that the A_2 parent was 80 years old which might suggest that the agglutinogen was A_1, but had become weaker from old age. Wiener and Rothberg (74) also found three exceptions to the first rule and reported a family A_1 X O with two children of group O, four of sub-group A_1 and one of sub-group A_2, which was contrary to the theory as shown in Table No. 43.

/118

Wolff & Jonsson (53) examined 500 mothers and their children in order to test the accuracy of this theory, according to which an A_1B parent cannot give rise to an A_2 child or vice versa, but found no exceptions of this kind.

Wiener (75) studying the statistical aspect found that the results supported this theory.

THE WRITER'S COMMENTS REGARDING THE VALIDITY OF THESE THEORIES.

From the above-mentioned discussions one may conclude that the Bernstein theory is the only one suited to explain the heredity of the four blood-groups, and that Thomsen's theory agrees with the statistical data and only requires some additional family observations in order to be accepted as a basis for the explanation of the neredity of the sub-groups. The discovery of the sub-groups B_1 and B_2 does not of course invalidate this theory, since, as Thomsen personally indicated to the writer, one can substitute the genes B_2 and B_1 in place of B.

With regard to the statistics of populations, one must admit that the Bernstein Theory is quite satisfactory. The reason seems to be that this theory represents the inheritance of three agglutinogens, namely, O, in addition to A and B. As the writer has shown, O is a positive factor, an agglutinogen, and therefore plays an essential part in constituting the system of 4 groups. In Dungern and Hirszfeld's view it is merely negative by the absence of A or B, accordingly the population statistics calculated on the basis of the Dungern and Hirszfeld Theory do not agree with the observed frequency.

Dungern and Hirszfeld's Theory differs from the Bernstein Theory in that, with the first, O children can be obtained from an AB parent, which is impossible according to the Bernstein Theory: therefore it

/119

explains such cases although the expected number of children is much higher statistically than has been observed in practice, for example, 19.7% of children of matings O X AB should be of Group O, while those observed is only 1.6% This shows that the Dungern and Hirszfeld theory is not yet satisfactory from the statistical point of view.

The reason why with the Bernstein theory, O children cannot be obtained from AB parents is that the O agglutinogen is not represented in group AB since the genotype of the latter group possess two genes A and B.

The basis on which the validity of the Bernstein theory is upheld in civil actions may be summed up in the following points:-

(1) In the great number of complete families examined as well as 4500 mothers and their children also examined, there was only one solitary exception proved. This was found by Haselhorst and the results were checked by other known workers.

(2) All the other exceptions against the father can be explained by illegitimacy or faulty technique. The other exceptions found against the mother can be explained also by faulty technique, especially by overlooking the A_2 agglutinogen on account of its weakness and therefore the child may be considered to belong to group O.

(3) The exception of Haselhorst was explained by Levine (76), as the result of non-disjunction and by Wiener (77) as the result of recessive mutation of the agglutinogens A and B.

Regarding the possibility of illegitimacy, a certain number of illegitimate children must be expected, and owing to the scarcity of group AB persons, one would also expect that the chances of exclusions will be higher when either parent belongs to this group than with any other groups.

/120

The writer, in order to ascertain whether all these exceptions were actually due to illegitimacy, studied this question, and made a comparative estimate between the number of exceptions found in matings where there was no AB parent, and the exceptions found in matings where one of the parents belonged to group AB. In this study the writer depended on the data collected by other workers namely Hirszfeld (78), Wellisch (79) and Lattes (80), which will now be considered.

Hirszfeld collected in one table the families studied by Rubaschkin, Hecker and Korotkin, Fischer, Ljachowieckij and Rosanowa, Haselhorst, Zabolotnij and Kosowitsch, whose tests were performed from 1927-1933, so that presumably they were acquainted with the Bernstein Theory.

The number of families and children together with the exceptions against the two rules of the Bernstein Theory in each mating are given in Table No.44.

From this Table one will see that the number of exceptions found in the first nine matings where there was no AB parent was 33 out of 7833 (.42%) children, and that the exceptions found in the last seven matings when one or both parents belonged to group AB, 22 out of 1217 (1.8%). While one may explain the 33 exceptions found in the first 9 matings given in this table by the possibility of illegitimacy, one cannot do likewise with the 22 exceptions found in the last 7 matings, because 9 of these were against the mother, and provided that these 9 cases were real exceptions, they should not exist if the Bernstein Theory is correct. Only the other 13 cases may be considered as illegitimate children, according to the Bernstein Theory.

/121

Table No.44.

GROUP OF PARENTS F		M	NUMBER OF FAMILIES	NUMBER OF CHILDREN	NUMBER OF EXCEPTIONS IN GROUP O	A	B	AB	TOTAL
O	X	O	518	1159		9	1		10
O	X	A	568	1235			4	1	5
A	X	O	568	1267			5		5
A	X	A	594	1346			1	1	2
O	X	B	234	551		6			6
B	X	O	234	598		4			4
B	X	B	145	324				1	1
A	X	B	282	668					
B	X	A	281	685					
O	X	AB	115	267	(5)			2	7
AB	X	O	48	179	4			(2)	6
A	X	AB	95	209	(2)				2
AB	X	A	92	229	3				3
B	X	AB	43	111					
AB	X	B	55	168	4				4
AB	X	AB	30	54					
TOTAL			3902	9050					
FIRST 9 MATINGS			3424	7833		19	11	3	33
LAST 7 MATINGS			478	1217	18			4	22

The numbers given between brackets represent the exceptions against the mother.

In order to study the relations between the exceptions against the fathers of different groups, the writer has arranged them in Table

/122

No. 45 to be more convenient for the purpose.

Table No.45.

F.	M.	NO. OF CHILDREN.	NO. OF EXCEPTIONS.	
O	O	1159	10	
O	A	1235	5	
O	B	551	6	
O	AB	297	2	
TOTAL		3242	23	0.7%
A	O	1267	5	
A	A	1346	2	
A	B	668	-	
A	AB	209	-	
TOTAL		3490	7	0.2%
B	O	598	4	
B	A	685	-	
B	B	324	1	
B	AB	111	-	
TOTAL		1718	5	0.3%
AB	O	179	4	
AB	A	229	3	
AB	B	168	4	
AB	AB	54	-	
TOTAL		630	11	

This table shows that the exceptions against O fathers are 23, (0.7%) those against A fathers 7 (0.2%), those against B fathers 5 (0.3%) and those against AB fathers 11 (1.75%).

As it is possible according to Bernstein theory to calculate the chances of excluding the putative father in different groups, this theory may be put to the test by comparing the expected and observed percentage of such exclusions.

The chances of excluding paternity in the European and American populations were calculated by Wiener (81) as given in Table No.46 which also shows the frequency of the four groups and the percentage of exceptions found in the families given in the previous table.

Table No. 46

	Frequency of groups.				% of chances of exclusions of Putative Father according to the theory			
Population	O	A	B	AB	O	A	B	AB
European & American	39	43	12	6	23.5	7.7	14.6	39.9
Ratio between $\frac{O + A + B}{AB}$			$\frac{94}{6}$				$\frac{60.1}{39.9}$	
					Per cent of exceptions found against the father			
Children obtained from the families collected by Hirszfeld	37	45	13	6	0.7	0.2	0.3	1.75
Ratio between $\frac{O + A + B}{AB}$			$\frac{94}{6}$				$\frac{1.2}{1.75}$	

As the frequency of the blood groups is nearly similar in the population used by Wiener, for calculating these chances, and that obtained from

/124

the families studied from 1927-1933 given by Hirszfeld, one may apply Wiener's calculations in order to study the exceptions found in these families. If we consider the exception found against fathers of group O, A and B, one will notice a marked agreement between the relations of the expected chances and that of the observed exceptions, especially in groups O and A. In respect of the relation between the chance and exception with group AB, one may see that they do not agree, especially when they are compared with the total chances and exceptions in the first 3 groups respectively. According to the theory, we have 60 chances of excluding paternity in groups O, A and B, compared with 40 chances in group AB; but with the observed exception we have 1.2% in the first three groups, compared with 1.75% in group AB. According to the calculated chance, the maximum number of exceptions in group AB should be 0.8 or perhaps less.

In order to exclude any doubt regarding the importance of testing the theory in this manner, we may study the relation between the exception against the father and those against the mother of group AB. These are given in Table No.47.

Table No.47

GROUP OF PARENTS FATHER	MOTHER	NUMBER OF FAMILIES	NUMBER OF CHILDREN	NUMBER OF EXCEPTIONS	%
AB	O	48	179	4	
AB	A	92	229	3	
AB	B	55	168	4	
AB	AB	30	54	-	
TOTAL		225	630	11	1.75%
O	AB	115	267	5	
A	AB	95	209	2	
B	AB	43	111		
AB	AB	30	54		
TOTAL		283	641	7	1.08%

/125

This table shows that the exceptions against the father (1.75%) are higher than those against the mother (1.08%). It was previously shown that the percentage of illegitimate children in group AB father should be 0.8 according to the chances made by Wiener: if we deduct this number from that observed 1.75, we obtain 0.95% exceptions, which cannot be explained on the grounds of illegitimacy and therefore, like the 1.08% exceptions against the mother, remain unexplained by the Bernstein theory. The similarity between these two figures is striking and supports the fact that such exceptions are accurate since they are practically equal in mothers and fathers. Incidentally one may quote here Wiener 's comment (82) on the seven exceptions found against the theory of a single pair of allelomorphic genes by which the factors M, and N are supposed to be inherited. "these apparent exceptions may all be attributed to illegitimacy. This is true for the seven exceptions to the second rule, since in each case the parent involved was the father. If the theory were at fault, similar exceptions should be found in which the mother is implicated." Why then should the Bernstein Theory not have been doubted on account of the presence of similar exceptions against both the father and the mother?

According to the data collected by Wellisch 15 exceptions were found in 2896 families where there was no AB present and 9 exceptions were found in 599 where one parent was AB. The number of children in these two series was 5684 and 1401 respectively. The ratio between the frequency of exceptions is $\frac{0.25}{0.65}$ which is similar to that found in Hirszfeld's table but different from the expected according to the theory.

/126

The writer has also collected the exceptions found by Leitschik, Kassovitsch, Oku, Salobotny and Wiener and Vaisberg, as given by Lattes (80) which were not included in the tables of Wellisch because they appeared later. The writer neglected all the exceptions found by other workers, the work of whom was not complete in Lattes' opinion. The exceptions found by those five workers are given in Table No.48.

Table No. 48.

MATING	NO. OF FAMILIES	NO. OF CHILDREN	EXCEPTIONS O	A	B	AB	%
O X O	99	317	-	-	-	-	
O X A	191	656			1	1	
A X A	82	306					
O X B	58	179		4			
B X B	23	55				1	
TOTAL	453	1913			7		% 0.03%
O X AB	31	103	4			1	
A X AB	41	105					
B X AB	28	88	2				
TOTAL	100	296			7		% 2.3%

In the material of these five workers the frequency of the exceptions in group AB parents is much higher than that in the other three groups. From this one may see that the results obtained from the data given by Hirszfeld are quite reliable and not so contradictory in relation to the Bernstein Theory, as the date collected in Table No . 48 and by Wellisch

It can, therefore, be concluded that the majority of exceptions found against the father cannot be explained by illegitimacy and hence remain, with those found against the mother, unexplained by the Theory.

Another important point against the Bernstein Theory is that in certain matings the frequency of the children obtained in each group is different from that expected according to the Bernstein theory. Table No.49 shows the expected and observed frequencies of children in the matings O X O and A X B, as calculated by Wellisch from the material examined after 1927.

Table No. 49.

MATING	NO. OF FAMILIES	NO. OF CHILDREN	FREQUENCY OF CHILDREN IN GROUP	O	A	B	AB
O X O	531	1045	Expected	100			
			Observed	99.62	0.38		
			Difference	-0.38	+0.38		
A X B	593	1187	Expected	18.16	26.27	22.72	32.85
			Observed	18.20	31.00	25.19	25.61
			Difference	+0.04	+4.73	+2.47	-7.24

The high difference noticed in mating A X B cannot be explained by the possibility of including illegitimate children, since in the mating O X O where the illegitimate children are included the difference is very small.

One would expect, if the Bernstein theory is correct, that the expected and observed frequencies would be similar, especially when the number of families examined is as high as shown in the table.

Schiff (83) and after him Wiener, laid much stress on the results of testing several mothers and their children, and inferred that the absence of exceptions in these cases was evidence of the validity of the theory.

Wiener (84) collected the materials examined by fourteen workers

/128

in which 627 mothers of group AB, and 3941 mothers of group O, were tested with their children which numbered 898 and 5010 respectively. Three exceptions were found in the first series and 5 in the second. Wiener doubts these results because they were only found in three instances out of 14 which were examined without the knowledge of the Bernstein Theory, despite the fact that one of these was made in 1928, and that their number is comparatively very small.

Table No. 44 shows that 9 exceptions against the mother were found amongst 9050 children, and in this set of tests 5 exceptions were found among 5908 children: the similarity between the frequency of the exceptions in the two groups of blood samples is so marked as to make one believe that there is something behind it.

If these 5 exceptions shown by Wiener are neglected, can the absence of exceptions in this material be explained?

The mothers of group AB given in Wiener's tables, were examined in small batches of different workers and in different places, therefore they are not so reliable as if they had been examined uniformly in one place to ensure that there was every chance for the exceptions to appear. For example in the material examined by 10 out of 14 workers, the maximum number of AB mothers examined was 16 and many examined only 7. On account of the low frequency of these exceptions, one may never meet a single exception in such a small number of mothers. The highest number examined by one worker, namely Buining was 227: in such a number one would expect that the mothers married to group O husbands would be 80 (35.5%). In Table No. 44 one will see that in 115 mothers married to husbands of group O there are 5 exceptions, therefore one may expect proportionally 3

/129

exceptions here, provided that an AB mother may give birth to an O child. The absence of these 3 exceptions cannot be taken as definite proof against the possibility of such exceptions because had Buining examined another 20 wives he might have met with even more than 3 exceptions. The assumption which is going to be suggested later by the writer, is that only a small percentage of group AB parents can give birth to O children and this may explain the rarity of these exceptions. On the same basis, one may explain the absence of exceptions against the 1274 mothers of group O examined by Buining. If we assume that the percentage of group AB in this population is 6% the mothers married to group AB fathers will be 48, so it is not unexpected to find no exceptions in such a small number. The same may be said of the material examined by other workers which was much less than that of Buining.

One may conclude that even if we neglect the 5 exceptions found by Ohnesorge and Kheringer-Guggenberger, the absence of these exceptions in the bloods thus examined does not help very much in supporting the Bernstein theory.

With regard to the mistakes in the technique one should expect with the improvement achieved during the last ten years, that these workers have excluded such a possibility, but it may be as Wiener and Thomsen have said that the A_2, especially in children, will be overlooked on account of its weakness and therefore the child will be considered of group O although its true group is A_2. The writer does not agree with them that this is of any value in support of the theory, because if the A_2 element had been overlooked in a child of group A, it would almost certainly have been overlooked in a child of Group A_2B. This is

/130

against the theory because one may assume that in the O X AB matings some of the children considered to belong to group B were really A_2B, which if detected would tend to add to the number of exceptions and not reduce them as these workers have assumed.

There is evidence to believe that this is quite possible, because as the writer has remarked, the frequency of the group B children obtained from matings where an AB parent is included is usually higher than that expected, while the AB children are usually less than expected, according to the theory. The writer gives in Table No.50 the expected and observed frequency of these matings, as calculated by Wellisch from the families examined from 1927-1930. This table shows that the observed frequency of group B children is similar to those expected in the first mating and a little less in mating No.3, but higher in mating No.2 and No.4. With AB children one may see the opposite, while with A children the difference is not so marked as with B. Therefore one may be justified in assuming that some A_2B children were wrongly grouped under B. If this be right, then it may explain why in the first mating the exceptions of group O (0.76) are higher than those of group AB (0.38). Consequently this possibility which was considered to explain some of the exceptions found seems to add further exceptions.

With regard to the Haselhorst exception, Levine has tried to explain it by assuming that the two genes A and B may be attached together and hence both may be transmitted to the child or both may fail to be transmitted. With the present knowledge one cannot deny that such attachment is possible, but it will be reasonable to assume that this attachment manifests itself in all the children obtained by this parent. If this is right, then the explanation will suit only a family of an

Table No.50.

MATING No.	PARENTS	FREQUENCY ACCORDING TO	CHILDREN GROUP O	A	B	AB
1	O X AB	Bernstein theory	0	50.0	50.0	0
		observed	0.76	48.86	50.0	0.38
		difference	+0.76	-1.14	0	+0.38
2	A X AB	Bernstein theory	0	50.0	20.44	29.56
		observed	0.35	50.0	24.22	25.43
		difference	+0.35	0	+3.78	-4.13
3	B X AB	Bernstein theory	0	22.21	50.0	27.79
		observed	0.34	21.21	49.16	29.29
		difference	+0.34	-1.00	-0.84	+1.50
4	AB X AB	Bernstein theory	0	25	25	50
		observed	0	24.42	30.23	45.35
		difference	0	-0.58	+5.23	-4.65

O X AB mating which gives children of either O or AB. Landsteiner and Levine reported that a family (O X AB) gave two children of group O and 4 of groups A or B: there is no reason to assume that the two genes of the AB parent will be attached in the first two children and appear separately in the last four.

The same may be said regarding the possibility of recessive mutation as mentioned by Wiener which, if it can happen in one or two children, should happen in the rest; if such mutation takes place we may expect that it manifests itself in the change of the group of the person concerned and therefore the exception will not be noticed.

However, even if these two theoretical explanations are correct, the fact remains that there are certain exceptions to the second rule of Bernstein's theory, and therefore it loses its medico-legal importance which all these workers are striving to establish.

/132

In regard to the sub-groups A_1, A_2, B_1 and B_2, one may mention that their constant existence cannot be explained by the three allelomorphic theory as stated by Bernstein. This, however, was explained later by Thomsen with his four allelomorphic theory, which is really the Bernstein theory slightly modified.

In order to discuss the validity of Thomsen's theory, in the first place it should be subjected to the same criticism as previously mentioned. With regard to the theory itself one may notice the high number of exceptions found in rather a small number of families examined. In 69 families examined by Landsteiner and Levine and 89 families examined by Wiener and Rothberg there were seven exceptions contrary to what was expected, according to Thomsen's theory, which number, as Wiener confessed, is very high.

If this theory is correct these unfortunate children would be considered as illegitimate.

It is true that Friedenreich and Zacho, who examined 103 families and Wolff and Jonsson, who examined 500 mothers and their children, did not find exceptions to this theory, but this seems scarcely to weigh in favour of the theory on account of the exceptions found in the families examined by the writer.

Personal Investigations:

In order to study the inheritance of the blood-groups, sub-groups, and the factors M and N, the writer has examined two series of families. The first, which was examined in Glasgow, consisted of 52 families and 168 children. The second, which was examined in Cairo, consisted of 100 families and 300 children. From the results given at the end of this Section, one will find the following exceptions with regard to

/133

the inheritance of the groups and their sub-groups according to Bernstein and Thomsen's Theories.

1. Family No.15 of the first series.

FO X MO = 1 child A_1 and 1 child O.

The first child is against the theories of Dungern and Hirszfeld, Bernstein (first rule) and also Thomsen's theory, because according to these, the child cannot have an A or A_1 agglutinogen, except if present in at least one of the parents.

Such exceptions are very rare, compared to the great number of families tested. In not a single case of these could the child be proved to be a legitimate one. For this reason, one may quite safely consider the first child of this family to be illegitimate.

2. Family No.37 of the first series:

Maternal Grandmother O

Father B X Mother A_1

1st Child (13 yrs.) A_2

2nd Child (11 yrs.) A_1

3rd Child (9 yrs.) A_2B

4th Child (5 mths.) A_1

When this family is studied without taking into consideration the group of the maternal-grandmother, there is no exception against any theory: when the groups of the grandmother and mother are studied together, no exception is found, as actually happened when the writer tested these bloods. Later, when he studied the two families together, he found that there is an exception against the Thomsen theory. This is shown as follows.

According to Thomsen, the genotype of an A_1 person may be one of three, namely A_1A_1, A_1A_2, and A_1O. As the group of the maternal grand-

/134

mother is O, the genotype of the mother will be A_1O, which, according to Thomsen's theory, should not give an A_2 child, except if the father be A_2 So the first child (A_2) will be considered as illegitimate, because the husband in this case cannot supply A_2.

The third child belonged to sub-group A_2B. If we assume that the B gene is transmitted from the father, which is quite reasonable, this exception will be against the mother, who, according to Thomsen's theory, should not give the A_2 gene. There is no doubt that this cannot be explained by saying that the A_2 has come from another man, because in this case the father must supply the two genes A_2 and B, both of which are absent in the mother, which is again against the theory.

Indirectly this proved that the first child is not illegitimate as we supposed. Therefore the two exceptions contradict the rules of Thomsen's theory.

One may try to explain this by saying that the real group of the grandmother was A_2, which was overlooked. Regarding this point the writer has sufficient reason to believe that on account of the precautions taken by him, this could not possibly arise. The writer has used test sera from two sources, both of which were strong enough to detect the A_2 in persons as old as 82 years and the A_1 in children as young as 3 months.

3. Family No.35 in the second series.

Mother (45 years) B_1
1st child (26 years) B_1
2nd child (24 years) O
3rd child (20 years) A_2B
4th child (17 years) O

According to Thomsen, the rules applied to A_1 and A_2 may be applied to B_1

and B_2, therefore this family is strong evidence against his theory on account of the following:-

The genotype of the mother may be one of three, namely:-

B_1B_1, B_1B_2, or B_1O.

As this mother has given birth to two children of group O, there is strong reason to believe that her genotype is B_1O. According to this, the possible children from this mother and an unknown husband will be shown in Table No.51.

Table No.51.

GENOTYPE Mother	Husband	Possible Children	GENOTYPE Mother	Husband	Possible Children
B_1O	X OO	= B_1 or O	B_1O	X	
	A_1O	= O, A_1, B_1, A_1B_1		A_2A_2	= A_2, A_2B_1
	A_1A_2	= A_2, A_1, A_1B_1, A_2B_1		A_2O	= O, A_2, B_1, A_2B_1
	A_1A_1	= A_1, A_1B_1			
	B_1O	= O, B_1		B_2B_2	= B_1, B_2
	B_1B_2	= B_1, B_2		B_2O	= O, B_2, B_1
	B_1B_1	= B_1			
	A_1B_1	= A_1, B_1, A_1B_1		A_2B_1	= A_2, B_1, A_2B_1
	A_1B_2	= A_1, B_2, B_1, A_1B_2		A_2B_2	= A_2, B_2, A_2B_1

If we compare the children obtained with each possible father with those actually obtained by this mother, one may see that there is no agreement between the latter and any of the former. The writer was able to form an opinion about the group of the father who was dead, by testing the family od his dead brother, which was found to be:

Mother B_1 : 2 children O : one child A_1 : 3 children B_1.

/136

According to this the father is A_1, of the genotype (A_1O); if we consider that the two brothers were similar one can explain the presence of the child B_1, and the two O children. There remains the A_1B_2 child which can be explained so far as the father is concerned, but not in the case of the mother. Therefore this family shows another exception which could not be explained by illegitimacy.

One may try to explain this by a mistake in recording the numbers of tubes or results or to some unspecific reactions. Regarding such possibility the writer would stress the fact that he adopted the greatest care to eliminate the occurrence of mistakes arising in this regard.

Another explanation may be that the mother and the first child belong to sub-group B_2, or that the third child belongs to sub-group A_1B_1 and owing to the slight difference between B_1 and B_2 the sub-group was falsely determined. The writer is willing to admit such a possibility, had the blood cells been tested only with (a_1) and (b_1) agglutinin, but as these results were controlled by the positive reaction obtained with anti-O serum every doubt on this point was eliminated. (Please see guide to the reactions with different sera attached to the families of the second series). Consequently, one may conclude that these two families are contrary to Thomsen's theory in every sense, the failure of which indicates an indirect failure of the Bernstein theory, since the object of the former was to complete the defect of the latter.

HOW TO EXPLAIN THESE EXCEPTIONS.

As previously mentioned in Section III, the Bernstein and Thomsen theories could not explain, from the serological point of view, the presence of four sub-groups A and B and also the existence of

agglutinogen O in certain bloods of group AB. These were explained by a hypothesis based on the assumption that the genotype of each person is composed of 4 genes which may represent each of the agglutinogens, O, B or A, or two of them, or the three together. The different sub-groups derived from such a hypothesis were found to be:-

GROUP O	GROUP A	GROUP B	GROUP AB.
1. OOOO	2. (AAAA	6. (BBBB	10. (A_1B_1) AABB
	3. (A_1) (AAAO	7. (B_1) (BBBO	11. (A_1B_2) AABO
	4. (AAOO	8. (BBOO	12. (A_2B_1) AOBB
	5. (A_2) AOOO	9. (B_2) BOOO	13. (A_2B_2) AOBO
			14. AAAB
			15. ABBB

The first thirteen types were found by the writer who has indicated the satisfactory manner in which this hypothesis has explained the serological results despite the absence of the last two sub-groups which was explained by him as shown in page 94.

In order to see how far this hypothesis could explain the previously-mentioned exceptions found against both theories, these will be discussed hereafter, separately.

1) Children of group O obtained from some AB parents, which is impossible according to the Bernstein theory.

As previously explained in Section III, two genes out of the four of each parent are transmitted to the child, therefore to make a child of group O, two genes (OO) are transmitted from the father and from the mother. According to this if an AB parent contains two genes of O, children of group O can be born, provided the other parent can also give two genes of O. In sub-group (A_2B_2) AOBO, we have two O and therefore this parent can give an O child when the other parent is of

/138

group OOOO, (A_1) AAOO, (A_2) AOOO, (B_1) BBOO, (B_2) BOOO or even ABOO. It has been noticed that, especially in the Haselhorst exception, the mother belonged to sub-group A_2B: the sub-division of B was not known at that time.

2) Children of sub-group A_1 obtained from mating A_2 X A_2 which is impossible according to Thomsen's theory.

According to this hypothesis the formulae of this mating will be AOOO X AOOO. If the first two genes of each parent were to be transmitted to the child, its formula would be AAOO, which corresponds to A_1.

3) Children of sub-group A_1 obtained from mating A_2 X O which is impossible according to Thomsen's theory.

Landsteiner, Thomsen and Wiener found 7 such exceptions. These may be explained in two ways:-

(a) According to this hypothesis there are three types of A_1 which differ quantitatively on account of the amount of A agglutinogen in them, namely AAAA, AAAO, AAOO, the last of which is not far from the (A_2) AOOO and probably this is the one which was called by Landsteiner the intermediate type because it reacted with both agglutinins (a_1) and (a_2). Consequently an (A_1) AAOO may be mistaken for (A_2) AOOO, especially if the agglutinin (a_1) used in the test is not strong enough, or if the sensitivity of the cells tested become less than the normal, owing to disease or old age, or even to keeping them for some time before being tested.

(b) If it is definitely proved that these parents belong to the type (A_2) AOOO by using efficient sera and taking every precaution against errors of technique, such A_1 children can only be explained by illegitimacy, especially if the parents are not old.

/139

4) Wiener's exception to Thomsen's Theory.

Wiener found a family A_1 X O with two children of group O, four of sub-group A_1, and one of sub-group A_2. If we assume that the A_1 parent is of the type AAOO, and the other parent is of the type OOOO, and two genes of each are transmitted to the child at random, one may obtain the following children:-

OOOO, AOOO (A_2) and AAOO (A_1)

If the AA in the parent AAOO are attached together of course the type AOOO will be impossible and therefore such a child will be illegitimate. As will be shown later in the families studied by the writer, there is reason to think that at least in some cases the two genes may be transmitted together (AA) or each separately (AO).

5) The writer 's first exception to Thomsen's Theory.

If we assume that the genotype of the members of this family were as follows:-

Maternal Grandmother	O	(OOOO)
Father B (BBOO) x Mother	A_1	(AAOO)
2 children	A_1	(AAOO)
1 child	A_2	(AOOO)
1 child	A_2B	(AOBB)

One can see that all the children can be explained according to this hypothesis. For the A_1 (AAOO) children, one may say that they took 2A from the mother and two O from the father: for the A_2 child one may say that it took two O from the father and one A and one O from the mother : for the A_2B child, one may say that it took two B from the father and one A and one O from the mother.

/140

From the above explanation, it appears that this hypothesis is quite satisfactory from the genetic point of view, as it is from the serological standpoint.

WHAT ELSE CAN THIS HYPOTHESIS EXPLAIN?

1. It is remarked by many workers that the exceptions to the Bernstein theory are so few in number that they may be neglected. According to this hypothesis the only possible sub-group of group AB which can give O children is A_2B_2, which is very rare. 4 such cases were found by the writer in more than 400 persons examined, which makes this type less than 1%. The chances of an individual of this sub-group meeting with one having two OO will be still smaller, therefore this hypothesis explains why these exceptions are rare and even absent in the material examined by some workers.

2. It was noticed that there were no O children obtained from all the matings examined of AB X AB, which was taken as an evidence that an AB parent does not give O children. According to Hirszfeld's Table, the exceptions found in different matings were as follows:-

MATING	No.of FAMILIES	No.of EXCEPTIONS
AB X O	163	13
AB X A	187	5
AB X B	98	4

From this one can see that the exceptions are more numerous when the other parent is O, than if A or B. The reason for such difference is that with an O parent the chances of giving two O are more than with A or B parents, since each of these have only two types out of four which can supply two O, namely AAOO, AOOO, BBOO and BOOO, and in these types the OO may or may not be transmitted to the child.

/141

In persons of group AB there are about 10% who belong to ABOO consequently the matings ABOO X ABOO will be much fewer than the other matings of group AB, namely AABB, AABO, ABBO, also even in the mating ABOO X ABOO it is not obligatory to have O children, since children A, B or AB may also be obtained.

Consequently, it is quite possible that O children may be absent in 67 families in which both parents are of group AB, even if these were examined together and certainly that would be more likely if, as it happened, these families were examined in batches in different populations, and the maximum number examined by a single worker was 7 families.

Testing the Efficiency of the New Hypothesis by Family Observations:

As previously mentioned, the basis of this hypothesis is that:

a) The genotype of a person is composed of four genes, which represent the three agglutinogens O, A and B in variable quantities, as shown in Section III.

b) The child receives two of these genes from the father and two from the mother.

If we assume that each of the two genes is taken at random from the four genes of each parent, the following rules can be deduced:

1. The child cannot have one gene of any of O, A or B agglutinogens except when at least one of the parents has at least one gene representing the agglutinogen concerned.

For example, with agglutinogen A:

CHILD	1st PARENT	2nd PARENT
A???	A???	????

2. The child cannot have two genes of O, A or B, except if two of these

/142

at least are found in one parent, or one at least is found in each parent.

CHILD		1st PARENT	2nd PARENT
AA??		AA??	????
	or	A???	A???

3. The child cannot have three genes of O, A or B, except when at least two of these genes are present in one parent and at least one present in the other parent.

For example:-

CHILD	1st PARENT	2nd PARENT
AAA?	AA??	A???

4. The child cannot have four genes of O, A or B, except when at least two of these genes are present in each parent.

For example:-

CHILD	1st PARENT	2nd PARENT
AAAA	AA??	AA??

Consequently, this hypothesis may be tested in the following ways:-

1. Observing the relation between the types of the children and their parents.
2. Observing the relation between the types of the children and their mothers.

In carrying out these two methods of testing the hypothesis in 100 families examined by the writer, the results of which are given in the Second Series at the end of this Section, it will be seen that there is only one exception to Rule 4, against the father in Family No. 87 namely :-

/143

Father	(55 years)	BBBO
1st child	(22 years)	BBOO
2nd child	(13 years)	BBOO
3rd child	(6 years)	OOOO

The third child can only be obtained if the father were BBOO, but not when BBBO. However, this child could be explained by illegitimacy on account of the mother having been divorced shortly after giving birth to this child, because of misconduct legally proved. Personal enquiries revealed that she afterwards married the man accused of being her lover. This exception, therefore, cannot be taken against the hypothesis.

Practically speaking, there is not a single exception against this hypothesis in all the families examined by the writer, and as he has previously shown, it explains all the exceptions found against both the Bernstein theory and the Thomsen theory, as well as the existence of agglutinogen O in certain bloods of group AB, and the existence of four sub-groups in each of groups A, B and AB; therefore one cannot but think that this is a fit hypothesis, at least in principle, to explain the heredity of the different groups and their sub-groups.

RELATION BETWEEN THE FOUR GENES COMPOSING THE GENOTYPE

Previously it was assumed that each of the two genes transmitted from each parent were taken at random. From the families studied one may form an opinion about the validity of this assumption.

As an example, we may study the families with the following mating:)

AAOO X OOOO

If the two genes are taken at random from each parent, one

would expect a higher number of children of A000 than of AA00. What actually happened was, in six families, six children belonged to the AA00 and two to the A000.

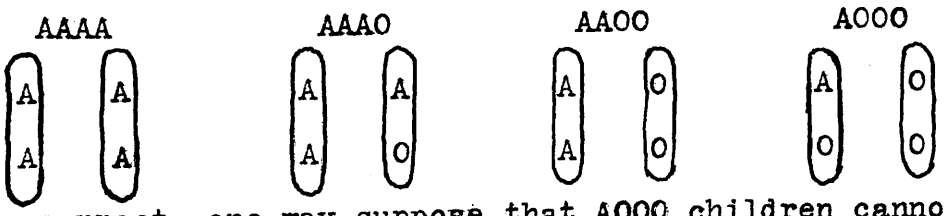
In the families where the parents were BB00 X 0000, there were four children of BB00 and none of B000. This suggests that there is a sort of linkage between the AA or BB and therefore they are more likely to be transmitted together. This suggestion is supported by the fact that in matings AA00 X AA00 and BB00 X BB00, there were 10 children of AA00, three of AAAA, two of BB00, and none of either A000 or B000, as shown in Table No.52.

Table No.52.

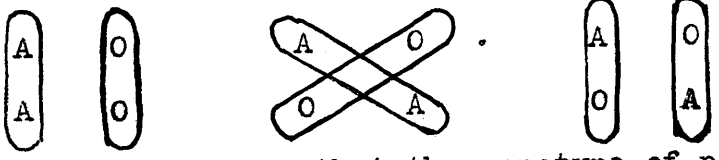
Family No.	AA00 X AA00 Group of Children AAAA	AA00	A000	0000	Family No.	AA00 X 0000 Group of Children AA00	A000	0000
3	-	1	-	-	12	1	-	2
23	1	3	-	1	31	3	-	1
27	1	2	-	2	40	2	2	-
34	1	3	-	1	47	-	-	2
46	-	1	-	-	49	-	-	2
					78			1
TOTAL	3	10	-	4	TOTAL	6	2	8

Family No.	BB00 X BB00 Group of Children BBBB	BB00	B000	0000	Family No.	BB00 X 0000 Group of Children BB00	B000	0000
4	-	2	-	1	90	-	-	1
					89	-	-	4
					79	1	-	-
					57	1	-	2
					19	2	-	4
					TOTAL	4	-	11

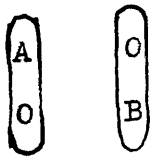
Provided that the future family studies prove the existence of such linkage, one may assume that each of the two genes are located in one chromosome, with regard to the types of group A, as shown in the following example:-



If this is correct, one may suppose that AOOO children cannot be obtained from a mating AAOO X OOOO: but this, of course, can be explained by crossing over, as shown in the following diagram:-



Accordingly, if we assume that the genotype of persons of the type AOBO is as represented in the following diagram:-



one may expect if such a person marries a person of group OOOO, the majority of children will be either A or B, since AB or O children are only possibly obtained when crossing over takes place, which occurs but seldom. That may explain the scarcity of such children in those matings.

In Table No.44 of Hirszfeld, we have seen that there are 7 children of Group O and two of group AB obtained from AB mothers. The comparatively small number of AB children, previously shown, may be partly due to overlooking the A_2 agglutinogen in A_2B children. Another theoretical explanation may be that there is a certain repulsion between the A and B genes and therefore they do not meet often in one

/146

chromosome, hence O children will be greater in number than AB children in the mating ABOO X OOOO.

If such repulsion actually exists, for some reasons which, at present, we do not know, it may explain the absence of the types AAAB and ABBB, in the cases examined by the writer. In such cases one parent must give AB and the other either AA or BB. However, one must admit that it is impossible from so few families to draw any definite conclusion, and therefore these assumptions together with the hypothesis itself should await further studies.

FAMILIES OF THE FIRST SERIES

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TECHNIQUE FOLLOWED IN FIRST SERIES.

The blood cells only were examined: a few drops of blood were taken from each person and transferred to 1 c.c. of citrated saline. The examination was made as a general rule on the same day, but in a few cases in which the bloods were collected in the evenings, the tests were performed next morning. Every precaution was taken to keep everything as sterile as possible, the tubes were numbered and recorded at the time of taking the blood in order to avoid mistaking one blood for another. All the cells were washed once in saline and made into approximately a 5% suspension. The sera used in testing these cells were as follows:-

1. Anti-A and anti-B sera supplied from the Sero-Theraputic Institute of Vienna, which were used as controls to the sera prepared by the writer.
2. Anti-A and anti-B sera taken from normal persons and prepared by leaving the whole blood overnight in the refrigerator before the serum was separated to exclude the cold and auto-agglutinins.
3. Natural (a_1) agglutinin found in group A_2B persons.
4. Anti-A serum absorbed with A_2 cells, which was used as a control to the natural (a_1).
5. Anti-M and N sera which were supplied by the Sero-Theraputic Institute. The titre of the sera used was as follows:

SERUM	TITRE
Anti-A	32-64 with A cells: 128-256 with A_1 cells
Anti-B	64-128 with 2 B cells.
Natural (a_1) agglutinin	4-16 with A_1B and A_1 cells
Prepared (a_1) agglutinin	16-32 with A_1B cells and A_1 cells
Anti-M	32 with M cells
Anti-N	16 with N cells

The following controls were made:-

1. Standard cells of all types to control the results of the daily

examined batches for the object of comparison.

2. O cells were tested at the same time with anti-A, B and A_1 sera. Known M and N cells were also tested with both anti-M and N sera.

3. All the cells to be tested were examined also with an AB serum free from agglutinins.

The object of the last two controls was to detedt any reaction due to unspecific phenomena of agglutinin.

The test was made either in tubes or on slides as described in Section II.

The reading was made first by the naked-eye and then by the aid of the low power of the microscope. The reactions obtained with anti)A and B sera from the Sero-Theraputic Institute were identical to those prepared by the writer. The reactions obtained with natural (a_1) were similar to those obtained with the prepared (a_1), but weaker.

The reactions obtained with the anti-M were stronger than those with anti-N, especially with the type MN.

There was not a single case where O cells were agglutinated by the test sera or the tested cells were agglutinated with AB serum, which contains no agglutinins.

The results given here show 3 exceptions in Families Nos.15 and 37 which were contrary to the heredity of the groups and sub-groups according to Bernstein's and Thomsen's theory, as previously described.

/ 150
Section V.

FAMILY No.	AGE	FAMILY MEMBER	GROUP	M or N
8.	45 years	Father	O	MN
	47 "	Mother	A_2	MN
	23 "	Child (a)	O	MN
	20 "	Child (b)	A_2	N
	11 "	Child (c)	A_2	M
9.	56 years	Father	O	MN
	39 "	Mother	O	M
	15 "	Child (a)	O	MN
	8 "	Child (b)	O	MN
	5 "	Child (c)	O	M
10.	66 years	M.G.Father	A_1	MN
	67 "	M.G.Mother	O	MN
	41 "	Mother	A_1	MN
	13 "	Child (a)	O	MN
	11 "	Child (b)	A_1	MN
11.	50 years	Father	O	MN
	41 "	Mother	O	MN
	11 "	Child (a)	O	MN
	9 "	Child (b)	O	MN
	8 "	Child (c)	O	MN
	4 "	Child (d)	O	N
12.	49 years	Father	B	MN
	45 "	Mother	O	MN
	14 "	Child (a)	B	MN
	10 "	Child (b)	B	N
	8 "	Child (c)	O	N
	4 "	Child (d)	O	M
13.	74 years	P.G.Father	A_2	M
	45 "	Father	A_2	M
	42 "	Mother	A_1	MN
	14 "	Child	A_2	MN

FAMILY No.	AGE	FAMILY MEMBER	GROUP	M and N
14.	66 years	M.G.Father	A_2	MN
	66 "	M.G.Mother	O	N
	34 "	Father	O	MN
	32 "	Mother	A_2	N
	6 "	Child	A_2	N
15.	62 years	Father	O	M
	59 "	Mother	O	M
	20 "	Child (a)	A_1 ?	M
	15 "	Child (b)	O	M
16.	63 years	Father	O	N
	61 "	Mother	O	MN
	31 "	Child (a)	O	N
	26 "	Child (b)	O	MN
	19 "	Child (c)	O	MN
17.	55 years	Father	A_1	M
	52 "	Mother	O	MN
	29 "	Child (a)	A_1	M
	18 "	Child (b)	O	M
	16 "	Child (c)	A_1	MN
18.	60 years	Father	A_2	M
	53 "	Mother	A_2B	MN
	25 "	Child (a)	B	M
	19 "	Child (b)	A_2	N ?
	14 "	Child (c)	A_2B	M
	12 "	Child (d)	B	M
	9 "	Child (e)	B	M
19.	43 years	Father	A_2	M
	36 "	Mother	O	M
	4 "	Child (a)	O	M
	2 "	Child (b)	A_2	M
20.	51 years	Father	B	M
	43 "	Mother	O	M
	18 "	Child (a)	B	M
	17 "	Child (b)	B	M
	12 "	Child (c)	O	M
	4 "	Child (d)	B	M

FAMILY No.	AGE	FAMILY MEMBER	GROUP	M and N
21.	64 years	Father	A_2	M
	43 "	Mother	O	M
	5 "	Child (a)	A_2	M
	3 "	Child (b)	O	M
22.	56 years	Father	O	M
	40 "	Mother	B	M
	11 "	Child (a)	O	M
	10 "	Child (b)	O	M
	9 "	Child (c)	O	M
	6 "	Child (d)	B	M
	1 "	Child (e)	O	M
23.	38 years	Father	A_1	M
	38 "	Mother	O	M
	14 "	Child (a)	O	M
	11 "	Child (b)	A_1	M
	6 "	Child (c)	A_1	M
24.	65 years	P.G.Mother	A_1	M
	38 "	Father	A_1	M
	33 "	Mother	A_1	M
	7 "	Child (a)	O	M
	5 "	Child (b)	A_1	M
	2 "	Child (c)	A_1	M
25.	50 years	Father	B	M
	49 "	Mother	O	MN
	19 "	Child (a)	O	M
	14 "	Child (b)	O	M
	12 "	Child (c)	O	M
26.	64 years	Father	O	M
	64 "	Mother	A_1	N
	36 "	Child (a)	A_1	MN
	34 "	Child (b)	A_2	MN
	23 "	Child (c)	A_2	MN

FAMILY No.	AGE	FAMILY MEMBER	GROUP	M and N
27.	58 years	Father	A_1	N
	56 "	Mother	O	M
	35 "	Child (a)	A_1	MN
	30 "	Child (b)	A_1	MN
	20 "	Child (c)	O	MN
	13 "	Child (d)	A_1	MN
	11 "	Child (e)	A_1	MN
28.	50 years	Father	A_1	MN
	46 "	Mother	A_1	MN
	17 "	Child (a)	A_1	MN
	14 "	Child (b)	A_1	MN
	12 "	Child (c)	A_1	MN
	9 "	Child (d)	A_1	M
	6 "	Child (e)	A_1	M
	3 months	Child (f)	A_1	MN
29.	47 years	Father	O	MN
	47 "	Mother	O	M
	20 "	Child (a)	O	MN
	18 "	Child (b)	O	MN
Twins.	17 "	Child (c)	O	M
	17 "	Child (d)	O	MN
	16 "	Child (e)	O	MN
	11 "	Child (f)	O	MN
	9 "	Child (g)	O	MN
	8 "	Child (h)	O	M
	7 "	Child (i)	O	MN
	5 "	Child (j)	O	MN
	1 "	Child (k)	O	MN
30.	84 years	M.G.Mother	O	N
	67 "	Father	O	M
	52 "	Mother	A_2	N
	26 "	Child (a)	A_2	MN
	23 "	Child (b)	A_2	MN

FAMILY No.	AGE	FAMILY MEMBER	GROUP	M and N
31.	41 years	Father	O	M
	40 "	Mother	A_1	M
	14 "	Child (a)	A_1	M
	7 "	Child (b)	A_1	M
	6 "	Child (c)	A_1	M
32.	47 years	Father	A_1	MN
	47 "	Mother	O	MN
	6 "	Child	A_1	MN
33.	72 years	M.G.Father	A_2	MN
	41 "	Father	A_1	M
	33 "	Mother	A_2	MN
	4 "	Child (a)	A_1	M
	2 "	Child (b)	A_1	MN
34.	76 years	M.G.Father	B	M
	49 "	Father	O	MN
	36 "	Mother	O	M
	12 "	Child (a)	O	M
	9 "	Child (b)	O	M
	7 weeks	Child (c)	O	M
35.	45 years	Father	O	MN
	52 "	Mother	O	MN
	22 "	Child (a)	O	MN
	20 "	Child (b)	O	MN
36.	65 years	Father	O	N
	60 "	Mother	O	MN
	30 "	Child (a)	O	MN
	25 "	Child (b)	O	N
	20 "	Child (c)	O	MN

FAMILY No.	AGE	FAMILY MEMBER	GROUP	M and N
37.	74 years	M.G.Mother	O	M
	38 "	Father	B	M
	35 "	Mother	A_1	M
	13 "	Child (a)	A_2?	M
	11 "	Child (b)	A_1	M
	9 "	Child (c)	A_2B?	M
	5 months	Child (d)	A_1	M
38.	36 years	Father	A_1	MN
	34 "	Mother	O	MN
	14 "	Child (a)	O	M
	12 "	Child (b)	A_1	MN
39.	65 years	M.G.Mother	O	N
	24 "	Father	O	N
	22 "	Mother	A_1	N
	4 "	Child (a)	O	N
	10 months	Child (b)	A_1	N
40.		Father	A_1	M
		Mother	O	M
		Child (a)	A_1	M
		Child (b)	O	M
41.	70 years	Father	O	M
	60 "	Mother	O	M
	22 "	Child	O	M
42.	52 years	Father	A_2B	MN
	45 "	Mother	O	M
	17 "	Child (a)	A_2	MN
	15 "	Child (b)	A_2	MN
	13 "	Child (c)	A_2	MN
	11 "	Child (d)	B	MN
	10 "	Child (e)	B	MN
	7 "	Child (f)	B	M
	6 "	Child (g)	B	MN

FAMILY No.	AGE	FAMILY MEMBER	GROUP	M and N
43	80 years	M.G.Mother	A_1	MN
	51 "	Father	O	M
	52 "	Mother	A_1	MN
	21 "	Child (a)	A_1	M
	18 "	Child (b)	A_1	M
	16 "	Child (c)	A_1	M
	13 "	Child (d)	A_1	M
44.	60 years	Father	A_1	M
	61 "	Mother	A_1	M
	34 "	Child (a)	O	MN
	27 "	Child (b)	A_1	MN
45.	50 years	Father	A_1	MN
	30 "	Mother	B	N
	6 "	Child (a)	A_1B	N
	4 "	Child (b)	A_1B	N
46.	40 years	Father	A_1B	MN
	34 "	Mother	A_1B	MN
	22 "	Child (a)	A_1	MN
	18 "	Child (b)	A_1B	MN
	16 "	Child (c)	A_1B	MN
	14 "	Child (d)	A_1B	MN
	10 "	Child (e)	A_1	N
	7 "	Child (f)	A_1B	MN
47.	43 years	Father	A_1	M
	44 "	Mother	A_1	MN
	19 "	Child (a)	A_1	MN
	16 "	Child (b)	A_1	MN
	12 "	Child (c)	A_1	M
	7 "	Child (d)	A_1	MN
	6 "	Child (e)	A_1	M

FAMILY No.	AGE	FAMILY MEMBER	GROUP	M and N
48.	40 years	Father	O	N
	40 "	Mother	O	M
	18 "	Child (a)	O	MN
	12 "	Child (b)	O	MN
	10 "	Child (c)	O	MN
	7 "	Child (d)	O	MN
	6 "	Child (e)	O	M ?
	3 "	Child (f)	O	MN
49.		Father	A_1	N
		Mother	A_1	N
		Child (a)	A_1	N
		Child (b)	A_1	N
		Child (c)	A_1	N
		Child (d)	A_1	N
50.	41 years	Father	O	N
	37 "	Mother	B	M
	6 "	Child (a)	O	MN
	1 "	Child (b)	O	MN
51		Father	B	MN
		Mother	O	M
		Child (a)	B	M
		Child (b)	B	M

FAMILIES OF THE SECOND SERIES.

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TECHNIQUE FOLLOWED IN SECOND SERIES.

The object of studying these families: which were examined in Egypt, was to study the inheritance of sub-groups of A, B and AB and principally to test the new hypothesis in order to ascertain the rules governing the inheritance of the three agglutinogens O, A and B.

The bloods were always collected by the writer himself in the evenings and kept in an ice-box overnight for examination the following day.

In order to differentiate the four groups, the cells were tested with:-

1. Immune anti-O serum.
2. Immune anti-A serum, and serum of group B .
3. Immune anti-B serum, and serum of group A.

In order to differentiate A_1 from A_2, the cells of groups A and AB were tested with:-

1. Serum of sub-group A_2B_1 which contained a strong (a_1) agglutinin.
2. Serum of group B absorbed with A_2 cells.
3. Serum of sub-group A_1B_1 which contained an anti-O agglutinin (a_2)

In order to differentiate B_1 and B_2, the cells of group B and AB were tested with:-

1. Serum of sub-group A_1B_1 which contained an anti-O agglutinin (b_2), and in addition cells of group B were tested with
2. Serum of sub-group B_2 containing (b_1) agglutinin, and in addition the cells were tested with
3. Serum of group A which was absorbed with AB_2 cells. This serum gave positive reactions with cells of sub-groups A_1B_1, and A_2B_1, and negative reactions with cells of sub-groups A_1B_2, and A_2B_2.

In order to differentiate the types according to the classification given in Section III, all the cells which gave position reactions with immune anti-O were titrated against this serum. The results were compared with the reactions obtained by (a_1) and (b_1) and natural anti-O (a_2 and b_2). The types were classified according to the results given in the following guide.

	CLASSIFICATION OLD	CLASSIFICATION NEW	IMMUNE TITRE	ANTI-O REACTION	NATURAL ANTI-O	(a_1)	(b_1)
0	0	0000	64-128	+++	+	-	-
A	A_1	(AAAA	.....	-	-	+	-
		(AAA0	2-4	tr	-	±	-
		(AA00	8-16	±	-	±	-
	A_2	A000	32	++	±	-	-
B	B_1	(BBBB	.....	-	-	-	±
		(BBB0	2-4	tr	-	-	±
		(BB00	8-16	±	-	-	tr
	B_2	B000	32	++	±	-	tr
AB	A_1B_1	AABB	....	-	-	±	tr
	A_1B_2	AAB0	4	tr	-	±	-
	A_2B_1	A0BB	2-4	tr	-	-	±
	A_2B_2	A0B0	16	±	f.tr.	-	-

In order to differentiate the types M, N and MN, the cells were tested with anti-M and N sera prepared by the writer, as well as by sera obtained from the Sero-Theraputic Institute of Vienna.

The tests were either performed in tubes or on slides according to the technique mentioned in the first series. The room temperature varied from 25° to 30°C. All records, tests, and readings were

made by the writer himself in order to avoid any discrepancy. Every precaution was taken to avoid any possible source of mistake whatever, in the technique itself. The results are recorded in Table below The results obtained by testing the bloods with anti-M and anti-N sera are given in this Table for the purpose of reference for the inheritance of the M and N factors which are going to be discussed in Section V.

In these families there was one exception to the Thomsen's theory, also one exception to the new hypothesis, which have been already discussed.

Note: The families have been so arranged as to include the grandparents and in the case where any member of the family was previously examined, such member is underlined for ready referencel

FAMILY No.	AGE	SEX	MEMBER	GROUP CLASSIFICATION OLD	NEW	M and N
1.	58		Mother	A_1	AACO	MN
	35	F.	Child (a)	A_1	AACO	MN
	32	M.	Child (b)	O	OOOO	MN
	30	F.	Child (c)	O	OOOO	MN
2.	58		Mother	A_1B_1	AABB	MN
	38	M.	Child (a)	A_1	AACO	N
	30	M.	Child (b)	B_1	BBOO	N
3.	58		M.G.M.	A_1	AACO	MN
	58		P.G.M.	A_1B_1	AABB	MN
	38		Father	A_1	AACO	N
	35		Mother	A_1	AACO	MN
	9	M.	Child (a)	A_1	AACO	MN
4.	58		P.G.M.	A_1B_1	AABB	MN
	30		Father	B_1	BBOO	N
	30		Mother	B_1	BBOO	MN
	6	F.	Child (a)	B_1	BBOO	MN
	4	F.	Child (b)	B_1	BBOO	MN
	2	M.	Child (c)	O	OOOO	MN
5.	59		Father	A_1	AAAO	MN
	57		Mother	A_1B_2	AABO	MN
	28	F.	Child (a)	A_1	AAAA	N
	24	F.	Child (b)	A_1B_2	AABO	MN
	22	M.	Child (c)	A_1	AAAO	MN
	18	F.	Child (d)	A_1	AAAO	MN
6.	60		Mother	O	OOOO	MN
	40	F.	Child (a)	B_1	BBOO	MN
	24	M.	Child (b)	B_1	BBOO	N
	20	M.	Child (c)	O	OOOO	N
	18	F.	Child (d)	B_1	BBOO	N
	16	F.	Child (e)	B_1	BBOO	N
	14	F.	Child (f)	B_1	BBOO	N

The underlined members are repeated

FAMILY No.	AGE	SEX	MEMBER	GROUP CLASSIFICATION OLD	NEW	M and N
13.	50		Father	A_2	AOOO	M
	45		Mother	O	OOOO	MN
	22	M.	Child (a)	A_2	AOOO	M
	20	F.	Child (b)	A_2	AOOO	M
	18	F.	Child (c)	O	OOOO	MN
	16	M.	Child (d)	A_2	AOOO	M
14.	50		M.G.F.	A_2	AOOO	M
	45		M.G.M.	O	OOOO	MN
	20		Mother	A_2	AOOO	M
	1	F.	Child	A_2B_1	AOBB	M
15.	55		Mother	A_1	AAOO	MN
	30	M.	Child (a)	A_1	AAOO	N
	28	M.	Child (b)	O	OOOO	N
	26	F.	Child (c)	O	OOOO	N
16.	45		Father	O	OOOO	MN
	40		Mother	O	OOOO	M
	10	F.	Child (a)	O	OOOO	MN
	8	F.	Child (b)	O	OOOO	M
	2	M.	Child (c)	O	OOOO	M
17.	40		Father	A_1B_2	AABO	N
	35		Mother	O	OOOO	MN
	14	M.	Child (a)	A_1	AAOO	N
	12	M.	Child (b)	A_1	AAOO	N
	9	M.	Child (c)	A_1	AAOO	N
	7	F.	Child (d)	A_1	AAOO	N
	6	F.	Child (e)	B_2	BOOO	N
	1	F.	Child (f)	B_2	BOOO	N
18.	35		Father	A_1B_2	AABO	MN
	32		Mother	B_1	BBBO	M
	5	M.	Child (a)	A_1B_2	AABO	MN
	9m.	F.	Child (b)	A_1B_2	AABO	M

Note: The Fathers of Families 17 and 18 are brothers.

FAMILY No.	AGE	SEX	MEMBER	GROUP CLASSIFICATION OLD	NEW	M and N
19.	46		Father	O	OOOO	MN
	40	-	Mother	B_1	BBOO	N
	21	M.	Child (a)	O	OOOO	MN
	19	M.	Child (b)	O	OOOO	MN
	17	F.	Child (c)	O	OOOO	MN
	14	M.	Child (d)	B_1	BBOO	MN
	11	M.	Child (e)	B_1	BBOO	MN
	6	M.	Child (f)	O	OOOO	MN
20.	40		Father	B_2	BOOO	N
	33	-	Mother	A_1B_2	AABO	N
	7	F.	Child (a)	A_1B_2	AABO	N
	5	F.	Child (b)	A_1	AAOO	N
21.	45		Father	A_1	AAAO	M
	45	-	Mother	O	OOOO	N
	16	F.	Child (a)	A_1	AAOO	MN
	15	F.	Child (b)	A_1	AAOO	MN
	13	M.	Child (c)	A_1	AAOO	MN
	12	F.	Child (d)	A_1	AAOO	MN
	9	F.	Child (e)	A_1	AAOO	MN
	7	F.	Child (f)	A_1	AAOO	MN
22.	72		Father	A_2B_1	AOBB	N
	65	-	Mother	A_1	AAOO	MN
	35	M.	Child (a)	A_2B_1	AOBB	N
	22	M.	Child (b)	A_2	AOOO	N
	20	M.	Child (c)	A_1	AAOO	N
	18	F.	Child (d)	A_1	AAOO	N
23.	49		Father	A_1	AAOO	MN
	45	-	Mother	A_1	AAOO	M
	21	M.	Child (a)	A_1	AAAA	MN
	18	M.	Child (b)	A_1	AAOO	M
	14	M.	Child (c)	O	OOOO	M
	8	F.	Child (d)	A_1	AAOO	MN
	2	F.	Child (e)	A_2	AAOO	MN
24.	85	-	Mother	O	OOOO	N
	60	M.	Child	O	OOOO	MN

FAMILY No.	AGE	SEX	MEMBER	GROUP CLASSIFICATION OLD	NEW	M and N
25.	60		Father	O	OOOO	MN
	58		Mother	O	OOOO	MN
	30	M.	Child	O	OOOO	MN
26.	30		Father	O	OOOO	MN
	30		Mother	O	OOOO	M
	3	M.	Child (a)	O	OOOO	MN
	1	F.	Child (b)	O	OOOO	M
27.	45		Father	A_1	AAOO	MN
	38		Mother	A_1	AAOO	MN
	16	M.	Child (a)	A_1	AAOO	MN
	14	F.	Child (b)	O	OOOO	MN
	8	F.	Child (c)	A_1	AAOO	MN
	6	M.	Child (d)	O	OOOO	MN
	2	F.	Child (e)	A_1	AAAA	MN
28.	48		Father	A_1	AAOO	MN
	18	F.	Child (a)	A_1	AAOO	M
	11	F.	Child (b)	O	OOOO	M
	9	M.	Child (c)	O	OOOO	MN
	5	F.	Child (d)	O	OOOO	N
29.	35		Father	A_2	AOOO	M
	30		Mother	O	OOOO	M
	2	F.	Child (a)	A_2	AOOO	M
	3m.	M.	Child (b)	A_2	AOOO	M
30.	40		Mother	A_1	AAAA	N
	12	M.	Child (a)	A_1	AAAA	MN
	10	M.	Child (b)	A_1	AAOO	MN
	7	M.	Child (c)	A_1	AAAA	MN

Families Nos. 24, 25 and 26 represent three generations of one pedigree.

In Family No. 30 the brother of the husband was AAOO.

FAMILY No.	AGE	SEX	MEMBER	GROUP CLASSIFICATION OLD	NEW	M and N
31.	36		Father	O	OOOO	MN
	35	-	Mother	A_1	AAOO	MN
	7	M.	Child (a)	O	OOOO	MN
	6	F.	Child (b)	A_1	AAOO	MN
	3	M.	Child (c)	A_1	AAOO	MN
	4m.	F.	Child (d)	A_1	AAOO	MN
32.	35		Father	B_1	BBOO	M
	30		Mother	A_1	AAAA	MN
	8	F.	Child (a)	A_1B_1	AABB	M
	6	F.	Child (b)	A_1B_1	AABB	M
33.	40	-	Mother	A_1	AAOO	MN
	19	M.	Child (a)	O	OOOO	MN
	16	F.	Child (b)	A_1	AAOO	MN
	14	F.	Child (c)	B_1	BBOO	N
	12	F.	Child (d)	A_1B_1	AABB	M
34.	46		Father	A_1	AAOO	MN
	40	-	Mother	A_1	AAOO	MN
	16	M.	Child (a)	A_1	AAOO	MN
	14	M.	Child (b)	A_1	AAOO	MN
	9	F.	Child (c)	A_1	AAOO	MN
	8	M.	Child (d)	O	OOOO	MN
	2	M.	Child (e)	A_1	AAAA	MN
35.	45	-	Mother	B_1	BBOO	MN
	26	M.	Child (a)	B_1	BBOO	M
	24	F.	Child (b)	O	OOOO	N
	20	M.	Child (c)	A_1B_2?	AABO	MN
	17	F.	Child (d)	O	OOOO	MN
36.	45		M.G.M.	B_1	BBOO	MN
	24	-	Mother	O	OOOO	N
	2	F.	Child (a)	O	OOOO	N

FAMILY No.	AGE	SEX	MEMBER	GROUP CLASSIFICATION OLD	NEW	M and N
37.	36		Father	A_1	AACO	M
	30		Mother	A_1	AAAO	MN
	10	M.	Child (a)	A_1	AACO	MN
	8	M.	Child (b)	A_1	AAAA	MN
	4	M.	Child (c)	A_1	AAAA	MN
	2	M.	Child (d)	A_1	AACO	MN
38.	42		Mother	B_1	BBOO	MN
	24	F.	Child (a)	O	OOOO	MN
	22	M.	Child (b)	O	OOOO	M
	16	F.	Child (c)	A_1	AAOO	M
	10	F.	Child (d)	B_1	BBOO	MN
	8	M.	Child (e)	B_1	BBOO	M
	5	M.	Child (f)	B_1	BBOO	MN
39.	39		Mother	A_2	AOOO	MN
	2	M.	Child	A_1	AACO	M
40.	43		Father	A_1	AAOO	N
	40		Mother	O	OOOO	N
	18	M.	Child (a)	A_2	AOOO	N
	16	F.	Child (b)	A_1	AACO	N
	14	M.	Child (c)	A_2	AOOO	N (1)
	12	F.	Child (d)	A_1	AACO	N
41.	40		Father	B_1	BBOO	MN
	36		Mother	O	OOOO	N
	6	M.	Child (a)	O	OOOO	N
	4	M.	Child (b)	O	OOOO	N
42.	40		Father	A_1	AAOO	M
	39		Mother	B_1	BBBB	MN
	18	M.	Child (a)	B_1	BBOO	M
	16	F.	Child (b)	A_1B_1	AABB	M
	14	M.	Child (c)	B_1	BBOO	MN

In Families Nos. 35 and 38 the two husbands, who were brothers, were dead. The exception noticed in Family No. 35 was discussed previously.

(1) This child gave negative reaction with anti-M and N of the S.T.I. and a moderate reaction with anti-N prepared by the writer.

FAMILY No.	AGE	SEX	MEMBER	GROUP CLASSIFICATION OLD	NEW	M and N
43.	36		Mother	A_2B_1	AOBB	N
	16	M.	Child (a)	A_2	AOOO	N
	14	F.	Child (b)	A_2	AOOO	N
	12	F.	Child (c)	A_2	AOOO	N
	6	F.	Child (d)	A_2	AOOO	N
44.	46		Father	O	OOOO	MN? (1)
	34		Mother	A_2B_1	AOBB	N
	11	F.	Child (a)	B_1	BBOO	N
	10	F.	Child (b)	B_1	BBOO	N
	9	F.	Child (c)	A_2	AOOO	MN
	7	F.	Child (d)	A_2	AOOO	MN
45.	24		Mother	A_1	AAOO	M
	7m.	F.	Child	B_2	BOOO	M
46.	60		Father	A_1	AAOO	M
	58		Mother	A_1	AAOO	M
	30	F.	Child	A_1	AAOO	M
47.	38		Father	O	OOOO	MN
	30		Mother	A_1	AAOO	M
	3	M.	Child (a)	O	OOOO	MN
	1	M.	Child (b)	O	OOOO	MN
48.	36		Father	A_2	AOOO	M
	26		Mother	O	OOOO	M
	5	M.	Child (a)	A_2	AOOO	M
	3	M.	Child (b)	A_2	AOOO	M
	1	F.	Child (c)	O	OOOO	M

Note: The mothers of Families Nos. 43 and 44 are sisters.

(1) The cells gave negative reaction with anti-N serum of the Sero-Theraputic Institute but positive with the writer's.

FAMILY No.	AGE	SEX	MEMBER	GROUP CLASSIFICATION OLD	NEW	M and N
49.	32		Father	A_1	AACO	M
	30		Mother	O	OOOO	M
	7	M.	Child (a)	O	OOOO	M
	5	F.	Child (b)	O	OOOO	M
50.	32		Mother	O	OOOO	M
	7	F.	Child (a)	O	OOOO	M
	4	M.	Child (b)	O	OOOO	M
51.	28		Mother	O	OOOO	M
	1	F.	Child	A_2	AOOO	M
52.	42		Father	O	OOOO	MN
	40		Mother	A_1	AAAA	N
	18	F.	Child (a)	A_1	AACO	N
	16	F.	Child (b)	A_1	AAOO	N
	14	F.	Child (c)	A_1	AACO	N
	6	M.	Child (d)	A_1	AAOO	MN
53.	48		Father	A_1	AACO	N
	40		Mother	A_1B_1	AABB	MN
	20	F.	Child (a)	A_1	AAOO	N
	19	M.	Child (b)	A_1	AACO	N
	17	F.	Child (c)	B_1	BBOO	MN
	12	F.	Child (d)	A_1B_1	AABB	MN
	10	F.	Child (e)	A_1	AACO	N
	8	F.	Child (f)	A_1	AACO	MN
54.	33		Father	O	OOOO	MN
	30		Mother	O	OOOO	M
	1m.	M.	Child	O	OOOO	M
55.	68		Father	B_1	BBOO	MN
	39	M.	Child (a)	O	OOOO	M
	32	M.	Child (b)	B_1	BBOO	MN
	19	F.	Child (c)	B_1	BBOO	MN

FAMILY No.	AGE	SEX	MEMBER	GROUP CLASSIFICATION OLD	NEW	M and N
56.	34		Father	A_1	AACO	M
	34		Mother	B_2	BOOO	MN
	15	M.	Child (a)	A_1	AACO	M
	13	M.	Child (b)	A_1	AACO	M
	8	M.	Child (c)	B_2	BOOO	MN
	6	M.	Child (d)	A_1B_2	AABO	MN
57.	38		Father	B_1	BBOO	N
	35		Mother	O	OOOO	N
	9	M.	Child (a)	O	OOOO	N
	7	M.	Child (b)	B_1	BBOO	N
	3	F.	Child (c)	O	OOOO	N
58.	35		Father	B_1	BBOO	MN
	32		Mother	A_1B_1	AABB	MN
	9	F.	Child (a)	A_1	AACO	N
	6	M.	Child (b)	A_1B_1	AABB	MN
	3	F.	Child (c)	B_1	BBBB	MN
59.	72		Father	B_1	BBOO	M
	65		Mother	A_1	AAOO	M
	30	F.	Child (a)	O	OOOO	M
60.	30		Mother	O	OOOO	M
	5	F.	Child (b)	B_1	BBOO	M
	3	F.	Child (c)	B_1	BBOO	M
	10m.	M.	Child (d)	O	OOOO	M
61.	40		Father	A_1B_1	AABB	N
	11	M.	Child	A_1B_1	AABB	N
62.	39		Mother	B_2	BOOO	MN
	22	F.	Child (a)	B_2	BOOO	MN
	18	M.	Child (b)	A_2B_2	AOBO	N
	12	M.	Child (c)	B_2	BOOO	N
	8	M.	Child (d)	A_2B_2	AOBO	N
	6	M.	Child (e)	A_2	AOOO	M

FAMILY No.	AGE	SEX	MEMBER	GROUP CLASSIFICATION OLD	NEW	M and N
63.	55		Father	A_2	AOOO	N
	50		Mother	A_1	AAOO	MN
	30	M.	Child (a)	A_1	AAOO	MN
	20	M.	Child (b)	A_1	AAOO	N
	16	F.	Child (c)	A_1	AAOO	N
	14	M.	Child (d)	A_1	AAOO	N
64.	30		Mother	O	OOOO	N
	1	F.	Child	O	OOOO	N
65.	28		Mother	A_1	AAOO	N
	1	M.	Child	A_1	AAOO	N
66.	24		Mother	A_1	AAOO	N
	3	F.	Child	A_1	AAOO	N
67.	35		Father	A_1	AAOO	N
	32		Mother	A_1	AAAA	M
	6	M.	Child (a)	A_1	AAOO	MN
	4	M.	Child (b)	A_1	AAAA	MN
	3	M.	Child (c)	A_1	AAAA	MN
68.	60		Father	A_2	AOOO	M
	30	F.	Child (a)	A_2	AOOO	M
	20	F.	Child (b)	B_1	BBOO	MN
69.	30		Mother	A_2	AOOO	M
	11	M.	Child (a)	A_1	AAOO	M
	4	F.	Child (b)	A_1	AAOO	M
	3	F.	Child (c)	A_1	AAOO	M
	6m.	F.	Child (d)	A_1	AAOO	M
70.	52		Mother	A_2	AOOO	MN
	30	F.	Child (a)	B_1	BBOO	MN
	28	M.	Child (b)	O	OOOO	M

Note: The husband of family No. 63 is the brother of the husband of No. 62 who was dead.

FAMILY No.	AGE	SEX	MEMBER	GROUP CLASSIFICATION OLD	NEW	M and N
71.	30		Mother	B_1	BBOO	MN
	4	F.	Child (a)	B_1	BBOO	MN
	3	M.	Child (b)	A_2	AOOO	M
72.	55		Mother	A_1	AAOO	MN
	32	M.	Child (a)	A_1B_1	AABB	MN
	26	M.	Child (b)	B_1	BBOO	MN
	22	M.	Child (c)	B_1	BBOO	M
73.	32		Father	A_1B_1	AABB	MN
	26		Mother	O	OOOO	M
	9m.	F.	Child	B_1	BBOO	M
74.	60		Father	A_1	AAOO	M
	18	F.	Child (a)	A_1	AAOO	M
	16	M.	Child (b)	A_1	AAOO	M
75.	36		Mother	O	OOOO	M
	8	M.	Child (a)	B_1	BBOO	MN
	6	F.	Child (b)	O	OOOO	M
	2	M.	Child (c)	O	OOOO	M
76.	50		Mother	A_1	AAAO	MN
	24	F.	Child (a)	A_2B_2	AOBO	N
	22	M.	Child (b)	A_2B_2	AOBO	N
	20	F.	Child (c)	A_1B_2	AABO	N
77.	45		Father	A_1B_2	AABO	MN
	40		Mother	B_2	BOOO	N
	19	M.	Child	B_2	BOOO	N
78.	40		Father	O	OOOO	N
	35		Mother	A_1	AAOO	N
	13	F.	Child	O	OOOO	N
79.	45		Father	O	OOOO	M
	38		Mother	B_1	BBOO	N
	2	F.	Child	B_1	BBOO	MN

FAMILY No.	AGE	SEX	MEMBER	GROUP CLASSIFICATION OLD	NEW	M and N
80.	45		Father	O	OOOO	M
	14	F.	Child (a)	B_1	BBOO	MN
	12	F.	Child (b)	A_1	AAOO	M
	10	F.	Child (c)	B_1	BBOO	MN
81.	40		Mother	B_2	BOOO	MN
	22	M.	Child (a)	A_1B_2	AABO	MN
	19	F.	Child (b)	A_1B_2	AABO	M
	17	M.	Child (c)	A_1	AAOO	N
82.	45		Father	A_1	AAAO	MN
	40		Mother	A_1	AAOO	MN
	24	M.	Child (a)	A_1	AAAO	N
	20	M.	Child (b)	A_1	AAAO	N
	16	F.	Child (c)	A_1	AAAO	M
	12	M.	Child (d)	A_1	AAAO	M
	10	M.	Child (e)	A_1	AAOO	MN
83.	50		Father	B_1	BBBO	M
	40		Mother	B_1	BBOO	M
	20	M.	Child (a)	B_1	BBBO	M
	18	M.	Child (b)	B_1	BBBO	M
84.	55		Father	A_1B_2	AABO	M
	45		Mother	B_2	BOOO	MN
	22	M.	Child	A_1B_2	AABO	M
85.	60		Mother	A_1	AAOO	N
	30	M.	Child (a)	A_1	AAOO	MN
	25	F.	Child (b)	O	OOOO	MN
	20	F.	Child (c)	A_1	AAOO	N
86.	34		Father	A_1	AAAO	M
	30		Mother	B_1	BBOO	M
	2	M.	Child	A_1B_1	AABB	M

FAMILY No.	AGE	SEX	MEMBER	GROUP CLASSIFICATION OLD	NEW	M and N
87.	55		Father	B_1	BBBO	MN
	22	F.	Child (a)	B_1	BBOO	MN
	13	F.	Child (b)	B_1	BBOO	MN
	6	M.	Child (c)	O	OOOO?	M
88.	22		Mother	B_1	BBOO	MN
	1	F.	Child	A_1	AAOO	M
89.	40		Father	B_1	BBOO	MN
	30		Mother	O	OOOO	M
	5	F.	Child (a)	O	OOOO	M
	3	F.	Child (b)	O	OOOO	M
	1	F.	Child (c)	O	OOOO	M
90.	35		Father	O	OOOO	N
	22		Mother	B_1	BBOO	N
	4	M.	Child (a)	O	OOOO	N
91.	55		Mother	B_1	BBBO	MN
	36	F.	Child (a)	B_1	BBOO	MN
	26	F.	Child (b)	A_1B_2	AABO	MN
	24	M.	Child (c)	A_1B_2	AABO	MN
	22	F.	Child (d)	A_1B_2	AABO	MN
92.	36		Mother	B_1	BBOO	MN
	18	F.	Child	O	OOOO	MN
93.	22		Mother	A_1B_2	AABO	MN
	1	M.	Child	A_1	AAOO	MN
94.	60		Mother	B_2	BOOO	M
	34	M.	Child	A_1B_2	AABO	M

FAMILY No.	AGE	SEX	MEMBER	GROUP CLASSIFICATION OLD	NEW	M and N
95.	34		Father	A_1B_2	AABO	M
	29		Mother	A_1	AAAO	N
	8	F.	Child (a)	A_1	AAAA	MN
	4	F.	Child (b)	A_1	AAAA	MN
	2	F.	Child (c)	A_1B_2	AABO	MN
96.	45		Mother	B_1	BBBB	
	22	M.	Child (a)	A_2B_1	AOBB	
	20	M.	Child (b)	A_2B_1	AOBB	
97.	50		Mother	O	OOOO	
	30	M.	Child (a)	O	OOOO	
	28	F.	Child (b)	O	OOOO	
98.			Father	B_2	BOOO	
			Mother	B_1	BBOO	
			Child (a)	O	OOOO	
			Child (b)	B_1	BBOO	
			Child (c)	B_2	BOOO	
99.	40		Father	O	OOOO	
	14	M.	Child (a)	A_2	AOOO	
	5	F.	Child (b)	O	OOOO	
	3	F.	Child (c)	O	OOOO	

S E C T I O N V

FURTHER CLASSIFICATION OF HUMAN BLOODS.

The classification of human bloods into further types besides those previously mentioned has been suggested by several workers.

These classifications depend on the presence or absence of certain human blood cells. Their presence was detected by the aid of sera, either prepared by immunizing animals or found in normal animals or even in certain human beings. The different new classifications are summed up, as follows:-

1. The three types M, N and MN, found by Landsteiner and Levine (41) to be equally distributed amongst the four original blood-groups. The presence of these can only be differentiated by immune sera.
2. The two types P (+) and P (-) found also by these workers (35) who

/151

noticed the scarcity of P in the White Americans. The factor P may be detected by immune sera, certain animal sera and certain normal human sera.

3. The two types G (+) and G (-) found by Schiff (85), the factor concerned was detected by immune serum and its presence in group O could only be demonstrated by absorption and not by direct agglutination.
4. The two types H (+) and H (-) found by the same worker, by the aid of sheep's serum.
5. The two types Q (+) and Q (-) found by Imamura (42) who differentiated them by the aid of pig serum.
6. The two types E (large) and E (small) found by Sugushita (43) by the aid of eel's serum.
7. The secretary and non-secretory types claimed to exist by Schiff (46), in the first of which the saliva contains the group specific substances in great quantities while in the second these are found only in small traces or may be altogether absent.

There are still further types accidentally observed, as that reported by Ottenberg and Johnson (36), in which a serum of group B agglutinated some B cells and O cells. Another case was reported by Landsteiner, Levine and James (86), in which a patient of group O, after being twice transfused developed an agglutinin which could agglutinate some bloods and not others.

Out of all the above-mentioned classifications only the first seems to have been studied in detail by several workers who, after confirming the results of Landsteiner and Levine, suggested its application in medico-legal work. Therefore the writer has concentrated on

/152

its study.

THE AGGLUTINOGENS M AND N.

Landsteiner and Levine, by immunising rabbits with certain human blood cells and absorbing the resulting anti-sera with some other cells of the same group as those used in immunization, found two agglutinogens which they named M and N. They examined a number of bloods of all groups with the anti-M and N sera and also families. The results led to the following conclusions:-

1. There are three types of human blood cells, namely M, N and MN, and in no blood were both M and N absent.
2. These agglutinogens are independent of A and B, since they are equally distributed in the four groups.
3. These two agglutinogens are inherited according to a single pair of allelomorphic genes M and N, both of which are Mendelian dominants. Consequently there are three types M, N and MN.

Thomsen and his school (87), Schiff (88) and Lattes (89), by the use of specimens of blood sent by Landsteiner, were able to prepare such sera and study the distribution and the heredity of these factors in Europe. Their results confirmed those of Landsteiner and Levine.

Wiener, Vaisberg and Rothberg (90) in America, Clausen (91), Wolff (92), Mayser (93), Crome (94), Laubenheimer (95), Maureau (96), Meixner (97) in Europe, came to the same conclusions and finally the test was recommended for medico-legal application.

PERSONAL INVESTIGATIONS.

A preliminary study of these factors was carried out in Glasgow by the use of anti-sera purchased from the Sero-Therapeutic Institute

/153

of Vienna: their inheritance was also studied by examining the bloods of 52 families. In Cairo, the writer prepared immune sera, by which he studied the distribution and inheritance of these factors there.

PREPARATION OF IMMUNE ANTI-M and N SERA:

12 rabbits and 5 goats were immunized with M, N and MN cells, as shown in Section III where the different methods of immunization were described and that followed by the writer was given in detail. The types of the cells used were determined by anti-M and N sera obtained from (a) the Sero-Theraputic Institute of Vienna prepared with blood specimens supplied by Schiff, and (b) Professor Boyd of Boston University (U.S.A.) who was studying the blood groups in Egypt at that time.

PREPARATION OF THE TESTING FLUIDS FOR M and N:

The sera of the immunized rabbits and goats were inactivated and then diluted 1:4 - 1:12, according to their titres. The diluted anti-M sera were mixed with half their volume of washed packed cells which were prepared by mixing equal volumes of cells of groups O, A and B which were of the type N, in order to remove the species, as well as other agglutinins, thus leaving the anti-M. After standing for 2 hours at 25° - 30°C., the tubes were centrifuged and the clear fluid tested with cells of different types. Some of the absorbed sera still gave unspecific reactions with regard to M and N: these were re-absorbed with another quantity of fresh cells of type N. The results are given in Table No.53.

Out of the 8 rabbits immunized with M or N cells, the sera from only two of the rabbits (7 and 13) produced strong anti-M while the other 6 rabbits gave either weak or negative reactions. The three

/154

goats which were immunised with M or MN yielded no anti-M, and the same results were obtained with all animals immunised with N cells.

Table No.53.

SERUM OF	ANIMAL IMMUNISED WITH CELLS	ABSORBED WITH CELLS	TESTED WITH CELLS					
			OM	ON	A_1M	A_1N	BM	BN
R1	OM	(O, A_1 & B) N	tr	-	-	-	-	-
R2	OM	" "	±	-	tr	-	tr	-
R5	A_2M	" "	tr	-	tr	-	tr	-
R7	A_2M	" "	+++	-	+++	-	+++	-
G1	OM	" "	-	-	-	-	-	-
G3	A_2M	" "	-	-	-	-	-	-
R3	OMN	" "	-	-	-	-	-	-
R4	OMN	" "	±	-	±	-	±	-
R12	A_2MN	" "	tr	-	tr	-	-	-
R13	A_2MN	" "	++	-	++	-	++	-
G2	OMN	" "	-	-	-	-	-	-
R8	A_1N	" "	-	-	-	-	-	-
R9	A_1N	" "	-	-	-	-	-	-
R10	BN	" "	-	-	-	-	-	-
R11	BN	" "	-	-	-	-	-	-
G4	A_1N	" "	-	-	-	-	-	-
G5	BN	" "	-	-	-	-	-	-

All the sera were absorbed in a similar manner with a mixture of cells OM, A_1M and BM, in order to remove all other agglutinins than anti-N, and the results are given in Table No.54.

/155

Table No.54.

SERUM OF	ANIMAL IMMUNISED WITH CELLS	ABSORBED WITH CELLS	TESTED WITH CELLS					
			OM	ON	A_1M	A_1N	BM	BN
R8	A_1N	(O, A & B) M	-	++	-	++	-	++
R9	A_1N	" "		+		+		+
R10	BN	" "		±		±		±
R11	BN	" "		±		±		±
G4	A_1N	" "		-	-	-	-	-
G5	BN	" "		tr		tr		tr
R3	OMN	" "		±		±		±
R4	OMN	" "		±		±		±
R12	A_2MN	" "		+++		+++		+++
R13	A_2MN	" "		tr		tr		tr
G2	OMN	" "		-		-		-
R2	OM	" "		tr		tr		tr
R1	OM	" "		-		-		-
R5	A_2M	" "		tr		-		-
R7	A_2M	" "		-		-		-
G1	OM	" "		-		-		-

Although the sera of all the rabbits immunised with N or MN gave specific reactions with N cells, only two of these gave strong reactions (R8 and R12) while the others were weak. Goat 5 which was immunised with BN cells produced a very weak anti-N which did not occur in the serum of Goat 4 immunised with A_1N.

EFFECT OF ABSORPTION WITH THE CELLS OF THE HETEROLOGOUS TYPE:

Landsteiner and Levine found that when an anti-N serum is

/156

repeatedly absorbed with cells of the type M, it lost its activity towards N cells.

That did not occur when the anti-M was repeatedly absorbed with N cells. In order to study this point, the writer absorbed each anti-M and N sera prepared from R7 and R12 respectively with M and N cells. The absorption was repeated with the cells of the heterologous type using in each case a quantity of the packed cells equal to half the volume of the serum. The results are given in Table No.55.

Table No.55.

SERUM		TITRE WITH M CELLS				
		1:2	4	8	16	32
Anti-M	before absorption	+++	++	+	±	±
Absorbed with M cells	1st absorption	-	-	-	-	-
absorbed with N cells	1st absorption	+++	++	+	±	tr
	2nd absorption	++	++	+	±	-
	3rd absorption	++	++	+	tr	-
		TITRE WITH N CELLS				
		1:1	2	4	8	16	32
Anti-N	before absorption	++	++	+	±	tr	-
Absorbed with N cells	1st absorption	-	-	-	-	-	-
Absorbed with M cells	1st absorption	++	++	+	±	tr	-
	2nd absorption	++	+	-	-	-	-
	3rd absorption	+	-	-	-	-	-
	4th absorption	-	-	-	-	-	-

This table shows the following:

1) M or N cells can exhaust completely their respective anti-sera.

/157

2) N cells are incapable of absorbing anti-M serum even after repeated absorption.

3) M cells can reduce the action of anti-N serum, which may become inactive if the absorption is repeated three times.

Levine, in explaining the third observation to the writer, assumed that the anti-N agglutinin may be attached to the species agglutinin found in the immune serum and therefore that when M cells are used in absorbing a serum containing the two agglutinins, they will not only absorb the species agglutinin but also that specific for N. This may be a reasonable explanation if these cells stopped absorbing the anti-N once the species agglutinins were removed from the serum but it was found that they could exhaust an anti-N serum purified from the species agglutinins. Another important point against this explanation was that the serum of R2 immunized with OM cells produced a weak anti-N which agglutinated all N bloods (see table No.54). One may conclude that there is a certain relation between the M and N agglutinogens since one of them (M) can produce immune serum against the other (N) and also can exhaust this serum if the absorption is repeated.

This makes it more difficult to prepare anti-N than anti-M. Landsteiner and Levine suggested that in order to prepare pure anti-N serum, the absorption with M blood cells should be carried out at 37°C. at which temperature only the unspecific anti-bodies will be absorbed. Hirszfeld (98) suggested that the absorption should be made for a short time at room temperature. In order to avoid the reactions obtained by the unspecific anti-bodies which may remain in the serum after such incomplete absorption, he recommends putting the tubes at a temperature of 40°C. for 2 to 3 minutes before reading the results. As the species

/158

agglutinin does not act at such a temperature only the specific reaction will remain and thus the type of the blood cells will be properly determined.

The writer carried out the absorptions and tests at a room temperature of 30°C. or sometimes higher and did not find much difficulty in obtaining a serum which acted on N and not on M cells.

A large number of tests made by several workers have resulted in a clear-cut classification of the red cells into three groups, M, N and MN, the last being agglutinated by both anti-sera. Schoukaert (99) met with a few exceptions apparently due to the use of weak test sera.

The writer studied the distribution of these types amongst the populations of both Glasgow and Cairo.

TECHNIQUE.

The cells were examined on the same day in which the blood samples were taken after washing them once with saline and making a suspension of 3-5% in citrated saline. The tests were performed in tubes by adding two drops of the testing fluids for M and N and one drop of the cell suspension. After standing one hour at room temperature the results were read by the naked-eye and then by examining all the negative tubes under the low power of the microscope. This method, originally suggested by Landsteiner, was compared with the centrifuge method recommended by Schiff, and the slide method. The first was found to be more reliable than the second, which may give weak pseudo-positive reactions. The slide method is also reliable, provided that the testing fluid is strong enough to produce agglutination quickly - within 10 minutes. There is no danger in the last method of pseudo-agglutination, since the sera are already

/159

the different populations given. It also shows that the frequency of the MN type is higher in the Egyptians tested by Boyd than in those tested by the writer, and vice versa, with the frequency of the type M. This can be explained by the fact that the anti-N serum of Professor Boyd was not completely freed from the unspecific agglutinins and therefore some of the M cells which gave positive reactions were considered to belong to the type MN. This is supported by the fact that the frequency of the type N was equal in the total number examined by each of them.

In order to show the distrubution of the three types in each of the four blood groups, the writer gives his results separately in Tables No. 57 and No.58, the first of which contains bloods examined in Glasgow and the second, those examined in Cairo.

Table No.57.
DISTRIBUTION IN THE DIFFERENT GROUPS IN GLASGOW.

	FREQUENCY						
BLOOD GROUP	M		N		MN		TOTAL
O	85	(38%)	39	(18%)	93	(43%)	217
A	56	(32%)	27	(15.4%)	92	(52.6%)	175
B	15	(36.6%)	8	(19.5%)	18	(43.9%)	41
AB	4	(17.4%)	4	(17.4%)	15	(65.2%)	23
TOTAL	160		78		218		456
PERCENTAGE	35%		17.1%		47.9%		

Table No.58/

/161

Table No.58.
DISTRIBUTION IN THE BLOOD GROUPS IN CAIRO.

BLOOD GROUP			FREQUENCY M	FREQUENCY N	FREQUENCY MN	TOTAL
O			56 (40.1%)	28 (20.5%)	53 (39.4%)	137
A	A_1	46	59 (31.9%)	29 36 (19.4%)	17 90 (48.7%)	185
	A_2	13		7	15	
B			40 (28.5%)	31 (22.1%)	69 (49.4%)	140
AB	A_1B	11	21 (38.8%)	5 7 (12.9%)	20 26 (48.2%)	54
	A_2B	10		2	6	
TOTAL			176	102	238	516
PERCENTAGE			34.1	19.8	46.1	

These two tables show that the factors M and N are independent of A and B agglutinogens since they may be equally absent or present in each group.

It has been already pointed out (1) that these two factors are independent of the O agglutinogen and in order to show this clearly the results obtained by testing the bloods of families of the Second Series given at the end of Section IV are classified in Table No.59.

Table No.59.

GROUP	TYPE	M	N	MN
O	OOOO	40	25	32
A_1	AAAA	1	3	12
	AAAO	4	4	5
	AAOO	24	39	44
A_2	AOOO	17	8	4
B_1	BBBB	-	-	2
	BBBO	1	-	2
	BBOO	9	14	35
B_2	BOOO	5	3	5
AB	AABB	5	3	7
	AABO	4	2	12
	AOBB	1	4	1
	AOBO	-	4	1

(1) See Section III.

/162.

The fact that out of 97 bloods of group O, 40 contain only M and 25 contain only N shows that these two factors are independent of the O agglutinogen.

In the bloods examined both in Glasgow and in Cairo, there was not a single specimen which did not react with either anti-M or N, although in Glasgow, one MN blood did not show agglutination until undiluted sera were used. In Cairo two bloods gave positive reaction with the anti-M but negative with the undiluted anti-N of the Sero-Theraputic Institute of Vienna, but these two bloods were positive with both anti-M and N prepared by the writer. As a control they were tested with Professor Boyd's anti-M and N sera, and with both, they gave positive reactions, although the anti-N reaction was weaker than usual.

When the cells of these two bloods which belonged to two medical students (K and H) were re-tested, they gave the same results. They were then titrated against the anti-M and N sera prepared by the writer and the results are given in Table No.60, which also shows the reactions obtained with standard cells of the three types.

Table No.60.

BLOOD CELLS	ANTI-M				ANTI-N				
	1:4	8	16	32	1	2	4	8	10
Standard M	+	+	+	±	-	-	-	-	-
Standard N	-	-	-	-	+	+	+	±	±
STANDARD MN	+	+	+	tr	+	+	±	tr	-
(K) MN	+	+	+	tr	±	?			
(H) MN	+	+	+	tr	±	?			

The weak sensitivity of the N factors in these two bloods could not be explained by assuming that these cells are less sensitive than the other

/163

cells of MN because if this were the case, the reactions with anti-M should also have been weak, but as can be seen from this table, they were as strong as that given with the standard MN cells. A possible explanation of the weak reactions obtained with anti-N may be, as Friedenreich (101) has suggested, i.e. that there are two types of N, namely N_1 and N_2 the latter being less sensitive than the former. Levine[1] contradicted this by saying that it happens sometimes that certain anti-N sera after being purified from other agglutinin still react with bloods of M types; which, in his opinion, renders such sera unsuitable for use. Although the writer has not yet sufficiently investigated this point to form a definite opinion, he would mention that in Family No.44 of the Second Series, the father was of the type M according to the reaction obtained with the sera of the Sero-Theraputic Institute of Viena, but according to the sera of the writer he belonged to the type MN. If this father is considered to be M, two of his children will be taken as illegitimate. Another case in the same series is Child (c) of Family No.40, which gave a negative reaction with the commercial anti-M and N sera but a weak positive reaction with the anti-N serum of the writer. Therefore if we assume with Levine that the writer's anti-N serum is not suitable because it reacted with some M cells (of the two students), this child will be considered of the type (M-N-) which does not exist according to the theory to be described later, or even according to actual observation. However, the assumption of Friedenreich needs further study before a conclusion can be arrived at.

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(1) In his reply to Thomsen's paper, read in IInd Congress of the International Society of Microbiology, July 1936.

INHERITANCE OF M AND N.

Landsteiner and Levine studied the inheritance of M and N in 62 families and came to the following conclusions:-

1. The factors M and N are inherited as Mendelian dominants.
2. They are inherited independently of the agglutinogens A and B.
3. Their inheritance is based on a single pair of allelomorphic genes M and N.

Later Schiff, Wiener and his co-workers, Thomsen and Clausen, and many others studied this question, and came to the same conclusions. Accordingly the possible three types are shown in the following illustration:

	M	N
M	MM	MN
N	MN	NN

In various populations tested by a number of workers only three three types were found.

FAMILY OBSERVATIONS.

The genotypes possible according to this theory are as follows:

Phenotype	M	N	MN
Genotype	MM	NN	MN

If one gene is transmitted from each parent to the child one may easily find out the possible and impossible children to be obtained from each of the six possible matings. These are given in Table No.61.

Table No.61

PARENTS	POSSIBLE CHILDREN	IMPOSSIBLE CHILDREN
1 M X M	M	N and MN
2 N X N	N	M and MN
3 M X N	MN	M and N
4 MN X M	M and MN	N
5 MN X N	N and MN	M
6 MN X MN	M, N and MN	.....

/165

This may be summarised in the following two rules:-

1. M and N cannot appear in the child's blood except when present in the blood of at least one parent.
2. A parent of the type M cannot produce a child N and a parent N cannot produce a child M.

The writer examined 151 families - 52 in Glasgow and 99 in Cairo. In the first series only the commercial sera were used in testing the bloods. In the 99 families examined in Cairo anti-sera prepared by the writer were also used in parallel. The results are shown in detail in the tables given at the end of Section IV. The study of the inheritance of M and N besides that of O, A and B indicates how the two former are inherited independently of the latter three. The results of testing the families of the first series are summed up in Table No.62.

Table No.62.

PARENTS	NO. OF FAMILIES	TOTAL NO.OF CHILDREN	CHILDREN IN EACH TYPE		
			M	N	MN
M X M	12	35	35	-	-
N X N	2	7	-	7	-
M X N	5	18	1	-	17
MN X M	13	51	26	1	24
MN X N	7	12	-	8	4
MN X MN	12	35	6	6	23
TOTAL	52	158	68	22	68

According to the second rule of this theory, there are two exceptions, namely:-

1. Family No.18.

Father M, mother MN and child (b) N and the other four children M.

/166

According to the theory the genotype of the father is MM and that of the mother MN, therefore all children should be either MM or MN, but not NN. Thus one may conclude that this child is illegitimate.

2. Family No.48.

Father N, mother M and child (e) M and the other 5 children MN. According to the theory the genotype of the parents is NN X MM, therefore all children should be MN, thus one may conclude that child (e) is illegitimate.

52 families, in which both parents were examined, 39 in which the mother only were examined and 6 in which the fathers only were examined, were investigated in Cairo. The results obtained with these families are summed up in Table No.63 which shows no exceptions to this theory.

Table No.63

PARENTS	NO. OF FAMILY	CHILDREN IN EACH TYPE M	N	MN	TOTAL NO. OF CHILDREN.
M X M	7	12			12
N X N	6		14		14
M X N	4			13	13
MN X M	14	20		18	38
MN X N	13		30	21	51
MN X MN	8	3	4	22	29
TOTAL	52	35	48	74	157
Father M	3	4	-	3	10
N	1	-	1	-	2
MN	2	4	1	1	8
Mother M	9	15	-	1	25
N	11	-	15	6	32
MN	19	12	19	23	73
TOTAL	45	35	36	34	150

One may mention here that if these families were tested only with the commercial sera two children of Family No.44 would have been considered

/167

illegitimate since according to those sera the father was M and two children N. With the writer's sera the cells of this father were agglutinated with anti-N therefore there was no reason to consider these children illegitimate. Another case is that of child (c) in Family No. 40, which was negative with both the commercial anti-M and N but positive with anti-N prepared by the writer:

Accordingly one may doubt that child (b) in Family No.18 of the First Series was illegitimate because if we assume, after Friedenreich, that the father was of the type MN_2 and the N was not detected owing to its weakness, such a child could possibly be obtained from such a family. Wiener (102) collected the families examined by several workers and found the following exceptions in 932 families including 2415 children.

MN X N = 2 children (M)

MN X M = 3 children (N)

M X N = 2 children (N)

M X M = 1 child (MN)

The fact that in 6 out of 8 exceptions the factor N was implicated may suggest that some of the 6 N children are not illegitimate according to Friedenreich's assumption regarding the existence of another factor which he called N_2 and which may be overlooked owing to its weakness. Wiener also compared the observed frequencies of the children of the three types in each mating with those expected according to the theory as shown in Table No.64.

Table No.64

PARENTS		PERCENTAGE CHILDREN OF TYPES MN	M	N
MN X MN	(Observed	53.83 ± 1.34	24.45 ± 1.16	21.72 ± 1.13
	(Theoretical	50.	25.	25.
	(Deviation	3.83 ± 1.34	0.45 ± 1.16	3.28 ± 1.13

/168

Table No.64 (cont.)

PARENTS		PERCENT. CHILDREN OF TYPES MN	M	N
MN X N	(Observed	49.37 ± 1.43		50.63 ± 1.43
	(Theoretical	50.		50.
	(Deviation	0.63 ± 1.43		0.63 ± 1.43
MN X M	(Observed	53.16 ± 1.28	46.84 ± 1.28	
	(Theoretical	50.	50.	
	(Deviation	3.16 ± 1.28	3.16 ± 1.28	

He explained the deviations shown in this table by the possibility of the inclusion of some illegitimate children or by errors in the technique, and therefore the results may be considered in favour of the theory. It is interesting to note that in the mating MN X N the observed and expected children were equal while in mating MN X M the children of the type M were less than expected.

In 2376 mothers and their 6071 children there was no exception found against the theory, moreover Schiff has calculated the expected frequencies of the children obtained from mothers of each type and by comparing these to those observed found that they are similar.

Therefore this theory was considered to be suitable as a basis on which these factors are inherited.

AGGLUTINOGEN P.

Landsteiner and Levine found another factor which they called P. This was detected by the following three sera:-

1. Normal human sera of different groups which can agglutinate different cells to various extents.
2. Normal animal sera like the horse, rabbit and pig, which were freed from the species agglutinin as well as those acting on A or B by absorption with cells lacking the factor P.

/169

3. Immune sera prepared by immunising rabbits with cells containing this factor and then removing the other agglutinins by absorption.

The reactions obtained by these sera were not similar in strength, since various degrees of agglutination were obtained with different cells. However these were independent of those acting on A, B, M or N. Landsteiner and Levine explained the difference in the strength of these reactions by assuming that this factor is composed of a group of several agglutinable substances mixed together in various quantities. Hence it was difficult to study this factor thoroughly, as was the case with M and N. Its distribution varied in the negros and the whites, since it was more commonly found in the former. The heredity of this factor was studied by these workers who found that it was inheritable like the other factors previously mentioned.

However, this factor has not been yet thoroughly studied since it occurs but seldom in the white populations and therefore it was difficult for other workers on the Continent to confirm the results.

Landsteiner, Stutton and Chase (103), by immunising rabbits with cells of group O which contain the factor P, could isolate from one of the sera an agglutinin which acted on certain P cells and not on the others and also on the cells of two bloods (P-).

There was no similarity between the reactions obtained with the anti-P sera and those obtained with that found by Ottenberg and Johnson.

Other individual differences were claimed to exist by several workers according to reactions obtained with normal human sera, but these have not been thoroughly studied specially in their relation to the minor agglutinins (a_1) and (a_2) found by Landsteiner and Levine, and (b_1) and

/170

(b_2) found by the writer.

Incidentally the writer gives here his studies on some normal human sera which are thought to be different from those previously defined.

1) SERUM OF A BLOOD OF SUB-GROUP A_1B_2(AABO).

This serum agglutinated all cells of group O, A_2, B_2 and B_1 and gave very weak reactions with the cells of one blood of sub-group A_2B_2 (ABOO). The reactions with O cells were stronger than those with A_2 and B_2 and those with B_1 were a little weaker.

When this serum was absorbed with O cells, it agglutinated only B_1 cells, and when absorbed with B_1 cells, it agglutinated O, A_2 and B_2 cells. Therefore one may conclude that this serum contains an anti-O and B agglutinins. This is quite reasonable since the cells contain only a small quantity of B and O agglutinogens and therefore their presence does not conflict with the presence of the two agglutinins which act on O and B agglutinogens when found in large quantities.

2) SERUM OF A BLOOD OF SUB-GROUP A_2B (1)

This serum agglutinated certain cells of each of the four groups and this reaction bore no relation to the blood being of A_1 or A_2 sub-groups, and therefore was thought by the writer to be similar to that of anti-P found by Landsteiner and Levine. The strength of the reactions varied with different cells even with those which belonged to the same group. It was active at a temperature of 25-30°C. Four rabbits and 2 goats were immunised with cells OM and OMN, both of which were agglutinated with this serum. No immune agglutinin could be isolated corres-

(1) The sub-group of the B was not determined since the two types, B_1 and B_2 had not been distinguished at that time.

ponding to that found in the serum.

After the discovery mentioned before that anti-O agglutinin may be present along with (b_1) agglutinin, the writer thought of re-examining this serum to find out whether it contained a weak fraction of (a), (b) and anti-O agglutinins beside each other, but unfortunately the person refused to give another sample of his blood. However this might possibly be obtained in the future.

The writer has discussed these observations in regard to the anti-P with Levine, who mentioned that there is no such possibility because some anti-P agglutinins were found in sera of all groups.

The factors G and H found by Schiff seem to be difficult to demonstrate, as he indicated to the writer during his visit to him.

The factor Q found by Imamura by the aid of pig serum and E found by Sugushita, have not yet been found by other workers and despite this, Furuhata suggests their application in legal cases. The writer has not made any effort to investigate these factors, because Japanese papers dealing with these matters did not come to his hand until after he finished his experiments.

With regard to the secretory and non-secretory types of Schiff, the writer has examined about 70 samples of saliva of persons of different groups. He never found a single case where the agglutinogen O, A or B were missing in the saliva when present in the blood cells. It is true that the saliva of certain persons contains larger quantities of the agglutinogens than the saliva of others, but a sharp differentiation between these two types was found impossible since there was gradual variation in the inhibiting power of the saliva on the corresponding sera.

/172

Moreover the quantity of the agglutinogen secreted in the saliva of one person was found to be variable from time to time. Hence it is difficult to draw definite conclusions from such results.

S E C T I O N VI

THE MEDICO-LEGAL APPLICATION OF BLOOD-GROUPING.

Shortly after the discovery of the four blood groups and their inheritability their application to certain medico-legal problems was suggested. It seems unnecessary to deal with the history of the test in this regard, since it has already been accepted by most courts as evidence in certain cases. The applicability of the test was acknowledged on account of the stability of the blood groups and their immunity from the influence of pathological phenomena.(1) The application of the test in solving medico-legal problems is based on the following fundamental observations related to the original four groups:-

(1) That the blood retains for a certain period of time its agglutinating and agglutinable properties whether in the blood of cadavers or in the form of blood-stains.

(2) That the last mentioned property is also found in certain organs and secretions of the body.

(3) That the agglutinable properties of blood are transmittable from the parents to their children according to Mendelian laws.

(1) See Section I, page /174

Further individual differences have been discovered during the last ten years. If these are definitely proved to be as applicable as those on which the system of the four groups was founded, the field of medico-legal application of the test will inevitably be greatly extended.

The object of the present research was to investigate the applicability of the original four blood-groups, their sub-groups, the M, and N factors and the inheritance[1] of blood-groups, in relation to their medico-legal value.

The three aspects of this subject will be discussed in the following order:-

I. The determination of the blood group of human blood stains.

II. The determination of the blood group of human secretions.

III. The determination of blood groups in relation to questions of maternity or paternity.

I. THE DETERMINATION OF THE BLOOD GROUP OF A BLOOD STAIN.

The principal question to be determined in regard to blood stains proved to be of a human nature, is whether the blood group of such a stain is similar to, or different from, the group of another stain or that of particular person.

The results of the test are accepted by Courts of Law as evidence. The following Continental cases have been described by Raestrup (104).

(1) Martin and Rochaix have reported a case in which blood stains of human origin were found in the room of a person accused of murder. The accused claimed that the blood originated from personal injury. The blood group of the accused, the victim and the blood stain on the

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(1) This is described in the previous five sections.

accused were determined when it was found that the group of the latter was similar to that of the murdered person, and different from that of the accused, who was convicted.

(2) Popoff reported that a woman was murdered. Two persons were accused. A knife bearing a blood stain was found in the possession of one of them which was suspected of having produced the wounds found on the murdered woman. The blood group of the stain was proved to be different from that of the woman, which suggested the innocence of the accused. Later the real murderers were arrested and they confessed.

(3) Muller-Hess reported a case of murder in which blood stains were found on the shoes and sleeves of an accused person. The two blood stains were found to belong to different groups. The former was similar to that of the victim and this fact played an important part in securing the conviction of the accused.

From these few examples, it can be clearly appreciated that the test has yielded evidence of value since the blood groups of the accused and the victims were different. When the blood of the victim and the suspect belong to the same group the test of course becomes valueless. The medico-legal application of the blood-grouping test is limited to certain stains, namely those which contain sufficient blood with which to perform the test and to those in which the properties of the blood have not been destroyed by external factors.

The method by which the blood group of a blood stain is determined differs from that used with fresh blood, since the presence of the agglutinins and agglutinogens in a dry form necessitates the application of certain methods for their detection. The various methods which may

be applied are given in order together with the various classifications of the groups and their sub-groups.

The Original Four Groups

In order to determine which of the four groups a blood stain belongs to, the dry blood is tested for the presence or absence of the agglutinins (a) and (b) and their respective agglutinogens.

The Detection of the Agglutinins (a) and (b).

Although the presence of these agglutinins is determined on the same principle as with fresh blood, it has been found necessary to adopt certain methods to meet the different circumstances associated with blood stains. The fact that many blood stains contain but small amounts of blood necessitates a certain modification of the test as applied with the fresh serum. This difficulty has been overcome by the utilisation of the technique used by Lattes (105).

A small drop of a weak suspension of the cells of each of groups O,A and B is put on a glass slide. A trace of the dried blood under examination is placed in the centre of each drop of stain extract. After 5-10 minutes, it is examined under the microscope for the presence or absence of agglutination. This method is considered to be useful only when the blood has been formed into crust. When the blood has soaked into the material on which the stain is present, Lattes and Canuto (106) suggest the preparation of an artificial crust by dissolving the blood in the smallest amount of distilled water, then dropping the fluid obtained on to a glass sheet and allowing the fluid to dry in a current of cold air. The crust thus prepared is examined as already described.

An alternative method for testing the agglutinating power of a dried blood stain is to dissolve the stain in distilled water equal

to four times its weight and then to test the resulting extract with known blood cells of groups O, A and B. Lattes recommends the addition of saline to facilitate extraction, which should be performed at 0°C in order to fix the auto-agglutinins and the test be made at a temperature of 25°. This method is only useful when the stain contains a sufficient quantity of blood.

If agglutinins (a) and (b) are present in the stain the cells of groups A and B will be agglutinated. If only (a) agglutinin is present, the cells of group A will be agglutinated when only (b) agglutinin is present, the cells of group B will be agglutinated.

The Detection of the Agglutinogens A and B.

As the blood cells lose their agglutinability after becoming dry, the agglutination test cannot be used for the detection of the agglutinogens A and B. The fact that the agglutinogens A and B resist drying and other deteriorating factors makes their demonstration possible by means other than the agglutination test. Therefore their presence in blood stains may be determined by the ability of these stains to absorb the agglutinins (a) and (b) or to inhibit the action of the sera containing these agglutinins to a certain extent.

The Absorption Method.

The ability of a blood stain to absorb the agglutinins can be demonstrated by allowing the dried blood to stand in contact with sera containing these agglutinins for some time at low temperature and then testing the serum with known cells of groups A and B.

By this method the agglutinogen content of a blood stain can be determined by the agglutinin which it can remove from the serum. If the stain contains agglutinogens A and B, both (a) and (b) agglutinins

/178

will be absorbed from the serum. If only agglutinogen A is present agglutinin (a) only will be absorbed and if only agglutinogen B is present, agglutinin (b) only will be absorbed.

The actual technique of the test depends on the state of the stain. When the blood is found in the form of crust, as in the case of hard objects, it can be scraped and powdered and then added to the serum. When the blood has soaked into the material, as in the case of fabrics, a piece of the blood stained fabric is soaked with the serum. In both cases the amount and titre of the serum must be adjusted to the weight of the blood in the stain so as to ensure complete absorption of the agglutinin, if the stain contains the corresponding agglutinogen. In order to test for the presence of both agglutinogens at the one time, and with the least quantity of stain extract, the use of group O serum (containing (a) and (b) agglutinins) has been suggested. But an objection to this method consists in the variation of the titres of the two agglutinins. Thomsen (11), Landsteiner and his assistants (12), and Harley(107), have also observed that the cells of groups A or B can absorb the heterolgous agglutinin to some extent. A mixture of equal volumes of anti-A and B sera of equal titres is used by some workers instead of group O. Other workers recommend that the absorption be carried out separately with each of the two sera.

According to Popoff (108) either the extract of the blood stain or the stained fabric moistened with saline should be heated for half-an-hour at 70°C-80°C, in order to inhibit the action of any agglutinin present. With regard to the relation between the quantity of the blood stain and that of the serum used, Schutze and Dolter (109) suggest that these should be equal in order to ensure that the absorption will

/179

be complete. Schiff (110) has suggested the use of diluted sera (1:5) so that the agglutinin contained in it may be more easily absorbed by the small qualtities of blood present in the stain. He also recommends that in order to prove that the agglutinin was absorbed, the stain should be heated to 55°C after the addition of a small amount of saline. If any agglutinin is absorbed it will be recovered in the saline.

The inhibition of the action of Anti-A and B sera by the extract of blood stains.

This method, recommended by Hirszfeld (111), is most useful when the blood is found in small traces and soaked into an absorbent material. In such cases the absorption method is not suitable, since it is difficult to obtain a sufficient quantity of blood from a small piece of fabric. In order to test stains of this character they are dissolved in the least amount of distilled water and the extract obtained is used in preparing an increasing dilution (1:2, 1:4 etc) of anti-A and B sera. Another set of dilutions of these sera prepared by the addition of saline are used as a control. The diluted sera thus prepared are tested with cells of groups A and B respectively.

If the stain does not contain agglutinogen A, the anti-A serum will agglutinate A cells at an equal titre whether diluted with saline or with the stain extract. If agglutinogen A is present in the extract, the portion of the serum diluted with the extract will agglutinate the cells at a much lower titre than the serum diluted with saline. The same holds with anti-B serum if the stain contains agglutinogen B. On account of the extract being turbid, the reading of the results is usually difficult. To overcome this difficulty Hirszfeld suggests that the agglutination test can be performed by centrifuging the tubes, in the case of the serum diluted

with the extract, the supernatant fluid is taken off and an equal amount of saline added to the cell sediment which, when shaken, will readily show the positive reaction if present. Hirszfeld considers that the blood stain contains a particular agglutinogen if the extract markedly inhibits the action of the serum on cells containing the same agglutinogen, i.e. if there is a difference of at least two tubes of successive dilutions between the titre of the stain diluted with saline and that diluted with stain extract.

In order to determine the group of a blood stain, some workers are inclined to depend on the test for agglutinins in the extract, while others, especially Hirszfeld, insist that the test for the agglutinogens should also be undertaken.

The former method is favoured owing to the small amounts of blood required for the test, while the latter is favoured for the fact that the agglutinogens are more stable than the agglutinins and that the results are more conclusive and reliable than with the former method.

PERSONAL INVESTIGATIONS.

In order to form an opinion on the value of testing for the agglutinins and agglutinogens, in addition to the various factors which might affect the application of the test in blood stains, the writer has tested 100 blood stains for both agglutinins and agglutinogens.

The blood group of all specimens was determined while the blood was fresh. The preparation of the stain was made by dropping a few drops of the blood before clotting on various objects, such as, sheets of glass, papers, wood and pieces of different fabrics of varying colour. The method used for testing the agglutinins varied according to the condition of the stain. When the stain was in the form of a

/181

crust, a piece of the crust was examined according to the technique devised by Lattes. When the stain was diffused, it was extracted and tested by preparing an artificial crust as suggested by Lattes and Canuto. In order to compare the results of the crust method with those obtained by testing the extract directly with the blood cells, this method was adopted in all cases when the crust method failed to demonstrate the presence of the agglutinins which were detected in the fresh blood. The cells of groups A and B used in the experiments were selected from the highly sensitive types A_1 and B_1

The absorption method was used for detecting the presence of agglutinogens. The blood powder was used except when the blood stain was diffused, as on certain fabrics, when a piece of the fabric densely soaked with blood was used in absorbing the serum. The powders or the stained fabrics were heated for 5 minutes in a dry oven at 100°C and then moistened with a few drops of distilled water and heated again for 10 minutes. This procedure was suggested by Lattes and it was found useful in rendering the blood insoluble so that when added to the serum, it remained more or less clear. The weight of the blood powder was estimated by the aid of the torsion balance, and in the case of diffused stains, it was roughly estimated by weighing a piece of the cloth containing the stain and an equal piece in size of the same fabric free from stain, the difference in the two weights representing the weight of the blood present in the stained fabric. For each 50 mmg. of the dried blood 0.2 c.c. of anti-A or B serum was absorbed separately after being diluted 4 or 5 times if the original titre was 128-256. The serum was separated by the centrifuge after 2 hours' standing at 0°C.

Anti-A serum was tested with the cells of group A and anti-B

serum was tested with the cells of group B. The tubes containing the absorbed serum and the cells were allowed to stand for 20 minutes, then centrifuged, the supernatant fluid removed, and substituted with saline; the tubes were then shaken and the results read by the naked eye.

The results obtained when the stains were from 3 months to 2 years old are given in Table No.65.

Table No.65.

Group of blood stain	Age of Stain when tested.	Number examined	No.showing presence of agglutinins			No.showing presence of agglutinogens		
			(a) only	(b) only	(a)&(b)	A only	B only	A & B
O	3 months	42	5	-	20	-	-	-
	6 months	20	4	-	8	-	-	-
	12 months	12	-	-	-	-	-	-
A	3 months	33	-	26	-	33	-	-
	6 months	26	-	12	-	26	-	-
	12 months	12	-	-	-	8	-	-
	2 years	8	-	-	-	3	-	-
B	3 months	16	12	-	-	-	16	-
	6 months	12	3	-	-	-	10	-
	12 months	3	-	-	-	-	2	-
AB	3 months	9	-	-	-	-	-	9
	6 months	6	-	-	-	-	1	4
	12 months	2	-	-	-	-	-	1

These results show that the agglutinins in the blood stains are less stable than the agglutinogens and that in certain cases of group O, one of the

/183

agglutinins may be detected and not the other. Also in stains of group AB one of the agglutinogens may be detected and the other not.

Therefore one cannot depend on the testing for the agglutinins alone or the agglutinogens alone, sinceone cannot tell if the presence of agglutinin (a) is due to the stain being of group B or of group O, the other agglutinin being destroyed. Similarly, if the agglutinogens B is present the stain may be of group B or of group AB, the agglutinogen A being destroyed.

Taking such possibilities into consideration, the determination of the blood group of a stain should only be carried out by testing for both the agglutinins and agglutinogens. Accordingly, the blood group can only be determined in the following cases as shown in Table No.66.

Table No.66.

AGGLUTININ CONTENT	AGGLUTINOGEN CONTENT	GROUP OF STAIN
(a) and (b)	Neither A nor B	O
(b) only	A only	A
(a) only	B only	B
neither (a) nor (b)	A and B	AB

The stains which were kept in dry form and not exposed to sunlight retained their agglutinins and agglutinogens for a longer period than those which were so exposed. A sample of the cloth free from bloodstains was used as a control in absorbing the serum when it was found that although the dye weakened the effect of the serum it was unable to absorb the agglutinin completely and no unspecific agglutination was produced. Table No.67 shows the effect of absorption of anti-A serum with cotton and silk fabrics dyed with different dyes. This does not tell much since

/184

one colour may be due to dyes of different chemical constitution.

Table No.67

Diluted anti-A Serum absorbed with fabrics.	Titration of the serum with A_1 cells						Undiluted serum tested with B cells
	1:1	1:2	1:4	1:8	1:16	1:32	
White (cotton)	+	+	+	±	tr	-	-
White (silk)	+	+	+	±	f.tr	-	-
Red (silk)	+	+	+	±	-	-	-
Green (silk)	+	+	+	±	tr	-	-
Light blue (cotton)	+	+	+	tr	-	-	-
Dark blue (cotton)	+	+	+	tr	-	-	-
Serum unabsorbed	+	+	+	±	tr	-	-
Serum absorbed with A cells	-	-	-	-	-	-	-

With regard to the different methods applied, the writer has found that the crust method devised by Lattes is as reliable in detecting the presence of the agglutinins as testing the extracted stain directly with blood cells. The former is more convenient when the blood stain is of a small size. The latter is preferable if the stain is of large size since with the former method confusion may result from the common occurrence of pseudo-agglutination. The writer did not have successful results in the cases he examined by artificial crust.

The absorption method was found to be efficient in most cases examined. The stains which showed the presence of the agglutinogens A and B as found in the fresh blood were examined by the inhibition test but these agglutinogens could not be detected.

/185

SOURCES OF MISTAKES IN THE BLOOD GROUPING OF STAINS.

Mistakes are more liable to occur in testing blood stains than with fresh blood. The sources of such mistakes and the measures which should be taken to avoid their occurrence are the following:-

(1) The presence of animal blood in the stain.

It is known that the blood of animals reacts in different ways towards human blood since species agglutinins are present in the serum of both. It has been found that certain animals' blood, for example sheep's blood, contains similar agglutinogen to that found in group A. Hence it is important, in the first instance, to prove that the blood stain under examination consists of human blood only.

(2) The presence of human secretions on the fabrics stained with blood.

It is an important fact that the agglutinins and agglutinogens are found in certain secretions, namely saliva, milk, tears, urine, sweat, etc. may produce mistakes in testing blood stains found on the clothes (especially on underwear). This, however, can be avoided by testing another piece of unstained cloth which will serve as a control. If the unstained piece gives the same reactions as that stained with blood, the results should be neglected.

(3) Presence of dyes.

Certain dyes (indigo-blue, as met with in the dye used by the native Egyptians in preparing their cotton cloth) may interfere with the reactions, but mistakes can be avoided by the employment of controls.

(4) Unspecific phenomena of agglutination.

(a) Pseudo-agglutination. This phenomenon is very common in testing blood stain for the presence of agglutinins. On account of the concentration of the serum in the crust test the red cells may show pseudo-

/186

Fig. 1
Real agglutination with B.cells.

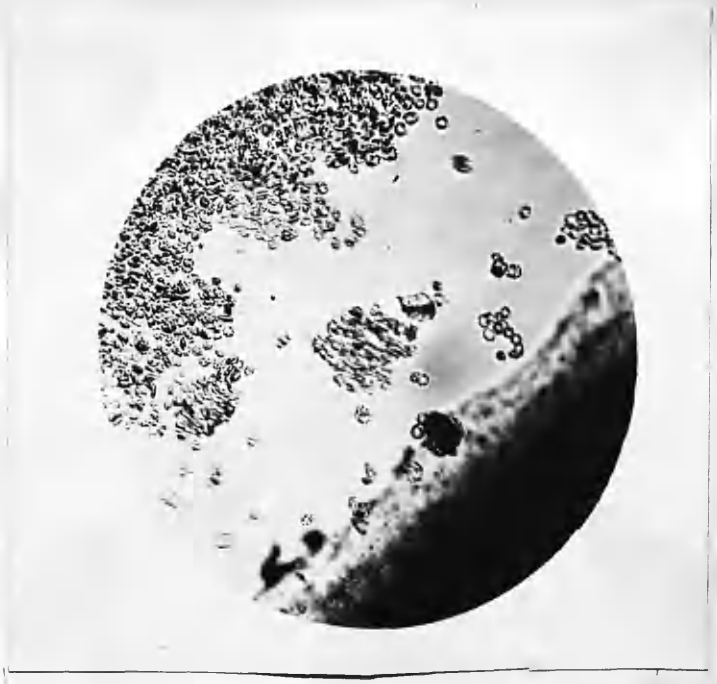

Fig. 2
Pseudo agglutination with A Cells

These micro - photographs show the effect of a dried blood stain of group A on the cells of group B, A and O respectively.

In the fresh state the serum agglutinated only B cells.

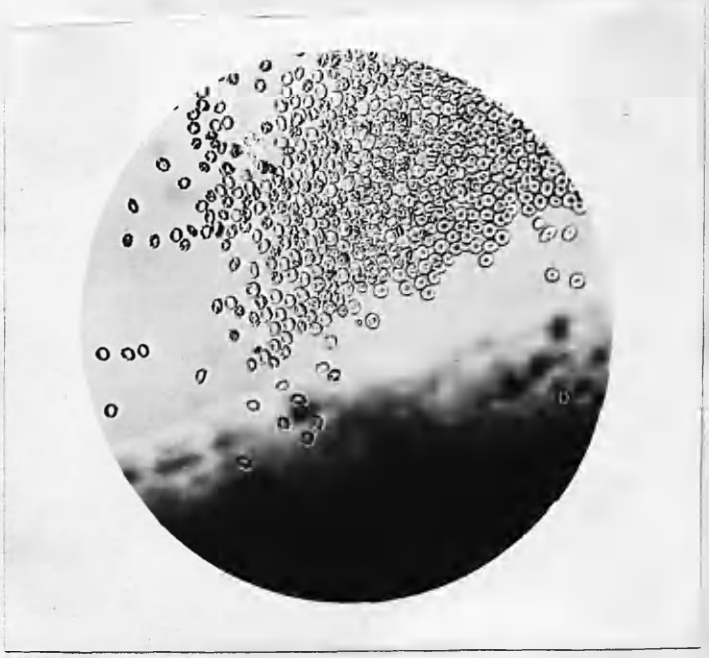

Fig. 3

Negative reaction with O cells.

N.B. See page 186.

agglutination and thus simulate proper agglutination. In the experiments carried out by the writer, it was possible to differentiate between pseudo-agglutination and proper agglutination by either moving the cover glass or lifting it up and then replacing it by which method the pseudo-agglutination disappears.

In one case, even after removing the cover slip, the clump persisted but at the same time some rouleaux appeared which indicated the presence of pseudo-agglutination. However it is impossible to assert that the presence of rouleaux formation indicates that the reaction is due to pseudo-agglutination since both pseudo and real agglutination may occur simultaneously. Usually the clumps of cells in pseudo-agglutination are smaller and less firm than those seen in real agglutination. In such cases it is advisable to neglect the results.

Figures 1, 2 and 3 show the actual micro-photographs of real agglutination, pseudo-agglutination and a negative reaction. The use of group O cells as a control is not of much value since pseudo-agglutination may occur only with A or B cells and not with O cells.

However, it is possible to evade drawing false conclusions if the stain is tested for the presence of agglutinogens, which affords a control for the results obtained.

(b) Reactions due to the presence of abnormal agglutinins. The auto-cold- and other abnormal agglutinins are very seldom met with in blood stain examinations, especially if the test is performed at a temperature higher than 25°C. If occasionally found, these reactions can be ruled out by comparing the results of testing for the agglutinin with those obtained by testing for the agglutinogens.

(c) Unspecific agglutination due to bacteria. Thomsen has demonstrated that bacteria which produce such unspecific reactions can be

detected in dried blood stains. One, however, should expect their presence more frequently in stains than in fresh blood on account of the liability to contamination being greater in the former instance. Usually precautions are taken against this contamination when testing for the agglutinogen by heating the stain to 100°C which destroys the bacteria.

THE DETERMINATION OF THE SUB-GROUP TO WHICH THE DRIED BLOOD BELONGS.

The sub-groups A_1, A_2, B_1, B_2, A_1B_1, A_2B_1 and A_2B_2 are differentiated by the aid of the agglutinins (a_1), (b_1) and (a_2) or (b_2). Such agglutinins are very weak compared to the (a) and (b) agglutinins and therefore could not be demonstrated by the writer in several blood stains composed of bloods containing them. In view of the fact that the difference between the agglutinogens A and B in these sub-groups is of a quantitative nature,(1) the absorption method is not reliable for differentiating them, on account of the difficulty in judging whether a weak reaction is due to original or acquired weakness of the agglutinogens found in the stain. Consequently the application of these sub-groups in blood stains is not considered practical.

THE APPLICATION OF THE NEW CLASSIFICATION OF SUB-GROUPS IN RELATION TO TESTING BLOOD STAINS.

The application of the thirteen sub-groups described by the writer in Section III is also limited in regard to blood stains since the difference is mostly quantitative. The only point of importance about these sub-groups is the possibility of detecting the presence of the agglutinogen O in certain types of blood cells. This observation should prove valuable in detecting the agglutinogen present in blood stains. If

(1) See Section II.

these are used in absorbing the immune anti-O serum,(1) the demonstration of the agglutinogen O in cells of group O affords a positive diagnosis of these cells. This method will increase the chances of determining whether the blood stain did not originate from a certain individual. This is shown in the following example: If a blood stain which belong to groups A, B or AB is proved to contain the agglutinogen O, while the blood of the accused who belongs to the same group as the stain, proves to lack this agglutinogen, one can decide that the blood stain has not originated from this individual.

The writer has examined fifteen blood stains, the group of which was determined in the fresh state according to the results obtained by testing the cells with anti-O, A and B sera. The blood stains were used in absorbing these three sera after a period of 6 weeks, according to the technique followed in the 100 stains previously mentioned. The results are given in Table No.68.

These results show that the application of the test for agglutinogen O is as reliable as that for the agglutinogens A and B; consequently its application may be used in differentiating the stain originating from heterozygous individuals and as a confirmatory test for the diagnosis of blood stains of group O.

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(1) See Section III.

Table No.68.

No.	FRESH BLOOD Group	Type	Agglutinogen present in the blood stain according to the effect of an extract in absorbing anti-O, anti-A and anti-B sera.
3	O	OOOO (1)	Only agglutinogen O
1	A_1	AAAO	A and O
1	A_1	AAOO	A and O
1	A_2	AOOO	A and O
1	B_1	BBBB	Only agglutinogen B
1	B_1	BBBO	B and O
1	B_1	BBOO	B and O
1	B_2	BOOO	B and O
1	A_1B_1	AABB	A and B
1	A_1B_2	AABO	O, A and B
1	A_2B_1	ABBO	
1	A_2B_2	ABOO	

(1) The blood of the type AAAA was not available at the time when these stains were prepared.

THE APPLICATION OF THE M, N AND MN TYPES.

Landsteiner and Levine (41) demonstrated the presence of the agglutinogens M and N in blood stains and hence their application was suggested. The determination of the type to which the stain belongs is based on the results of absorbing the anti-M and N sera with the dried blood.

PERSONAL INVESTIGATION.

Two years ago when the writer was working in the Medico-legal Institute in Pavia, he studied the question of the detection of the agglutinogen M and N in blood stains. Two blood stains of the type M and

/190

six of the type N were examined by absorbing the anti-M sera as prepared by Professor Lattes, the Director of this Institute. The method of absorption used was the same as described previously, with the exception that the period of absorption was one hour in this case.

The sera were absorbed with both stained and unstained cloth as well as powdered dried blood. The serum was titrated before and after absorption with its respective cells. The results are given in Table No.69.

Table No.69.

Anti-M and N sera absorbed with		Titre of anti-M serum tested with M cells				Titre of anti-N serum tested with N cells			
		1:1	1:2	1:4	1:8	1:1	1:2	1:4	1:8
Unabsorbed serum		+++	++	+	+	++	++	±	-
Unstained cloth		+++	++	+	tr	++	++	tr	-
M stain powder		±	-	-	-	++	+	tr	-
N stain powder		+++	++	+	tr	-	-	-	-
M stain on cloth	1	+	-	-	-	++	++	tr	-
M stain on cloth	2	tr	-	-	-	++	+	tr	-
N stain on cloth	3	+++	++	+	tr	+	-	-	-
N stain on cloth	4	+++	++	+	tr	±	-	-	-
N stain on cloth	5	+++	++	+	tr	tr	-	-	-
N stain on cloth	6	+++	++	+	tr	tr	-	-	-
N stain on cloth	7	+++	++	+	tr	tr	-	-	-
N stain on cloth	8	+++	++	+	tr	tr	-	-	-

These results show that the M and N agglutinogens could be demonstrated in the blood stains. In order to show that the agglutinin was absorbed the powdered stain used in absorption was washed with ice-cold saline, mixed with 0.2 c.c. of saline, heated for 5 minutes at 55°C and then

/191

sedimented by the centrifuge. Then the saline was separated and tested with M and N cells it gave the results shown in Table No.70.

Table No.70

FLUID SEPARATED FROM POWDERED STAIN	TESTED WITH CELLS M	N
M	tr	-
N	-	tr

Later some other stains representing the three types M, N and MN were tested with this method and it was possible to determine the type to which the blood stain belonged.

However one ought to be careful in utilising the test for the M and N agglutinogens for practical application before certain points are elucidated. These have been discussed in Section V, namely:-

1. That certain MN cells react very weakly with anti-N serum.
2. That certain M cells can absorb unspecifically the anti-N serum.

The absence of agglutinins corresponding to these agglutinogens in human blood serum renders the test more doubtful, since one cannot control the results obtained by testing the agglutinogen by those of the agglutinins as is the case with the agglutinogens A and B and their respective agglutinins. The main sources of mistakes here are that an MN stain may only absorb the anti-M serum and consequently will be considered as M and also that an M stain may absorb anti-N serum and consequently will be considered as MN.

THE DETERMINATION OF THE BLOOD GROUP OF HUMAN SECRETIONS.

The fact that the agglutinogens A and B and their respective agglutinins are found in the body tissues and secretions provided an

excellent method of the identity of human organs and secretions whether fresh or dry. The agglutinogens A and B are more useful in this respect since they are more commonly and constantly found outside the blood than the agglutinins.

The test for detecting these agglutinogens in human remains is performed in the same manner as with blood stains, namely, by the power of absorbing or inhibiting the action of the agglutinins (a) or (b).

The test has actually been applied in the identity of stains produced by saliva, urine, sweat or sminal stains. Schiff (112) has determined the group of saliva used for closing an envelope and Lattes (113) states that he has determined the group of traces of saliva found on the stumps of cigarettes.

PERSONAL INVESTIGATIONS.

The writer has studied the question of the presence of the agglutinogens A and B in the seminal fluid of seventy men of different groups. The method of inhibition was used in detecting the agglutinogen found in the seminal fluid. The technique was as follows:

Dilutions 1:2, 1:4, 1:8, 1:16 etc. were prepared from anti-A and anti-B sera using saline as a diluting factor. 0.1 c.c. of the dilution of each of anti-A and anti-B sera was added to 0.1 c.c. of the seminal fluid in a small tube. After leaving the tubes to stand for 2 hours at room temperature, they were centrifuged and the supernatant fluid was tested with cells of groups A and B respectively. An example of the results obtained with the seminal fluid of each group is given in Table No.71.

/193

Table No.71.

DILUTIONS OF ANTI-A Serum	Tested with A cells	0.1 c.c. of serum absorbed with 0.1 c.c. of seminal fluid of group. O	A	B	AB
1:2	++	++	tr	++	+
1:4	++	++	-	++	tr
1:8	++	++	-	++	-
1:16	+	+	-	+	-
1:32	±	±	-	±	-
DILUTIONS OF ANTI-B					
Serum	Tested with B cells				
1:2	++	++	++	-	tr
1:4	++	++	++	-	-
1:8	++	++	++	-	-
1:16	+	+	+	-	-
1:32	tr	tr	tr	-	-

These results show that the seminal fluid of group O has no inhibiting action on either serum. The seminal fluid of group A inhibits anti-A serum, the seminal fluid of group B inhibits anti-B serum, and the seminal fluid of group AB inhibits both anti-A and anti-B sera. This can only be explained by the presence of the agglutinogens A and B in the seminal fluid in the same order as in the blood cells.

Stains were prepared from the 70 samples of seminal fluid on various kinds of fabrics. After a period of two months the stains were examined by the absorption methods used in testing the blood stains. The

/194

results are given in Table No.72.

Table No.72.

No.of Specimens	Group of Blood	Agglutinogens found in the fresh state.	Agglutinogens found in the stain after a period of 2 months
26	O	Neither A nor B	Neither A nor B
29	A	Only A	Only A found in all specimens.
9	B	Only B	Only B found in all specimens.
6	AB	Both A and B	Both A and B found in all specimens.

These results show that the agglutinogens A and B are stable for a long time in the seminal stains and therefore this test may be applied in the identity of such stains found in cases of rape. It should, however, be proved before the determination of the group of a seminal stain that such a stain is actually due to seminal fluid by the ordinary chemical and microscopic methods. It is also advisable to determine the species nature of these stains, before an opinion is given.

The writer has examined several fresh samples of seminal fluid which belonged to the typed M, N and MN and could not trace the presence of the agglutinogen M and N. It was also impossible to differentiate between the samples which belonged to A_2 and A_1 individuals.

No attempt has been made to study the presence of the agglutinogen O in the seminal fluid.

THE DEMONSTRATION OF THE PRESENCE OF THE AGGLUTINOGEN IN THE SALIVA.

The agglutinogens found in the blood cells of the individual are usually secreted in the saliva. This, however, is not a constant property since in certain individuals the agglutinogens can hardly be demonstrated.

Thirty samples of saliva from individuals of groups A, B and AB were examined with anti-A and anti-B sera and all were found to contain the agglutinogen found in the blood cells. Two samples of group A and one and sample of group B showed the presence of small quantities of the respective agglutinogens. Stains were prepared from these samples on various fabrics and all the stains were tested after one month with the method applied with the blood stains. 10 stains out of 17 of group A showed the presence of agglutinogen A, 8 stains out of 9 of group B showed the presence of agglutinogen B and the four stains of group AB showed the presence of agglutinogen B, but in only three of them was agglutinogen A found.

The differentiation between the sub-groups A_1 and A_2 and B_1 and B_2 was not possible.

The presence of the agglutinogen O in saliva was studied in 18 fresh samples. Immune anti-A, B and O sera were diluted so as to agglutinate their corresponding cells at a titre of about 1:32. The diluted sera were absorbed with each sample of the fresh saliva by adding 0.1 c.c. of the saliva to 0.1 c.c. of the diluted serum in a tube. After 2 hours' standing at $0^{\circ}C$ the tubes were centrifuged to clear the fluid from any turbidity and then each serum was tested with its respective cells. As a control each of the three sera was diluted with an equal volume of saline and tested with the same cells. The results are given in Table No.73.

/196

Table No.73.

			Anti-O Serum + O cells	Anti-A Serum + A cells	Anti B Serum + B cells
0.1 c.c. of serum + 0.1 c.c. saline			++	++	++
0.1 c.c. serum + 0.1 c.c. saliva of a person of group					
No.					
1.	O	0000	-	++	++
2.	O	0000	-	++	++
3.	O	0000	-	++	++
4.	A_2	(AOOO)	-	tr	++
5.	A_2	(AOOO)	-	-	++
6.	A_1	(AAOO)	-	-	++
7.	A_1	(AAOO)	tr	-	++
8.	A_1	(AAOO)	-	-	++
9.	A_1	(AAOO)	tr	tr	++
10.	A_1	(AAAA)	++	-	++
11.	B_2	(BOOO)	-	++	-
12.	B_1	(BBOO)	±	++	±
13.	B_1	(BBOO)	-	++	-
14.	B_1	(BBOO)	tr	++	-
15.	B_1	(BBOO)	-	++	-
16.	B_1	(BBOO)	±	++	±
17.	B_1	(BBOO)	-	++	-
18.	A_2B_1	(AOBB)	±	-	-

These results show that the agglutinogen O can be demonstrated in saliva of the individuals of the four groups. It is interesting to note that

/197

the saliva of specimen No.10 which belongs to the type (AAAA) did not absorb the anti-O serum while the specimen No.18 (AOBB) which contained a small quantity of the O agglutinogen weakened the action of the serum to a marked extent.

Schiff (46) and Moureaux (114) and others have also demonstrated the presence of the agglutinogen O in the saliva of group O individuals. This indicates that the agglutinogen O is similar to the A and B in that it is found in the body secretions. Consequently one may look forward to its application in the identity of stains and human remains.

MEDICO-LEGAL APPLICATION OF THE BLOOD-GROUPING TEST IN PATERNITY QUESTIONS.

The fact that the agglutinogens found in the blood cells of the parents are inherited by their children according to certain definite Mendelian laws is utilised in solving many problems with regard to the relation between children and their reputed parents.

The main question to be solved by the test is whether a certain child is the offspring of a certain parent or parents, or vice versa. This can only be answered in a negative manner and only in certain cases, as when a child's blood is found to contain an agglutinogen which is not found in the blood of its parents, in which case the child cannot belong to these parents. On the other hand, if the agglutinogens found in the child's blood are present in one or both parents that does not mean that the child belongs to these parents, since other parents of the same group can give birth to a child belonging to a similar blood group.

The following examples show the different cases in which the tests of blood-grouping is applied:-

/198

(1) The question of illegitimacy of children.

When the husband doubts the legitimacy of the child, the test may prove valuable in proving that this husband is not the father.

(2) The question of disputed paternity.

If a woman claims that a certain man is the father of her child, the test may show that this man cannot be the father of the child.

(3) The question of disputed maternity.

When a woman claims that she has given birth to a child and this statement is doubted, the test may prove that she could not have given birth to such a child.

(4) The interchange of children.

When two children of two families are interchanged the test may prove valuable in deciding to which family each child belongs.

THE LAWS OF INHERITANCE OF THE ORIGINAL FOUR GROUPS.

The four theories, of Dungern and Hirszfeld, Bauer, Furuhata and Bernstein which are discussed in Section IV agree in that the agglutinogens A and B are Mendelian dominants and therefore do not appear in the child's blood except when present in at least one of the parents. The theories of Furuhata and Bernstein add another rule to the above-mentioned one, namely that on account of the presence of genes A and B in an individual of group AB, such individual cannot have a child of group O, since either A or B must be transmitted to the child. Also an AB child cannot come from an O parent, since each parent must supply either the A or B agglutinogen. The four theories have been discussed in detail in Sections III and IV from observations of the serological and statistical aspects. The conclusions of this study can be summed up as follows:

(1) The Dungern and Hirszfeld theory does not agree with the family and population statistics.

(2) The same holds for the Bauer theory.

(3) The Bernstein theory, and similarly that of Furuhata, agree with population statistics, but not definitely with the family statistics. Moreover, they do not afford an explanation of the presence of O children of group AB parents.

Consequently, one might assume that all these theories are unsuitable as bases for the inheritance of the four blood groups.

However, this does not invalidate certain facts based on family observations with regard to the dominance of the agglutinogens A and B. In about 9000 families with 19088 children examined by many workers, only 59 children showed the presence of either A or B agglutinogens in their blood when these were absent from both parents. This comparatively small number does not interfere with the assumption that these agglutinins are Mendelian dominants. In not a single case of these 59 could any proof be given that the child was actually the offspring of the parents in question.

One may account for the presence of such children by either faulty technique or illegitimacy. These results afford strong evidence for the justification of the application of this simple Mendelian law for the time being in the previously mentioned question of illegitimacy, disputed paternity and interchange of children.

According to the simple Mendelian law as stated above, the children possible and impossible to be obtained from each mating are given in Table No.74.

/200

Table No.74.

MATING	POSSIBLE CHILDREN	IMPOSSIBLE CHILDREN
O X O	O	A, B and AB
O X A A X A	O or A O or A	B and AB B and AB
O X B B X B	O or B O or B	A and AB A and AB
AB X O) AB X A) AB X B) AB X AB)	O, A, B or AB	None

In order to test the manner in which this system of inheritance operates in solving the above-mentioned question, each question will be discussed separately in the following way:-

(1) Whether a child is illegitimate or not.

This question is solved by the aid of the data given in Table No.74. Whenever the group of the child is similar to the impossible children, it is considered to be illegitimate. If it is similar to the group of possible children, it cannot be decided whether it be legitimate or not.

(2) Interchange of children.

This is solved on the same basis as given in Table No.74. For example, if one family is OXA and another is OXB and one of the children claimed to be interchanged belongs to group A and the other belongs to group B, one can decide that the first child cannot belong to the second family but may belong to the first, similarly the second child cannot belong to the first family but may belong to the second.

/201

(3) Disputed paternity or maternity.

If the group of one parent and that of the child are known, it can be decided in certain cases whether the alleged other parent is the genuine one or not. Table No.75 shows that possible group of one parent if that of the child and the other parent are known.

Table No.75.

Group of One Parent	THE POSSIBLE GROUP OF THE OTHER PARENT WHEN			
	Child of Gr.O	Child of Gr.A	Child of Gr.B	Child of Gr.AB
O	any group	A or AB	B or AB	AB
A	any group	any group	B or AB	B or AB
B	any group	A or AB	any group	A or AB
AB	any group			

This law corresponds to the only rule of the Dungern and Hirszfeld theory and to the first rule of Bernstein's theory, since both theories are based on the assumption that the agglutinogens A and B are inherited as Mendelian dominants. Consequently the chances of exclusion according to this law are less than those obtained by applying the whole Bernstein theory, since according to the latter, still further exclusions can be made on account of the second rule, namely, an AB parent cannot give birth to an O child or vice versa. Certain authorities on the subject, such as Landsteiner and Hirszfeld and others (1) do not accept the additional rule of Bernstein as definite evidence of illegitimacy, but consider it as highly probable. However, the defence can always deny this probability on the basis of the fact that it has been definitely proved at least in one case (2)

(1) See Section IV, page 113
(2) The Haselhorst Case.

that a mother of group AB has given birth to a child of group O. Certainly there is no one who can deny that a father of group AB may similarly give rise to a child of group O since that has happened, though rarely. Consequently precedents rendered this rule inapplicable from the legal standpoint just as it fails from the scientific point of view.

THE APPLICATION OF THE INHERITANCE OF THE SUB-GROUPS OF GROUPS A, B, and AB.

Thomsen's theory which is suggested as a basis for the inheritance of A sub-groups has been criticised in Section IV and on account of family observations as unsuitable for the following reasons:

(1) A mother of sub-group A_1 with a genotype A_1O was found to give birth to a child of A_2 and another of A_2B, the second of which cannot be explained by illegitimacy.

(2) A mother of sub-group B_1 gave birth to two children of group O, hence her genotype is B_1, and she has also given birth to a child of sub-group A_1B_2 which cannot be explained by illegitimacy.

These exceptions, as has been previously shown, indicate clearly that the difference between A_1 and A_2 and similarly between B_1 and B_2 is not qualitative, as assumed by Thomsen, but quantitative. Consequently the theory should be considered unsuitable for explaining the inheritance of these sub-groups.

THE APPLICATION OF THE INHERITANCE OF THE SUB-GROUPS ACCORDING TO THE NEW HYPOTHESIS.

A new hypothesis has been suggested by the writer to explain the classification of the four groups into 13 sub-groups as well as the inheritance of the agglutinogens O, A and B. This hypothesis has been studied from the serological and heredity aspects and has been found to explain the results so far obtained by the writer It has also explained

/203

certain points which could not be explained by Bernstein's or Thomsen's theories, as has already been mentioned in Sections III and IV.

The genetic bases of this hypothesis are as follows:-

(1) The inheritance of the blood groups depends on three Mendelian dominant factors, namely: the agglutinogens O, A and B.

(2) The genotype of a person is composed of four genes which may represent one, two or three agglutinogens.

(3) Two of these genes are transmitted from each parent to the child.

Accordingly the genotypes of the different individuals in each group will be as given in Table No.76.

Table No.76.

GROUP	GENOTYPES			
O	OOOO			
A	AAAA,	AAAO,	AAOO,	AOOO
B	BBBB,	BBBO,	BBOO,	BOOO
AB	AABB AAAB ABBB	AABO ABBO	ABOO	

Although it is yet too early to discuss the applicability of this hypothesis from the practical point of view, it is opportune to suggest its usefulness in the event of its validity being subsequently established.

In the first place it should be mentioned that according to this hypothesis the agglutinogens O, A and B cannot appear in the child's blood except when present in at least one of the parents, and therefore the exclusions shown in Table No.77 can be brought forward.

/204

Table No.77

GROUP OF PARENTS	CHILDREN CANNOT HAVE THE AGGLUTINOGEN INDICATED BELOW IN ANY QUANTITY WHATEVER.
0000 x 0000	A or B
AAAA X AAAA	0 or B
BBBB X BBBB	0 or A
AAAA x BBBB or AABB	0
AABB X AABB	0

Moreover, according to this hypothesis two genes are transmitted from each parent to the child. Consequently, if the group of the parents is known, the group of their children can be determined; a few examples showing this are given in Table No.78.

Table No.78

GROUP OF PARENTS	POSSIBLE GROUPS OF CHILDREN
0000 X 0000	0000
0000 X AAAA	AA00
0000 X AAA0	AA00 or A000
0000 X AA00	AA00, A000 or 0000
0000 X A000	A000 or 0000

If the group of one parent and that of the child are known the group of the other parent can also be determined as shown in Table No.79.

Table No.79.

GROUP OF ONE PARENT	GROUP OF CHILD	THE SECOND PARENT MAY BELONG TO GROUP
0000	0000	0000 AA00 A000 BB00 B000 AB00
0000	AA00	AAAA AAA0 AA00 AABB AAB0

If the second presumptive parent was found to belong to a group other than those given in the Table, that individual can be excluded as a parent of the child in question.

Further exclusion can be made in certain cases when the group of one parent and that of the child are known. This depends on the fact that two of the child's genes must be traced in each parent. The exclusions made on this assumption are given in Table No.80.

Table No.80

WHEN ONE OF THE PARENTS IS OF THE TYPE	CHILDREN CANNOT BE OF THE TYPES WHATEVER THE OTHER PARENT BE.
AOOO	AAAA BBBO or BBBB
BOOO	BBBB AAAO or AAAA
ABBO	AAAA
AABO	BBBB
AAAA or AAAO BBBB or BBBO AABB, AABO or ABBO	OOOO

The chances of exclusion according to this hypothesis appear to be greater than on the Bernstein theory, hence its application should prove more valuable if it is found suitable from the genetic and statistical standpoints.

THE APPLICATION OF THE INHERITANCE OF THE AGGLUTINOGENS M AND N.

The inheritance of the agglutinogens M and N has actually been applied in a certain number of cases of questionable paternity (vide Wiener) The medico-legal application of these agglutinogens is based on the theory of a single pair of allelomorphic genes previously discussed in Section V.

/206

According to this theory the following two rules should hold:-

(1) Non-parentage is proved if the child's blood shows the presence of the agglutinogen M or N and if this agglutinogen is not present in at least one of the child's parents.

(2) If one of the parents is M and the child N, or, if one parent is N and the child M, non-parentage is proved.

According to this theory the chance of exclusions in parentage questions should be increased, since it is possible that an illegitimate child cannot be detected according to the rule governing the inheritance of the agglutinogens A and B, but discrepancy can be shown by the test for the N and M agglutinogens. This is shown in the following example:-

In a mating A X A, if the child belongs to group A its legitimacy cannot be doubted on the basis of the test. But, if the two parents belong to the type M and the child belongs to the type MN its illegitimacy is proved on the part of one parent. If the child belongs to the type N, non-paternity can be established on the part of both parents.

The writer has not found any exceptions to these rules which could not be explained by illegitimacy. However the possibility of the existence of another type of weak N named N_2 has been mentioned by Friedenreich. This type was actually noticed by the writer (see Section V) and could only be demonstrated by the aid of strong anti-N serum.

The question might arise then if an M parent can give birth to a child of the type N, since this parent might belong to the type MN_2 and the N_2 could not be diagnosed because of the weakness of the element. Such a possibility naturally makes one hesitate in putting this theory into practice in connection with such serious questions of domestic relationships. Further research is necessary before a categorical opinion can be expressed.

C O N C L U S I O N S

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As the result of the experimental investigations which the writer has undertaken, the following conclusions have been reached:-

(1) That the confirmation of the serological bases on which human blood is differentiated into four main groups has been established.

(2) That proof has been obtained of the existence within group A of the sub-groups A_2 and A_1 and within group AB of sub-groups A_2B and A_1B. To these the writer has been able to add the sub-groups B_2, B_1, A_1B_1, A_1B_2, A_2B_1 and A_2B_2.

(3) That the nature of the difference which exists between the sub-groups of group A is quantitative, that is, the agglutinogen A is present in the respective cells in greater or less amount; the same applies to agglutinogen B, and to both agglutinogens in group AB.

(4) That Schiff's observation regarding the existence of the agglutinogen O has been confirmed and, further, that not only is it positive in character, but that it takes an equal part with the agglutinogens A and B in the foundation of the system of the four blood groups. Its quantitative variation in the cells of the various sub-groups has been utilised along with the distribution of A and B agglutinogens for the formulation of a new classification of 13 sub-

groups. Moreover, its presence in the cells of certain individuals belonging to group AB has been proved.

(5) That the results of actual observations while being readily explicable on the basis of the 13 sub-groups cannot be fully explained by the application of any of the four theories of heredity advanced by Dungern and Hirszfeld, Bauer, Furuhata and Bernstein.

(6) That the new hypothesis to explain the results has been based on the view that the agglutinogens O, A and B are Mendelian dominants and that the genotype of each individual is composed of four genes representing one, two or three agglutinogens, two of which are transmitted from each parent to the child.

(7) That a critical study of the theories of the heredity of the blood groups, suggested by Dungern and Hirszfeld, Bauer, Furuhata and Bernstein, has indicated that these theories are unsuitable as a basis for the inheritance of the blood properties, since they fail to agree either with the population or the family statistics and observations or with both.

(8) That the new hypothesis has up to the present, been tested only by family observations of the blood types of 99 families.

(9) That the study of the agglutinogens M and N has shown the existence of three blood types M, N and MN, to one of which all individuals belong. The study of the inheritance of these agglutinogens has indicated that they are inheritable independently of the agglutinogens O, A and B. The assumption of Friedenreich regarding the presence of a weak type of agglutinogen

N is considered to be probable on account of the results obtained by the writer.

(10) That the study of the medico-legal aspects of the blood-grouping test has shown that the test can be applied with safety in the solution of the question of the identity of blood stains in addition to stains from human secretion such as seminal fluid, saliva etc. This application of the test is based on the presence or absence in the stains of the agglutinogens A and B and their respective agglutinins (a) and (b). The test is also of much value in cases of disputed paternity. But the latter application should be restricted, in the meantime, to exclusions based on the simple Mendelian law regarding the dominance of the agglutinogens A and B, i.e. a child cannot possess an A or B unless at least one parent possesses the same agglutinogen. The application of sub-grouping, based on the assumption of the existence of two independent agglutinogens A_1 and A_2, appears impracticable since it has been shown by the writer that these agglutinogens are similar and only differ quantitatively.

The application of the new hypothesis will offer greater chances of exclusion than the other theories previously advanced. This, however, is not yet thought to be suitable for practical application in medico-legal work until corroborated by further research on a larger number of families.

The application of the agglutinogens M and N in blood tests, although likely to prove of value, should await further study

in order to clear up certain points in regard to the relation existing between the M and N agglutinogens and also the N_2 agglutinogen.

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B I B L I O G R A P H Y

References have been made only to the original works concerned with the problems studied in this thesis which have been personally read by the writer. In certain cases, however, reference has been made to text books by the workers whose opinions are discussed.
The text books thus utilised and which also contain a complete list of references on this subject, are the following:-

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