SOME OBSERVATIONS ON BLOOD GROUPING.*

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WHEN a person has lost blood and shows symptoms that he has not enough left to carry on his circulation efficiently, it would seem a wise procedure to attempt to give him some in place of that lost. In practice, however, this for long proved very disappointing in many cases on account of accidents which were often so dangerous that it was almost entirely given up.

Some thirty years ago, the cause of many of the accidents was discovered, and means were devised of preventing them to a great extent. The great number of lives that were saved during the War by transfusion brought it into prominence as a therapeutic procedure of great value. Yet, in spite of the increase in our knowledge of the various factors involved and of the vast amount of experience gained, both of the preliminary testing and the actual technique of the operation, its wonderful efficiency is still marred every now and then by catastrophes. Short of catastrophes, accidents still occur which are due to minor reactions hitherto neglected in our present practice.

Foremost among the causes of these is the phenomenon of agglutination. Often the blood of one person will not mix properly with that of another, either when transfused in this way, or even when mixed in a test tube. In such cases certain changes occur resulting in many of the corpuscles adhering to others and forming little masses which can generally be seen by the naked eye or with the slight magnification obtained by using a microscope eye-piece, but are always quite evident under a microscope. This clumping of the red blood cells is due to a reaction between the plasma of one person and the cells of the other, and when it happens it not only prevents any benefit coming to the recipient from the transfusion but often is harmful to him and may be the cause of his death.

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Some Observations on Blood Grouping

The importance of the problem attracted the attention of many investigators, and it was found that persons could be divided into groups in respect of their blood reactions. Of various systems of grouping suggested, that introduced by Moss [1] is used almost exclusively in this country.

TABLE I.

Donors.	Recipients.				Constituents.	
Group No.	I.	II.	III.	IV.	S.	C.
I.	o	+	+	+	o	AB
II.	o	o	+	+	α	B
III.	o	+	o	+	β	A
IV.	o	o	o	o	$\alpha\beta$	o

The four groups according to Moss—

α and β = Agglutinins in sera. S = Serum.
A and B = Agglutinogens in cells. C = Corpuscles.

According to this system, persons in Group I. can receive blood with impunity from all and sundry, but can give blood only to persons of Group I. Those in Group II. can receive blood from those of Group II. and Group IV. and can give blood only to those of Group I. and Group II. Persons in Group III. can receive blood from Group III. and Group IV. individuals and can give to those in Group I. or Group III. Persons in Group IV. can receive from Group IV. only and can give to all and sundry, hence these persons are called "universal donors."

To ascertain the group of any blood in this way, we proceed as follows:—A drop of standard or stock serum of a known

TABLE II.

Method of Grouping.

II.	III.	Group.
+	+	= I.
o	+	= II.
+	o	= III.
o	o	= IV.

Group II. is put at one end of a slide, and a drop from a known Group III. at the other end. With each of these, a drop of the patient's blood is mixed either undiluted, or diluted down

even to 2 per cent., and the reaction is allowed ten, fifteen, or thirty minutes to take place, and according as there is clumping in neither, either, or both, so the grouping is determined as shown in Table II.

Moss Grouping Unreliable.—This has proved a fairly workable scheme, but often, and particularly when using Group IV. as a "universal donor," it has led to trouble and even disaster. It has other serious fallacies. For instance the blood of two persons of the same Moss group may prove incompatible in a transfusion, a truly anomalous state of affairs. Again, when a person of a different group from the recipient has been used as a donor, even though according to the table they should be compatible, there may be an agglutination reaction. The theoretical reasons underlying these discrepancies will be seen later in this paper, and let it suffice meantime to state that the present régime of four groups is so fallacious that it is too dangerous a guide in practice.

Personal Investigation.—Testing blood by present methods is often inconclusive, for in not a few tests it is impossible to be certain whether agglutination has taken place or not. This fact led to an investigation in the course of which some 30,000 agglutination tests were performed. In the first half, we used corpuscles from three persons chiefly, though on occasions others were also employed. Sera were obtained mainly from the Royal Infirmary, and I have to thank Mr Lees and Dr Logan and other members of the staff for facilities and co-operation. and also Dr Goodall for inspiring the work and for numerous suggestions and criticisms.

At the outset, we tested the three persons, Owen, Brown, and Kerr in the orthodox way, as in Table II., with two tubes each of Group II. and Group III. sera obtained at the same time under the label of the same manufacturing chemist. We were surprised to find that one of the Group II. sera would always agglutinate Kerr cells strongly in one hour, whereas the other Group II. serum would not do so even in twelve hours. This can be seen in Table III.

The explanation of this was our first problem. The solution we obtained corroborates certain recent work [6] in this field which has thrown a new light on the whole subject of blood grouping and compatibility.

Bacteriologists tell us that agglutination of this nature depends on the interaction of substances called agglutinins

(in the serum) and corresponding substances agglutinable substances or agglutinogens (in the corpuscles). Also, we are assured that the reactions are specific for each pair.

TABLE III.

The grouping of Owen, Brown, and Kerr Cells.

Test No. / Sera.		1. II.	2. II.	3. III.	4. III.	Moss. Grouping.
Cells	Owen . .	+++	+++	o	o	III.
	Brown . .	o	o	+++	+++	II.
	Kerr . .	++	o	o	o	III. or IV.

To account for the four blood groups in this way it was assumed that two such pairs of agglutination elements existed and were distributed among the groups as in Table IV., the Greek letters α and β designating the agglutinins in the sera, and the Roman letters A and B the agglutinable substances in the cells.[2] Many other nomenclatures will be found in the literature.[1, 3, 4, 5, 6, 7] In fact their number is legion, causing great confusion (see Table IV.), and it is frequently impossible to correlate any one writer with others without extensive translation of terms.

TABLE IV.

Blood grouping according to various investigators.

Landsteiner, 1901.	De Castello, 1902.	Jansky, 1907.	Moss, 1909.	Moss. Early.		Moss. Later.		Von Dungern and Hirschfeldt.		Guthrie and Huck.	
Group.	No.	No.	No.	S.	C.	S.	C.	S.	C.	S.	C.
...	D	4	I.	o	a	o	abc	o	AB	o	ab
B	B	2	II.	AC	b	A	bc	α	B	A	b
A	A	3	III.	AB	c	B	ac	β	A	B	a
C	C	1	IV.	ABC	o	C	o	$\alpha\beta$	o	AB	o

S = Serum. C = Cells.

In following up our initial problem, we found that the crux of the matter was in the fact that there are more than these

two pairs of agglutination elements. Here is a composite table from our results, which indicates the presence of a third pair.

TABLE V.

Evidence of three pairs of agglutination elements.

		Sera.							
Test No.		1.	2.	3.	4.	5.	6.	7.	8.
Serial No.		784.	765.	785.	1304.	810.	1022.	1069.	1062.
Agglutinins.		α	β	$\alpha\beta$	$\alpha\gamma$	$\beta\gamma$	0	$\alpha\beta\gamma$	...
Cells	Owen .	+ + +	−	+ + +	+ + +	? +	−	+ + +	−
	Brown .	−	+ + +	+ + +	+	+ + +	−	+ + +	+ + +
	Kerr .	−	−	−	+ + +	+ +	−	+ + +	+

From the mass of our work we have conclusive proof of the existence of these three pairs, and also evidence of the existence of at least two other pairs. Now with three pairs there are possible biologically twenty-seven combinations giving twenty-seven groups.[6]

TABLE VI.

Combinations biologically possible with three pairs.

	S.	C.		S.	C.		S.	C.
1	0	A	10	α	C	19	0	ABC
2	β	A	11	β	C	20	0	0
3	γ	A	12	$\alpha\beta$	C	21	α	0
4	$\beta\gamma$	A	13	0	AB	22	β	0
5	0	B	14	γ	AB	23	γ	0
6	α	B	15	0	AC	24	$\alpha\beta$	0
7	γ	B	16	β	AC	25	$\alpha\gamma$	0
8	$\alpha\gamma$	B	17	0	BC	26	$\beta\gamma$	0
9	0	C	18	α	BC	27	$\alpha\beta\gamma$	0

S = Serum. C = Cells. (After Guthrie and Huck.)

Of these, we have identified fourteen in our work and we found reference to several others in the literature.[6] If six pairs are proved to exist there would likewise be possible over seven hundred groups. If all variations in each partner are taken into account the number of "groups" (if they can be called groups) may be for practical purposes infinite and at any rate

large enough to rule out grouping as ordinarily conceived. In practice, the whole question is one of the relative agglutination potentialities of the blood of two persons when mixed in a transfusion and the means of predetermining this. There are other reasons also why the present system of grouping should be cast aside. It has been pointed out already that the Moss grouping is a fallacious and dangerous guide and, in the light of later knowledge, the underlying reasons of this can be explained.

To begin with, in testing the donor's and recipient's blood prior to a transfusion as in Table II., we are apt to forget that we are only testing the cells of each person. It is taken for granted that the sera will contain agglutinins in accordance with the group to which its cells belong. Now in a transfusion, plasma is given as well as cells and it is often overlooked that the donor's plasma may react powerfully with the recipient's corpuscles. The degree of dilution of the donor's blood in the recipient's vessels after an average transfusion is round about one in ten,[8] and very often in doing tests we see examples of an agglutinin clumping cells in dilutions much above this. If then blood of a Group II. person (as on Table I.) is given to a Group I. person, it is quite possible that the serum of the former can agglutinate the cells of the latter so that whereas Table I. shows them quite compatible, they are in many cases really incompatible. Likewise also it can be shown that the serum in Group III. blood can agglutinate Group I. cells as also can that of Group IV. blood.

TABLE VII.

Person.	Constituents.		II.	III.	Group.
No. 18	α	BC	o	+	= II.
No. 8	$\alpha\gamma$	B	o	+	= II.

Again, take the case of two persons, say No. 18 and No. 8 in Table VI. Their sera contain respectively α and $\alpha\gamma$ and their cells BC and B. In the ordinary way they would be grouped in Group II. (Table I.). If one were donor, however, and the other recipient in a transfusion, there would likely be an agglutination reaction from the reactions of γ and C. Thus

we have two persons in the same group incompatible with each other.

Next let us examine the result which first attracted our attention in the investigation. One Group II. serum agglutinated Kerr corpuscles which when grouped in the ordinary way belonged to Group IV. The other Group II. serum would not agglutinate Kerr cells. In the first place, we see the Group II. sera are different, and in the second place cells in Group IV. Moss can contain an agglutinable substance other than A or B. Kerr would be a dangerous "universal donor" for this reason.

Thus the Moss system or any other four-group system does not contain the whole truth, and in many cases proves in practice a dangerous guide.

The Time Factor.—This has hitherto been largely overlooked or ignored in blood grouping, and neglect of it has led to fatalities. It is generally recommended to allow five to thirty minutes for agglutination to take place, and this we believe is far too short.

TABLE VIII.

Time Factor.

Minutes allowed for Test.		Dilutions of Sera.					
	1 in:	1	2	4	8	16
10	O	+++	+++	+++	++	+
	B	−	−	−	−	−
	K	+	?+	−	−	−
20	O	+++	+++	+++	+++	++
	B	+	−	−	−	−
	K	++	+	?+	−	−
40	O	+++	+++	+++	+++	+++
	B	++	+	−	−	−
	K	++	+	?+	−	−
80	O	+++	+++	+++	+++	+++
	B	+++	++	?+	−	−
	K	++	+	?+	−	−

When one partner of a pair is weak, it may take up to two hours or more to react with the other to cause clumping. The rate of the reaction does not always vary directly with the completeness of the end result, for in two tests done side by side under identical conditions one reaction may proceed very slowly, but eventually become more complete than another which proceeded much more quickly but stopped at

only moderate agglutination. This can be seen in Table VIII. which also gives the clue to the explanation. The γ in the first serum agglutinates Kerr cells at once but only moderately strongly; the weak β on the other hand agglutinates Brown's cells more slowly but nevertheless completely in time with the help of the reaction of the γ in the serum with the C in his cells.

The Age Factor.—Another point which emerged in our work was the variation with age of the strength of the agglutinogens in cells and the agglutinins in serum. In Table IX. can be seen the protocol of an experiment which shows this very well.

TABLE IX.

To show the effect of age on agglutination elements.
Time—30 minutes.

Dilution of Serum.	1 in:	1	2	4	8	16	32	64	128
5 days old	O	+++	+++	+++	++	+	?	–	–
	B	–	..	–	–	–	...	...	...
	K	+	?	–	–	–	...	...	...
3 days old	O	+++	+++	+++	+++	++	?+	–	–
	B	+	?	–	–	...	...	...	...
	K	++	?+	–	–	...	...	...	...
2 days old	O	+++	+++	+++	+++	+++	+	–	–
	B	++	+	–	–	...	...	...	...
	K	+++	?+	–	–	...	...	...	...
1 day old	O	+++	+++	+++	+++	+++	+	+	–
	B	++	+	–	–	...	...	...	...
	K	+++	+	–	–	...	...	...	...
Fresh	O	+++	+++	+++	+++	+++	+++	++	?+
	B	+++	+	?+	–	–	–	–	–
	K	+++	+	?	–	–	–	–	
Fresh Cells, serum 1 week old	O	+++	+++	+++	+++	+++	+	+	–
	B	++	+	–	–	–	–	...	...
	K	++	+	–	–	–	–	...	...

In it the cells Owen, Brown, and Kerr were used in suspensions of 2 per cent. varying in age from fresh to five days old, and in the lowest section is the result of a similar experiment using fresh cells and stale serum. The weakening in both the agglutinins and in the agglutinogens can be seen. Many workers deny this,[10, 11] and we believe some of their results have been vitiated thereby. It is possibly due to this that the weaker agglutinins remained so long undiscovered. Besides this, it had the effect of strengthening belief in the adequacy of the four-group theory.

The Dilution Factor.—We also investigated the importance of the strength of the suspension of cells and of the proportion of serum to cells used in a test. Normally in a transfusion, the donor's cells are present in the recipient's blood in a strength of about one in seven, *i.e.* 15 per cent. Many workers use this strength for their cell suspensions in doing tests, preferring to make a dilute suspension rather than to use a drop of whole blood. We found suspensions stronger than 10 per cent. gave results which in weakly positive specimens were exceedingly difficult to read and mostly unreadable on account of rouleaux formation, regular and irregular. Between this and 2 per cent. the difficulty diminished gradually, but the time required for completion of the reaction was longer. We used 2 per cent. suspensions and found this strength satisfactory when using drop of serum for drop of suspension. With regard to the sera it is better to use two or three drops in each test to prevent diluting, for the minor agglutinins do not stand diluting well. In these cases stronger suspensions of cells are an advantage up to 15 per cent., and we think this gave better results than those obtained by using drop for drop.

Effect of Temperature.—We were able to corroborate some of the results obtained by Guthrie and Huck in regard to the optimum temperature of the reaction. We found that the reactions mostly proceeded more quickly at lower temperature than 37° C. Especially is this so with γ and δ (and their corresponding agglutinogens) which sometimes will show a strong positive reaction in the ice-chest at a few degrees above zero, whereas the result is negative at 37° C. in one hour. As a compromise we did our tests at room temperature round about 15° C. on account of the ease of doing them at this temperature and in order to get results comparable in this respect.

Some Practical Suggestions.

In order to predetermine the compatibility of the blood of a prospective donor and recipient, the recipient's blood should be tested for its agglutination elements by using strong fresh standard or stock sera and fresh newly drawn cells of known constituents. It is imperative to use sera containing one agglutinin each. In our present state of knowledge there

would be α, β, γ. It is almost impossible to obtain δ alone. Corpuscles possessing agglutinogens A, B and C should be used and should be fresh. D is seldom found alone. These tests would give a pretty fair idea of the kinds of blood which might be compatible with the recipient's. If strong reagents are available half an hour would show this. Donors having a probable compatible blood should then be called up and specimens of their blood cross-matched with the recipient's, giving as long a time as possible, in this case at least two hours—and taking precautions to keep the preparations free from evaporation. This is best done by using hanging drop preparatious on a vaselined rubber ring, and keeping them in an ice-chest at 0° C. with frequent vigorous tapping. It can be done, however, quite well at room temperature. A simple short method of doing this test is to take about seven large drops of blood from the recipient and put them in the hollow of a hollow ground slide in which some powdered potassium oxalate has been placed and add one drop of donor's blood. Put on a vaseline ring and a coverslip and lay it in a cool place for two hours with occasional tapping. It can be examined with an eyepiece, or better still a drop taken from it can be diluted with saline and examined in the ordinary way with a microscope. This alone is usually an efficient and reliable test, but it should be compared if time permits with the separate agglutinating tests before deciding to accept the donor. The two procedures in conjunction would enable one to state definitely that a certain donor's blood was compatible.

In an emergency there might be insufficient time for this, in which case it might be expedient to give gum-saline as a temporary measure in order that efficient preliminary tests could be done before transfusion of blood.

It is, of course, possible to use preserved suspensions of red cells of known constitution in glucose saline or gum-saline after the method employed by Robertson[13] during the war. Indeed, this might obviate many of the difficulties inherent in ordinary arm-to-arm transfusion. It gets over the donor-serum difficulty and there is also a weakening of the agglutinogens in the cells and thus less likelihood of incompatibility from this cause. Cells can be kept satisfactorily on ice for three weeks at least.

Professional Donors.—The practice of using professional donors would seem to be a sound one if all the obvious precautions were taken in their choice. In some clinics they are used on a large scale with much success and there is a great deal to commend it.

Conclusions.—The conclusions we have formed as a result of our investigation are as follows.

(i) The four-group theory of Moss and others contains the truth but not the whole truth, and is a dangerous guide in practice. This is particularly so with regard to the universal donor who as such may prove a very dangerous person.

(ii) The effect of the donor's plasma must always be considered. It is not necessarily interfered with by the recipient's plasma.

(iii) In testing cells and sera ample time must be allowed for the completion of the reaction. Probably two hours is long enough.

(iv) Strong fresh sera corresponding to all common agglutinins should be used in testing. Stock sera weaken and do so unequally as regards the different agglutinins and sometimes lose their activity altogether on keeping. Cells should be obtained fresh for similar reasons.

(v) To ascertain whether a possible donor is suitable the recipient and proposed donor's blood should each be tested and also a cross-matching test done, *i.e.* the donor's corpuscles should be tested against the recipient's plasma and the recipient's corpuscles against the donor's serum.

(vi) In an emergency, it might be better to give gum-saline immediately or a stock glucose saline or gum-saline suspension of cells to tide the recipient over a critical period while his blood and that of the prospective donor is being tested.

(vii) The practice of employing professional donors would appear to be sound.

(viii) Even with all precautions it is probable that no two samples of blood will match any more exactly than any two sets of facial features, but with the procedure suggested, accidents should be reduced to a negligible minimum.

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DISCUSSION.

Dr Goodall said Dr Owen has taken us over a difficult subject, and one has regrets that he has dealt so severely with the Moss grouping. As he has said, almost nothing pleases a class of students more than to be told what blood group they belong to, but the fly in the ointment is that some of these exercises do not succeed. I need hardly enlarge on the sort of accident that may occur after transfusion. Every one who has seen a number of cases must be fairly familiar with them. A person who gets blood injected in a few hours starts to have a rigor, then a high temperature; in an hour or two more he has hæmaturia of the most alarming kind; sometimes he dies. Short of this, there are the innumerable little rises of temperature which are often a feature of these cases, and the explanation seems to be that while the Moss grouping contains the truth it does not contain the whole truth; and over and above the two pairs of agglutinins and agglutinogens which are present, there are various others of minor importance, but every now and again one of these minor ones will happen to be well represented in the particular blood being used and then accidents occur.

Dr Owen's work has been very impressive and some of the things he has shown have been exceedingly interesting. It is a remarkable thing that up to the present time the effect of the donor's serum on the recipient's corpuscles has been so little taken into account. The argument was that the recipient has so much plasma that it overwhelms the plasma of the donor. But the donor's plasma remains active. Then the time factor has been regulated.

Dr Owen quoted a case where a lady died after transfusion because her donor had agglutinins which acted very slowly—so slowly that their effect was not noticeable in the test—and the lady died of the delayed reaction. These delayed reactions may be greater than the prompt ones. Yet again certain agglutinins in stock sera may lose their activity while other agglutinins remain active.

Dr Owen has demonstrated that it is no longer a safe procedure to send a demand to a dispensing chemist for Group II. and Group III. sera and use these in tests, because they may be very different things; and if the test is going to be more elaborate in future, we shall just have to accept that. As Dr Owen has said, there is no emergency so very great, that it cannot be met with transfusion by salines while blood for transfusion is being selected on safe lines.

Dr Margaret Tod said—I undertook to try and find universal donors for the Sick Children's Hospital. I collected twenty-five healthy young men and arranged to carry out these tests. I did the test in rather a rough and ready way—one drop of donor's blood in 7 drops of Group II. or Group III. serum, which I had obtained from a reliable source. Out of these twenty-five I found eight who seemed, on the first test, to belong to Group IV. I asked them to come back and have further tests carried out. About two hours later I went back and had a look at the suspensions and found that three had agglutinised by that time. I repeated the tests on the eight donors with my own serum, which I know to belong to Group II., and with that of several other persons of known groups, and the results were so unsatisfactory that I had to tell them at the Hospital that I did not consider any of these young men a safe donor. Now I know the reason why.

Dr J. Lewis Owen (in reply) said—The point that Dr Goodall raised concerning the unequal keeping of the agglutinins is important. We found that α and β—the two originally discovered—keep very much better than γ and δ. Now if a specimen of serum had very weak α and strong γ, it might happen that the α would die out before the γ, so we can get in sera combinations different from the original one from one or other agglutinin dying out. We once found a serum with weak α, weak β, and strong γ in it in which we tested their keeping properties, and the agglutinins died out in the following order —first β then γ and finally α. That was over a matter of four months, but ordinarily they keep quite well for a month at least. Our serum lost strength rather rapidly inside the first week, but after that weakened more slowly. A good deal of course depends on the original strength as I have said. The cells, on the other hand, lose their power of being agglutinated more quickly. This is one reason why I like the idea of giving preserved cells in the way suggested by Robertson. It gets

over the donor's serum difficulty; and moreover the weakening of the agglutinogens in the cells lessens the chance of any agglutination, and apparently the results are as good as by giving fresh blood.

With regard to trouble with universal donors, that is more or less a universal trouble, both in the laboratory and in the wards. I think the time factor accounts for many such troubles. It may take over two hours for a weak γ to agglutinate a weak C. If then a blood containing weak C+B were tested with a serum containing weak γ and α (that is Group II. in the Moss system) you may not see agglutination for two hours, and it might be assigned to Group IV. when it should really be Group II. Many of the fatalities reported I believe are due to that sort of thing. It is not quite the same when persons in different groups are incompatible, for then it more frequently depends on the "donor's serum effect." For instance, a Group I. person, the so-called universal recipient, should take Group II. blood with impunity; but very frequently when Group II. blood is transfused into a Group I. person an agglutination reaction occurs. The mistake is in the system as well as the preliminary testing. In some laboratories, I admit, sera as well as the cells are tested, but if the four group system is rigidly adhered to there will always be reactions of this kind, for a system in which the blood of persons in the same group can be incompatible, cannot be relied upon to give safe guidance.