THE DETECTION OF A HEREDITARY ANTIGENIC DIFFERENCE IN THE BLOOD OF MICE BY MEANS OF HUMAN GROUP A SERUM.

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CONTENTS.

I. Introduction.

Up to the present no serological differences have been detected between the red cells of individual mice. McDowell and Hubbard (1922) failed to detect iso-agglutination reactions using a large number of different strains of mice. Boyd and Walker (1934) failed to induce the formation of antibodies by injecting blood from one mouse into another, a method used with success by Todd (1930) with blood of fowls. An attempt to detect individual differences by means of normal animal sera failed in the hands of Wünsch (1934).

During the present investigation all the above methods were tried. Natural iso-antibodies were not found in any sera, nor were injections of blood from one mouse to another able to induce their formation. Successful results were obtained by means of serum from immunised rabbits, but full details of these experiments will be reserved for a future communication. This paper deals with results obtained with normal human group A serum, by means of which it is possible to differentiate at least two types of mouse corpuscles.

The failure of previous observers to obtain positive results for mouse blood may be attributed to various causes. Any stock of laboratory

mice is likely to have been inbred to some extent and therefore may be as uniform as certain primitive tribes in respect of blood groups. Further, the present investigation has shown that the individual reactions of mouse blood are much weaker than the species reactions. Wünsch (*loc. cit.*) performed tests on slides for 3 min. at room temperature, by which technique individual differences would have been indetectable even if present in his stock.

It is not advisable to perform very long experiments with mouse blood for reasons to be stated later, and in order to detect individual differences it is necessary to employ a technique that is moderately quick and sensitive. Sera that give individual reactions, whether immune or normal, never give very intense agglutination although the titre may be moderately high.

Use was made of lines of mice, brother-sister inbred for a sufficient number of generations to ensure that they are a close approximation to a genetically uniform stock.

With a satisfactory technique for bleeding, it is possible to obtain sufficient cells from a single individual for absorption experiments, but for immunisation it is preferable to be able to use the blood of more than one individual. Since antigenic variation in animal tissues has not been shown to occur apart from genetic variation, the use of pure lines in this kind of work has obvious advantages.

II. Materials and technique.

The mice most thoroughly tested were drawn from the three pure lines (Little's black agouti, Dunn's self-black and the Bagg albino) and a very heterogeneous stock (the ZS stock). A few mice from other stocks and a few wild mice were also examined.

The animals were usually bled by cutting off the end of the tail and the blood collected in 4 per cent. sodium citrate. If the mouse is warmed in an incubator for upwards of half an hour and anaesthetised, it is possible to obtain $\frac{1}{2}$ c.c. of blood from one individual. If the tail is immersed in fluid during bleeding the flow of blood is much facilitated; mice 14 days old have been bled in this way and sufficient corpuscles obtained for making graduated suspensions. The death-rate by this procedure is negligible. Occasionally blood was obtained by cardiac puncture when tuberculin syringes and intradermal needles were used. The withdrawal of blood is much facilitated if the nozzle of the syringe is filled with saline or citrate. The animals were well anaesthetised with ether and steadied with the left hand whilst the plunger was withdrawn

with the right hand. It is frequently possible to obtain 1 c.c. of blood without killing the mouse.

One per cent. suspensions of cells in saline were used in all tests. The blood was spun and the requisite quantity of cells measured in a micro-pipette. Washing makes no difference to the agglutinability of the cells and was not invariably performed before making suspensions, but was always done in absorption experiments. Mouse cells are very easily lysed, especially in moderately dense suspensions (about 10 per cent.). Washing appeared to check the lysis to some extent, but it was liable to occur whatever precautions were taken.

Agglutination tests were performed by two methods. That used in earlier experiments was to put equal drops of cell suspension and serum in hollow-ground cells on glass plates, each cell being $\frac{1}{2}$ in. diameter. The plates were put in a moist chamber and readings taken after $1\frac{1}{2}$–2 hours.

This technique has the advantage that it is possible to take readings at various intervals without disturbing the test.

In all later work and in all absorption tests a centrifuge technique was used (see Wiener and Vaisberg, 1931). Equal volumes of cell suspension and serum were put up in small test-tubes (about $\frac{1}{4}$ in. internal diameter), left at the required temperature for $1\frac{1}{2}$–2 hours and then spun for $\frac{1}{2}$ min. If spinning is performed too fast or too long agglutination will take place in saline controls (or Ringer). Serum appears to exert some inhibitory effect on this type of agglutination, and one may find that the controls are agglutinated, whilst the tests are freely dispersed, if spinning is too violent. The optimum speed of spinning cannot be given. A small-angle centrifuge was used and the optimum rheostat position found empirically.

Provided spinning has not been too violent and the tubes are perfectly clean, controls may be resuspended by sucking the supernatant fluid into a capillary pipette and gently blowing it back on to the deposit. Occasionally a few cells stick to the glass, but it should not be necessary to pipette more than three times. In assessing the degree of agglutination it is necessary to observe the reaction when the supernatant fluid is blown on to the deposit, the degree to which clumps are broken up when sucked into the capillary and, in the case of weaker reactions, microscopically as well. Before microscopic examination each specimen is gently mixed three times. Graduations are performed as follows:

(1) + + +. The deposit breaks up to some extent. When viewed in the capillary after mixing three times, the suspension is seen to be coarsely granular.

(2) ++. The deposit breaks up into coarse granules. In the capillary the suspension is definitely granular.

(3) +. The deposit breaks up fairly readily, but is obviously granular. Microscopically, there are fewer large clumps than in the preceding stage, but plentiful small and medium-sized clumps.

(4) ±. The deposit breaks up readily. In favourable lights the suspension may be seen to be finely granular. Microscopically there are numerous small and a few medium-sized clumps.

(5) tr. = trace. Not detectible macroscopically. Microscopically moderately numerous small clumps.

(6) f.tr. = faint trace. A few small clumps detectable microscopically.

(7) v.f.tr. = very faint trace.

Occasionally it is difficult to classify individual tubes, for instance there may be very numerous small clumps. In these cases the test was repeated and was usually classifiable. Repeated tests showed that the error involved was plus or minus one degree. If tests are done in triplicate (which is very seldom necessary) it is extremely rare to find more than one test anomalous.

This method demands a certain amount of practice before reliable results are obtained, but is by far the most satisfactory method for use with mouse blood. Lengthy tests are impracticable as clumps tend to break up and haemolyse after some hours. The plate technique is far less sensitive, but it is preferable if it is desired to compare degrees of agglutination at a constant dilution.

The human group A serum came from the author in all experiments quoted. Others have been used and gave similar results, with the exception of one which was extremely weak (it had a high titre for human corpuscles). Human group B serum contains an antibody that reacts similarly on all mice. It has a higher titre than group A serum. No batch of serum was kept for any length of time, but it appears to keep best in ampoules without disinfectant, after being inactivated for 30 min. at 56° C.

Absorptions were performed with varying quantities of washed cells. Experiments were performed to ascertain the time required for absorption. There was no difference in results obtained between three-quarters of an hour and 4 hours. Since haemolysis always occurs after about 2 hours, absorptions were performed for about three-quarters of an hour.

Absorption on human cells of group B did not remove the agglutinin concerned in this reaction.

III. THE DETECTION OF INDIVIDUAL DIFFERENCES.

(*a*) *Effect of temperature. Direct agglutination and absorption.* Table I (*a*) and (*b*) shows the effect of temperature on the agglutination of red cells from mice of the four different groups of human group A serum.

TABLE I.

The effect of temperature on the agglutination of red cells from different groups of mice by human group A serum.

(*a*) Tests set up in duplicate at 20° C. and 37° C. Readings after 2 hours. Plate technique. Human group *A* serum diluted $\frac{1}{2}$.

	Tests on cells of							
	ZS5	ZS6	Ag51	Ag52	Al2	Al55	Bl1	Bl2
After 2 hours at 20° C.	+ + +	+ + +	tr.	+ + +	+	+ +	+	±
After 2 hours at 37° C.	+ + +	+ +	—	±	tr.	tr.	v.f.tr.	tr.

ZS = ZS stock described in text. Al = Bagg albino. Ag = Little's agouti. Bl = Dunn's black.

(*b*) Similar to above, but test done by centrifuge technique.

Tested on cells of	After 2 hours at 20° C. Serum dilutions				After 2 hours at 37° C. Serum dilutions			
	1/8	1/16	1/32	1/64	1/8	1/16	1/32	1/64
ZS4	+ +	+	±	±	+ +	+	±	tr.
ZS21	+ + +	+	±	tr.	+ +	+	±	±
Ag5 ♀	+ +	±	tr.	f.tr.	±	tr.	—	—
Ag6 ♂	+ + +	+ +	+	±	+ ±	+	±	—
Bl3*a*	±	tr.	—	—	f.tr.	f.tr.	—	—
Bl3*b*	+	+	—	—	tr.	v.f.tr.	—	—
Bl3*c*	+ +	+	tr.	—	—	—	—	—
Al1	+ +	+	+	tr.	+	±	v.f.tr.	—

If the plate technique is used one frequently finds that after about 15 min. at 37° C. all four groups are rather more strongly agglutinated than they were at room temperature, thereafter the clumps tend to break down, and after about 2 hours (or more in some cases) constant results may be obtained. After spinning the clumps do not break down very easily, but if the tests are put in the incubator with a minimum of delay prior to spinning it is possible to obtain fairly consistent results within half an hour.

Table II contains representative results for all four groups obtained by the centrifuge technique. It is clear that the ZS group reacts the strongest, whilst the blacks give only a weak reaction. In the case of the agoutis, the males appear to give rather stronger reactions than the females, and in four cases males gave reactions that were as strong as the weaker reactions of the ZS stock. None of the four were litter mates, and two were reared at the Lister Institute and two at University College

The albinos give weaker reactions than the ZS on the whole, but a certain number give intermediate reactions.

If absorption tests are performed, it can be shown that the pure lines fall into one group and the ZS stock into another. The results may be summarised as follows.

Undiluted serum absorbed with an equal volume of the cells of albinos,

TABLE II.

The variation in sensitivity of corpuscles of mice from different groups to agglutination by human group A serum. Centrifuge technique. Readings taken after 1½–2 hours at 37° C. All animals over 8 weeks old.

ZS cells of mouse No.	Cells of ZS. Serum dilutions 1/8	1/16	1/32	1/64	Cells of a mouse from litter No.	Cells of Dunn's blacks. Serum dilutions 1/8	1/16	1/32	1/64
17 ♂	+ +	+	±	tr.	3 ♂	f.tr.	—	—	—
1 ♀	+ +	+ ±	+	tr.	3 ♀	v.f.tr.	—	—	—
10 ♀	+ +	+	±	v.f.tr.	3 ♀	—	—	—	—
4 ♂	+ ±	±	tr.	f.tr.	3 ♂	—	—	—	—
62 ♂	+	±	tr.	—	10 ♂	tr.	—	—	—
2 ♂	+ +	+ +	+	tr.	10 ♀	tr.	f.tr.	—	—
3 ♂	+ +	+ +	+	±	10 ♀	tr.	v.f.tr.	—	—
10 ♀	+ +	+ ±	±	tr.	11 ♀	tr.	f.tr.	—	—
5 ♀	+ + +	+ +	+	±	56 ♂	±	tr.	f.tr.	—

Albino cells of mouse from litter No.	Cells of Bagg albino. Serum dilutions 1/8	1/16	1/32	1/64	Agouti cells of mouse from litter No.	Cells of Little's agouti. Serum dilutions 1/8	1/16	1/32	1/64
27 ♂	tr.	—	—	—	6 ♂*	+ ±	+	±	—
27 ♂	+ ±	±	f.tr.	v.f.tr.	6 ♂	±	v.f.tr.	—	—
27 ♂	+	±	f.tr.	—	6 ♀	±	—	—	—
15 ♀	±	tr.	f.tr.	—	6 ♀	—	—	—	—
15 ♂	+	tr.	—	—	6 ♀	f.tr.	—	—	—
18 ♂	+	f.tr.	?	—	18 ♂*	+ ±	±	tr.	—
13 ♀	+	tr.	f.tr.	—	18 ♂	±	f.tr.	—	—
14 ♀	+	±	f.tr.	v.f.tr.	18 ♀	tr.	—	—	—
14 ♀	+ ±	±	f.tr.	—	18 ♀	f.tr.	—	—	—
14 ♂	±	tr.	—	—	16 ♂	±	f.tr.	—	—

About fifty blacks, fifty ZS and fifteen albinos were tested in all. Thirty agoutis were tested; two other males gave reactions like those marked *.

blacks or agoutis (including the aberrant males mentioned above) will still agglutinate ZS cells.

Undiluted serum is completely exhausted for all groups by one-tenth its volume of ZS cells.

The titre is lowered by absorption with cells of any group. Diluted serum may be exhausted by fairly small quantities of cells from any group. Serum diluted to $\frac{1}{8}$ may be completely exhausted by one-tenth its volume of "black" cells.

The minimum quantity of cells required by each pure line to exhaust

the serum for itself has not been determined in detail. One volume of cells to five of serum may be safely used (see Table III (*a*) and (*b*)).

In one experiment it was found that a black had completely exhausted the serum *for itself* with one volume cells to twenty serum, whilst a ZS had exhausted the serum for itself and the black with the cell : serum ratio of 1 : 40.

From these results it was deduced that all three pure lines had approximately the same power of combining with the antibody, whilst the cells of certain individuals (albinos and certain agouti males) were

TABLE III†.

Absorption of human group A serum by cells from four groups of mice. The tests have been performed on several individuals from each group. The smallest number tested in any group is 6 (*from the agoutis*). N.B. *All absorptions and tests are performed at* 37° C.

(*a*)

Undiluted serum absorbed by cells of	Ratio of cells to serum	Tested on cells of ZS1	ZS5	Bl27	Bl43	Ag7♀	Ag5♀	Ag6*♂	Ag6♂
Black 27	1/2	±	+	—	—	—	—	—	—
Agouti 6* ♂	1/2	+	+	—	—	—	—	—	—
Agouti 6 ♂	1/2	±	+ ±	—	—	—	—	—	—

Agouti 6* gave an unusually strong reaction with unabsorbed serum. See Table II.
Ag = agouti, Bl = black, Al = albino.

(*b*)

Undiluted serum absorbed by cells of	Ratio of cells to serum	Tested on cells of Bl34	Bl36	Al24	Al25	Al26	ZS50	ZS6	ZS61
Blacks 34, 36	1/2	—	—	—	—	—	±	±	tr.
,, 34, 36	1/5	—	—	—	—	—	+ +	+ +	+
Albino 24	1/2	—	—	—	—	—	+	+ ±	±
,, 24	1/5	—	—	—	v.f.tr.	v.f.tr.	+ +	+ +	+ ±
ZS6 ♀	1/10	—	—	—	—	—	v.f.tr.	—	—

† The above results are typical of a large number of experiments. Sufficient mice from all four stocks were not available simultaneously to enable the results to be shown in the form of a single table.

agglutinated when sensitised by relatively small amounts of antibody. The ZS group is differentiated from any of the other groups by virtue of its greater power of combining with the antibody. Certain ZS individuals, such as No. 62 (Table II), are somewhat insensitive to agglutination but able to exhaust the serum in the same way as the more typical individuals.

(*b*) *The effect of age.* The results quoted above were all obtained from mice over 6 weeks old. In Table IV are shown the results obtained when very young mice are used. It will be seen that young blacks are more sensitive to agglutination than the adults from the ZS stock. After

about 6 weeks the sensitivity appears to remain constant throughout life; very old mice have not been studied, but typical strong reactions have been obtained from mice over a year old and from mice with carcinomata.

The young of other groups behave much the same as the blacks, and it is desirable to determine whether this enhanced sensitivity implies an enhanced power of absorbing antibody.

The results of absorption experiments (Table V (*a*) and (*b*)) show that:

(1) The cells of adult blacks will not exhaust undiluted serum for their offspring. If diluted serum is used, it is only possible to obtain complete exhaustion if equal volumes of cells and serum are used (Table V (*a*) and (*b*)).

TABLE IV.

The sensitivity of red cells of very young animals (from the black and ZS stocks) to agglutination by human group A serum.

Age	Cells of mouse	Dilutions of serum 1/8	1/16	1/32	1/64
Adult	Black ♀	tr.	f.tr.	—	—
,,	,, ♂	tr.	—	—	—
4 weeks	,, 121 ♀	±	f.tr.	—	—
,,	,, 111 ♀	+	±	tr.	f.tr.
2 weeks	,, ♀	+ +	+	+	tr.
,,	,, ♂	+ +	+ ±	±	tr.
,,	,, ♀	+ +	+ +	+	±
,,	,, ♂	+ + +	+ +	+	±
,,	,, ♀	+ +	+ +	+	tr.
Adult	ZS ♀	+ +—	+	±	tr.
2 weeks	ZS ♂	+ +	+	±	tr.

(2) ZS cells will exhaust undiluted serum for young blacks if equal volumes of cells and serum are used (Table V (*a*)). If smaller quantities of cells are used absorption is not complete.

(3) With a cell : serum ratio of 1 : 2, young blacks will not exhaust the serum of ZS cells but do so for themselves (Table V (*a*)).

(4) If a smaller proportion of cells is used the serum is not exhausted for either type (Table V (*c*)).

From this it would appear that the cells of young mice are very easily agglutinated by group A serum, but do not possess greater combining power for agglutinin than the adults of either group. It is possible that young mice may possess an antigen slightly different from that possessed by adults of the ZS group.

Young ZS individuals give the same type of reaction as young blacks. In some cases, animals tested over a period of 3 months, from the age of 4 weeks gave approximately the same type of reaction in all tests, others

appeared to give rather weak reactions when 4 weeks old and typical strong reaction when over 6 weeks old.

Tested against horse serum young blacks were not noticeably more sensitive than their parents.

TABLE V.

Reciprocal absorption tests, with human group A serum, between cells of young and adults of the black and ZS groups.

(*a*) Undiluted human group A serum.

Undiluted serum absorbed by cells of	Age	Ratio of cell to serum	Tested on cells of: Adult black	Adult ZS	Adult ZS	Adult ZS	2 weeks old black	2 weeks old black
Black	Adult	1/2	—	+	+	±	+ + +	+ + +
ZS	,,	1/1*	—	—	—	—	?v.f.tr.	—
Black	2 weeks	1/2*	—	+	+	±	—	v.f.tr

(*b*) Similar to above, but serum diluted 1/4.

Serum diluted 1/4 absorbed by cells of	Age	Ratio of cell to serum	Tested on cells of: Adult black	Adult ZS	2 weeks old ZS	2 weeks old ZS	2 weeks old black	2 weeks old black
Black	Adult	1/10	—	tr.	+ + +	+ + +	+ +	+ + +
,,	,,	1/1	—	—	v.f.tr.	—	—	—

(*c*) Demonstrating incomplete absorption of human group A serum by cells of very young blacks when the volume of cells used is relatively small.

Undiluted serum absorbed by cells of	Age	Ratio of cell to serum	Tested on cells of: Adult ZS	2 weeks old ZS	2 weeks old black
Black	2 weeks	1/5	±	+	tr.

* Approximate.

(*c*) *Reactions of miscellaneous stocks.* Individuals taken at random from other laboratory strains gave either weak or intermediate reactions. To classify mice from an untested stock it is advisable to perform direct titrations and absorption tests. Six wild mice were tested by direct titration and serum absorbed on the cells of albinos and of these, five gave strong reactions by both tests whilst one was intermediate by titration and negative by absorbed serum. Two others were tested by direct titration and by serum absorbed on blacks and ZS with the results shown in Table VI.

It appears that the type of reaction given by albinos occurs in wild mice. The reaction given by the male may either be a rather late manifestation of the type of reaction given by young mice or may be an entirely new type of reaction. It would be necessary to perform a fairly extensive

investigation before one could be certain on the point or generalise on the type of reaction given by the cells of wild mice (see below, section on "Genetics").

The experiments described in the preceding sections show that human group A serum is capable of differentiating between the red cells of mice coming from various stocks.

TABLE VI.

Absorption experiment demonstrating an abnormal reaction to human group A serum by a wild male mouse. The wild female is similar in its reactions to direct agglutination to a Bagg albino.

Undiluted serum absorbed by cells of	Ratio of cells to serum	Tested on cells of						
		Bl 56	Bl 35	ZS 2	ZS 1	*Wild* ♂	Wild ♀	Al 24
Black	1/2	—	—	+	+	+ + +	—	—
ZS 1	1/2	—	—	—	—	+ +	—	—
Wild ♀	1/10	—	f.tr.	+ +	+ ±	a.c.	v.f.tr.	—

Bl = black, Al = albino.

In some cases differentiation can be made by means of direct titration alone. The cells of adults from Dunn's blacks can be quite clearly distinguished from those of adult ZS mice in this way. Examination of other stocks and of young individuals from the blacks and ZS show that mouse corpuscles fall into a number of classes as far as their sensitivity to agglutination is concerned, but if their ability to *absorb agglutinin* is taken into account the red cells of mice from any of the laboratory stocks so far examined may be placed in one of two groups.

IV. Genetic investigation.

The foregoing investigations show that the different pure lines gave types of reactions characteristic of the line, whilst those individuals giving strong reactions were all drawn from the same stock. It should be mentioned that the ZS stock as a whole is extremely heterogeneous, not all individuals taken from it giving the type of reaction quoted as typical. It was found that animals taken from this stock tended to breed true for a given type of reaction. In order to make a thorough investigation of the inheritance of the reaction type typical strong reactors were crossed to blacks. (The cross was made ZS ♂ to black ♀ and *vice versa*.) Six litters were raised in the F_1 totalling forty-three mice. Of these thirty-nine were strong reactors. The remaining four all came from the same litter, the mother of which had given two weak and one strong when mated to her brother. Her F_1 litter tested by direct titration and

by absorbed sera was shown to contain four strong and four weak reactors. It was therefore presumed that this particular female was heterozygous. The strong reactors were indistinguishable from their ZS parent in agglutinability, or power of absorption.

The F_2 (from strong F_1 only) consisted of forty-four individuals of which thirty-two were strong, eleven weak and one was doubtful when tested by direct titration and was lost before it could be retested.

The back-cross to the blacks gave the following result: strong 22, weak 22, doubtful 1.

About half the back-cross was tested by means of absorbed serum and gave results entirely consistent with those obtained by direct agglutination. When first tested, some of the F_2 and back-cross were only about 4 weeks old. There were four doubtful cases in the F_2 which subsequent investigation showed quite clearly to be recessives. In the back-cross there were also four doubtful cases of which two were dominants, one recessive and one was lost. Most individuals of the F_2 gave slightly stronger reactions than the back-cross, however there was considerable overlap and the differences were not very great.

There appears to be little doubt that a strong reaction is determined by a single dominant gene. Modifiers do not appear to play a very prominent part in this particular cross but they would appear to account for the type of reaction given by the albinos. The fact that the blacks and albinos are pure lines is confirmation of this view. Similar modifiers would appear to be present in the wild female described earlier, if they are present together with the dominant gene present in the ZS stock this might account for the type of reaction given by the wild male. Alternatively, the reaction may be due to the presence of an unrelated agglutinin or a further allelomorph. These hypotheses are susceptible to experimental testing and it is hoped that this will be done at a future date.

V. REACTIONS WITH MISCELLANEOUS SERA.

The type of reaction that has been described, is specific for human group A serum; four samples of horse serum, six samples of rabbit serum and three of fowl serum were tested and in no case was any correlation observed between the type of reaction given by human group A serum and any of the above normal sera.

In the case of one sample of normal horse serum, the ZS stock appeared rather less sensitive than any members of the three pure lines when absorptions and tests were performed on ice (tests were all done by the plate technique). In a few cases it was found that serum absorbed

on ZS cells would agglutinate cells of the three pure lines and not those of ZS individuals. In many cases it was not possible to obtain clear-cut results, but the ZS cells were, as a rule, the least strongly agglutinated.

Experiments with rabbits immunised against cells from all four stocks are not yet completed but the following results are of interest. All react in a similar fashion prior to absorption.

An "anti-black" serum was absorbed with equal ease by the cells of all four groups, as was an "anti-ZS" serum.

An anti-albino serum after absorption on cells of blacks or agoutis reacted fairly strongly on the cells of albinos. A few weak reactions were obtained against ZS cells, but it will be necessary to perform further tests. After absorption on ZS cells there were still reactions with the cells of agoutis and albinos, but less strong than when the absorption was done on blacks. Repeated absorption with cells of ZS removes the agglutinin, repeated absorptions with the cells of blacks or agoutis does not lower the titre for albinos.

An anti-agouti serum could be completely exhausted by the cells of all four groups but most strongly by the agoutis and albinos, less strongly by the ZS and least strongly by the blacks.

VI. Discussion.

None of the phenomena described above is entirely new. The importance of temperature in determining specificity is well known and has been very well shown by the experiments of Dölter (1925) on the action of various normal animal sera on the four human blood groups.

The type of antigenic difference demonstrated bears a close resemblance to that existing between the varieties of the human group antigen A (*i.e.* A_1 and A_2). The resemblances are summarised below.

(1) Thomsen, Friedenreich and Worsaae (1930) showed that human corpuscles of type A_1 would completely exhaust human anti-A serum with a cell serum ratio of 1 : 32, whilst the A_2 corpuscles would not do so when there was an excess of corpuscles.

(2) Intermediate types exist if direct agglutination is used to differentiate the two types (Landsteiner and Levine, 1930) but not if absorption tests are employed (Thomsen and co-workers, *loc. cit.*).

The difference between the two human types is also determined by a single pair of allelomorphs, A_1 being dominant to A_2 (Thomsen and co-workers, *loc. cit.*; Friedenreich and Zacho, 1931).

The sensitivity of human cells has a more or less normal distribution for all groups, including group A. A stock of mice bred at random would,

given the same type of distribution as several factors (genetic and possibly environmental), contribute to the agglutinability by human group A serum, but in the case of the human and the mouse cells ability to *absorb agglutinin* appears to have a sharply bi-modal distribution and to be determined by a single gene. To classify a mouse coming from an untested stock, it is advisable to test (1) its reaction to serum absorbed on the cells of mice from tested stocks of both classes, (2) its ability to absorb serum for either class.

The second test is necessary in case an animal has abnormally agglutinable cells like those of very young mice.

There has been considerable controversy as to whether the difference between A_1 and A_2 is to be regarded as due to a quantitative or qualitative difference.

In favour of the latter hypothesis may be quoted the work of Landsteiner and Levine (1926, 1929) who found that a cold agglutinin acted more strongly on the A_2 cells (see also Landsteiner and Witt, 1926[1]), whilst more recently Schiff (1934) has shown that an anti-Shiga serum reacts more strongly on the same type of cell. It is rather difficult to reconcile these results with the hypothesis of a purely quantitative difference. Experiments with artificially modified proteins show that there may be considerable overlap in the reactions between proteins coupled with similar determinant groups (see Marrack, 1934). So far as erythrocytes are concerned, the only case we know of in which there is certainly a quantitative difference, is that of M and N when the reactions are slightly weaker in the heterozygotes (Wiener and co-workers, 1934), but there is far less difference than is observable in the case of the two types of A or the differences existing between the two types of mouse blood.

On these grounds the hypothesis is put forward that an antigenic difference exists in mice, similar to that existing between the types A_1 and A_2 present in human blood, and that in both cases the differences are referable to chemical differences.

It is true that as yet no reactions have been discovered comparable to those obtained with Shiga serum, although the reactions obtained on ice with horse serum are at any rate suggestive. It is not yet possible to say whether the reactions with immune sera bear any relationship to those obtained with normal human group A serum.

[1] Using an abnormal agglutinin with an intermediate temperature range.

VII. Summary.

1. By the use of human group A serum it is possible to distinguish various types of mouse blood. To obtain specific results tests must be performed at 37° C.

2. A pure line of black mice and individuals selected from a heterogeneous stock (ZS) are sharply divisible into two groups by direct agglutination alone.

3. Tests with other stocks show that intermediate types occur if direct agglutination is used as the sole criterion, but amongst laboratory stocks it is possible to distinguish two classes if ability to absorb agglutinin is taken into account.

4. An anomaly to the above rule was found in the case of a wild mouse. Explanations of this anomaly are suggested in the text.

5. Very young mice are very sensitive to *agglutination* by human group A serum, but they do not appear to possess enhanced powers of absorbing agglutinin.

6. The reaction is peculiar to human serum. A horse serum that gave somewhat indefinite results in the cold appeared to act in an opposite manner to human group A serum.

7. A cross between the blacks and ZS stock showed that a strong reaction was due to a single dominant gene. It is suggested that modifying factors may in part determine the reaction obtained by direct agglutination; the gene here studied determines the ability to absorb agglutinin.

8. Differences are detectable by immune sera which are different from that here described but may be correlated with it.

9. It is shown that a fairly close resemblance occurs between the factor here studied and the types A_1 and A_2 occurring in man.

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