FACTORS INFLUENCING THE AGGLUTINABILITY OF RED CELLS

VARIATION OF THE RED CELLS OF THE RABBIT IN SUSCEPTIBILITY TO AGGLUTINATION BY HOMOLOGOUS ISO-ANTISERA

By D. H. HEARD

Department of Pathology, University of Cambridge

(With 2 Figures in the Text)

INTRODUCTION

During work on the blood groups of the rabbit, one problem proved to be an ubiquitous nuisance: the red cells of some rabbits are agglutinated more strongly than the red cells of others, and to a higher titre, by a serum apparently containing only one iso-antibody. This phenomenon has been noted by many workers using other antigen-antibody systems; examples will be compared and discussed later. It was first described in association with the red cells of the rabbit by Knopfmacher in 1942. He noted that strongly reacting cells absorbed antibody from rabbit serum more efficiently than weakly reacting cells did, and suggested that the strongly reacting cells carried more antigenic substance. He linked this feature to whether the cells were homozygous or heterozygous for the antigen in question. Levine & Landsteiner (1929) also indicated that they encountered variation in the agglutinability of rabbit red cells; Kellner & Hedal (1953) met it too and drew attention to it. The problem is of practical as well as academic importance when working on rabbit blood groups, for the risk of using imperfectly absorbed antisera for crucial experiments and of drawing erroneous conclusions from them is considerable.

During the preparation of the sera which allowed four antigens temporarily labelled *Z*, *Y*, *X* and *W* to be recognized, the cells of a panel of rabbits were titrated against a number of different iso-antisera (Heard, 1955). Fig. 1 shows the results obtained after titrating anti-*Z*. The variation in the titration results for anti-*W* and anti-*X* were not so marked. It should be mentioned that cells which are strongly agglutinated by an antibody of one specificity are not necessarily strongly agglutinated by antibodies of other specificities.

One obvious explanation for this variation was that the serum contained two antibodies; but since there was good reason to believe that it contained only one (Heard, 1955), it seemed that there might be something in the structure of the red cell which allowed one cell to agglutinate better than others, and this hypothesis was considered.

The problem was investigated in three ways. One was by absorbing different samples of a serum several times, one sample with strongly agglutinating cells and another with exactly the same amounts of poorly agglutinating cells, to find any differences in antibody absorption. This method is spoken of as 'the method of comparative absorptions'.

https://doi.org/10.1017/S0022172400000917 Published online by Cambridge University Press

The second method of investigation was to titrate an antiserum, made by inoculating rabbits with poorly agglutinating cells, against both strongly and poorly agglutinating cells.

The third method, using serum labelled with radioactive iodine (^{131}I) and adapting the methods outlined by Boursnell, Coombs & Rizk (1953), is described in a separate paper (Boursnell, Heard & Rizk, 1955).

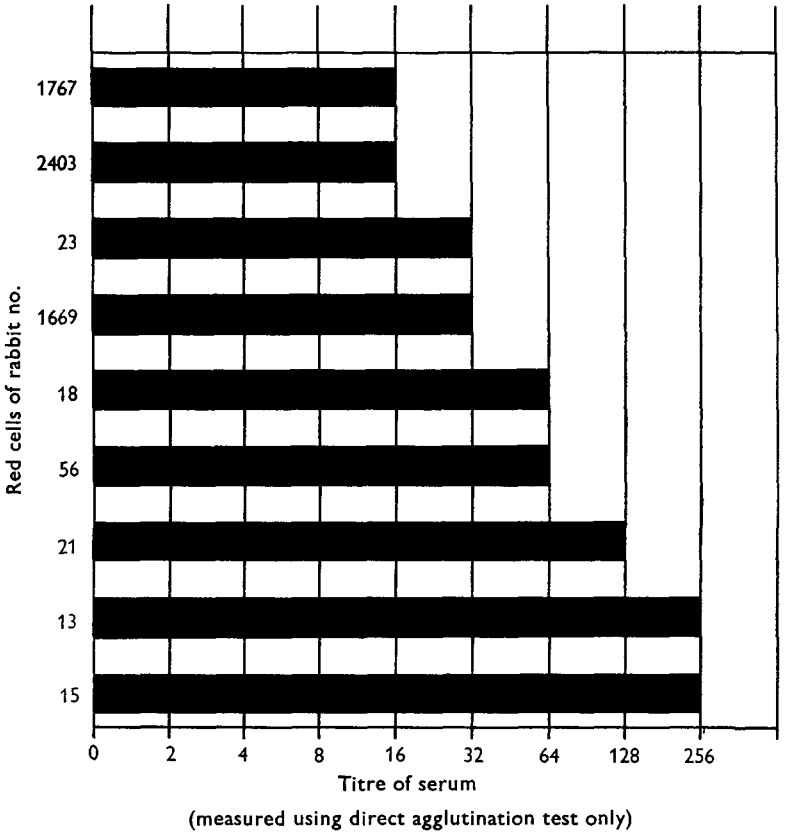

Fig. 1. Titration of anti-*Z* serum against the red cells of 9 *Z*-positive rabbits.

The results suggest that this variation in the agglutinability of the cells may well be due to a variation in the number of antigen sites available on the red cells for a particular antibody. Poorly agglutinating cells appear to have fewer sites for that particular antigen than strongly agglutinating cells.

EXPERIMENTAL

The methods and the materials used in this paper are the same as those fully given in the preceding paper on rabbit blood groups (Heard, 1955) and therefore are not described again.

https://doi.org/10.1017/S0022172400000917 Published online by Cambridge University Press

(1) *Comparative absorption experiments*

For each serum, cells were chosen to make contrasting pairs of strongly agglutinating and poorly agglutinating cells. Table 1 shows the pairs of cells chosen for investigation and the titres they gave when titrated against their respective antisera.

Table 1. *Titration of various antisera by direct agglutination against the pairs of cells chosen for comparative absorption experiments*

Serum	No. of rabbit carrying S.A. cell	Titre of serum using this cell (D.A. only)	No. of rabbit carrying P.A. cell	Titre of serum using this cell (D.A. only)
No. 1275 containing anti-*Z*	2156	128	1767	16
	1409	512	2403	16
No. 5 containing anti-*Y*	1	128	54	8
	9	128	1517	8
No. 1561 containing anti-*X*	1529	16	1408	8
No. 1471 containing anti-*X*	1529	64	1408	16

S.A. = strongly agglutinating.
P.A. = poorly agglutinating.
D.A. = direct agglutination.

(*a*) *Comparative absorptions of anti-Z serum*

Experiment 1. Two samples of anti-*Z* serum (1·5 ml. serum 1275 which was diluted 1 in 2) were absorbed under the same conditions with cells from rabbits 1767 and 2156 respectively six times, using 1, 0·5, 0·5, 0·5, 1 and 1 ml. carefully measured volumes of packed cells for each absorption. The samples were titrated after each absorption against the cells 1767 and 2156, using the direct agglutination test and the anti-globulin sensitization test previously described by Heard (1955).

The sample absorbed with cells 1767 (now measuring 1·5 ml. of serum 1 in 2) was then reabsorbed with nine doses of 1 ml. packed cells, fifteen doses in all. It was then used without further dilution to test, by direct agglutination and by the indirect sensitization test, the cells of rabbits on a panel which had reacted with the untreated serum. The sample absorbed six times with the cells 2156 was used without further reabsorption to test the cells of the same rabbits.

To control any non-specific absorption of antibody a sample of the serum was similarly absorbed with the cells of a rabbit (1971) which did not react with the untreated serum. This sample was titrated after fifteen absorptions against the cells of rabbits 1409 and 2156, and also it was tested against the cells of the panel rabbits.

Experiment 2. Two other samples of anti-*Z* serum (1·5 ml. serum 1275 diluted 1 in 2) were absorbed with the cells 1409 and 2403 respectively four times using 1·0, 0·5, 0·5, and 0·5 ml. volumes of packed cells. The samples were titrated after each absorption against the cells 1409 and 2403. The sample absorbed with the cells 2403 (now measuring 1·9 ml.) was reabsorbed three times using 1·0 ml. of packed cells for each absorption and then tested against sixteen of the panel rabbits whose cells reacted

https://doi.org/10.1017/S0022172400000917 Published online by Cambridge University Press

with the untreated serum. It was further reabsorbed (now measuring 1·0 ml. of serum 1 in 2) with 1·0 ml. doses of packed cells until it had had sixteen treatments altogether and then it was used at full strength to test the cells of the panel rabbits. The sample absorbed with the cells of 1409 was used without further reabsorption to test the cells of the panel rabbits.

(*b*) *Comparative absorptions of anti-Y serum*

Five samples of anti-Y serum (serum no. 5, 1·5 ml. diluted to 3 ml.) were absorbed with cells from rabbits 1, 9, 54, 1517 and 22 respectively. Rabbits 1 and 9 carried strongly agglutinating Y cells, rabbits 54 and 1517 poorly agglutinating Y cells, while the cells of rabbit 22 were not agglutinated by the serum and therefore acted as a control on the absorption procedure. The samples were absorbed six times using 1·0, 0·5, 0·5, 0·5, 1·0 and 1·0 ml. volumes of packed cells; after each absorption the samples were titrated against strongly and poorly agglutinating cells.

The samples absorbed with the cells of 54 and 1517 and 22—the control (now measuring 1·5 ml.)—were reabsorbed eight times with 1·5 ml. volumes of packed cells. They were then used at their full strength to test by the direct and indirect sensitization tests the cells of the rabbits on the panel which had reacted with the untreated serum. The samples absorbed six times with the cells from rabbits 1 and 9 were used without further reabsorption to test the cells of the panel.

(*c*) *Comparative absorption of anti-X sera*

Two sera (1471 and 1561) both contain two antibodies: anti-X, as a direct agglutinin, and anti-W, in the incomplete form. Samples of neat sera were absorbed with the cells of rabbits 1408 (poorly agglutinating cells) and 1529 (strongly agglutinating cells). Both cells carried the antigen X only and not W. Volumes of packed cells equal to the volumes of sera were used and the sera were absorbed nine times. After the second, third, fifth and seventh absorptions the samples of serum were titrated against red cells from rabbits 1408, 1775 and 1529, both carrying the antigen X, and also against the cells 1586 which carried W and 2471 which carried both antigens. The last two types of cell were included as a control on the process of absorption, and a further check that the two antibodies, anti-X and anti-W, would be absorbed out of the serum independently.

(2) *Preparation and testing of iso-antiserum to poorly agglutinating cells*

Two Z-positive rabbits (2403 and 1767) and two Y-positive (54 and 1517) rabbits whose red cells were poorly agglutinated by the appropriate antibody were chosen. Each animal was bled, the cells from each were washed thrice, made into a 50% suspension in saline and stored at $-20°$ C., giving four preparations which could be used as antigens. Four pairs of rabbits (two pairs of Z-negative and two pairs of Y-negative) were selected, and each pair was inoculated with the appropriate Z- or Y-positive frozen red cell material. Four courses were given consisting of six injections on alternate days of amounts ranging from 0·5 to 2 ml. The animals were bled 10 days after the second and fourth courses ended. One expects most

https://doi.org/10.1017/S0022172400000917 Published online by Cambridge University Press

rabbits to give a response to this course of inoculations when strongly agglutinating cells are used as the antigen. The resulting sera were tested neat against cells from the rabbits which had been bled for the antigen and against strongly agglutinating *Z*- or *Y*-positive cells. Any antibody found was titrated against strongly agglutinating cells and against the poorly agglutinating cells used as the antigen. The tests used were the direct agglutination test and the indirect sensitization test.

RESULTS

(1) *Comparative absorption experiments*

Anti-*Z* serum was absorbed with two different pairs of cells, each pair consisting of one strongly agglutinating cell and one poorly agglutinating cell. The results, one of which is given in Fig. 2, were very similar. The example given in Fig. 2

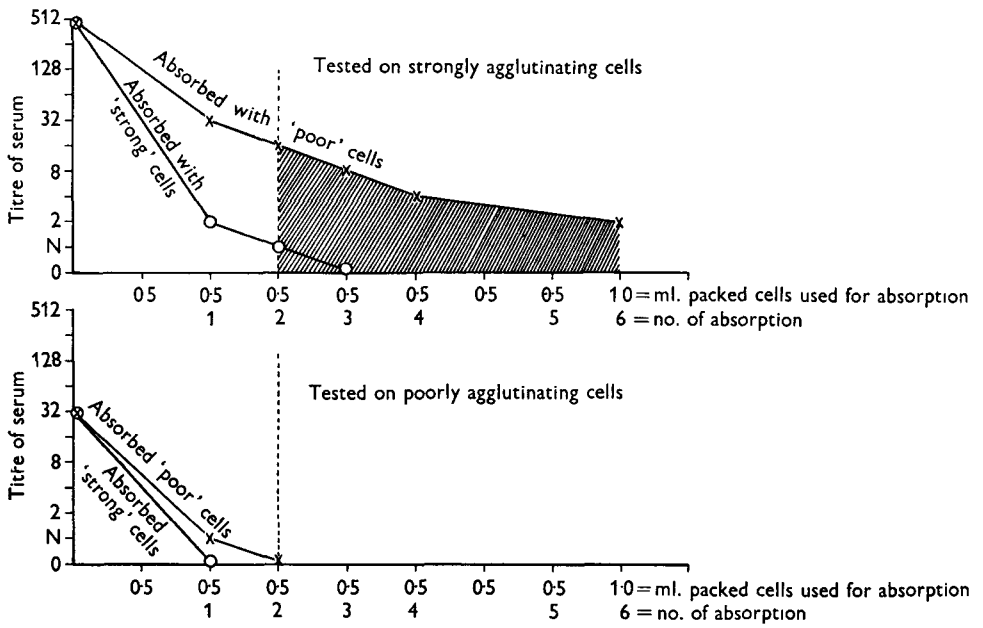

Fig. 2. Agglutination tests, following absorption of samples of a serum with strongly and poorly agglutinating red cells.

shows that serum absorbed with strongly agglutinating cells no longer agglutinated 'strong' cells after three absorptions, while poorly agglutinating cells were no longer agglutinated after one absorption. No cells from any of the rabbits on the panel were agglutinated by the serum absorbed six times with the strongly agglutinating cells.

However, the samples absorbed with the poorly agglutinating cells gave a rather different picture. They did not agglutinate 'poor' cells after two absorptions, but after six absorptions the strongly agglutinating cells were agglutinated quite markedly. The samples absorbed with poorly agglutinating cells were tested at

https://doi.org/10.1017/S0022172400000917 Published online by Cambridge University Press

intervals during their reabsorption against the cells of other rabbits on the panel; the poorly agglutinating cells ceased to be agglutinated after fewer absorptions than the strongly agglutinating cells. In fact, there was a 'hard core' of rabbits with 'strong' cells which continued to be agglutinated—albeit steadily more weakly—until the serum had had the full number of absorptions, by which time, however, the sample did not agglutinate any cells, whereas the sample absorbed with the control *Z*-negative cells had dropped in titre by only two serial dilutions. The poorly agglutinating cells had apparently absorbed out the antibody specifically. The shaded area in Fig. 2 shows the range over which the antibody can be detected only by strongly agglutinating cells.

The anti-*Y* serum was treated just as the anti-*Z* serum. The results were so like those obtained from the anti-*Z* serum that Fig. 2 can be taken as being representative of these too. The poorly agglutinating cells removed all the antibody after reabsorption, and after the same treatment the titre of the sample absorbed with the control cells was reduced by only two serial dilutions of serum.

The absorption of anti-*X* sera in the main followed the reactions of the other two sera, but though more poorly agglutinating cells were needed to remove the antibody, the 'zone' on Fig. 2, in which strongly agglutinating cells are agglutinated whilst 'poor' cells are not, did not appear. Two explanations are possible. One is that the poorly agglutinating cells used in this experiment were not so 'poor', or perhaps the strongly agglutinating cells were not so 'strong', as in the experiments on the anti-*Z* and anti-*Y* sera. There was a difference of only two serial dilutions of serum between the titres of the strongly and the poorly agglutinating cells, whereas with the anti-*Z* serum there was a difference of three dilutions and with the anti-*Y* serum the difference was four dilutions. The second explanation may be that in the experiment with anti-*X* sera equal volumes of packed cells and sera were used for the absorptions while in the experiments with the anti-*Z* and anti-*Y* sera the ratio of cells to serum was lower, so that more absorptions were needed to produce a comparable effect. The titre of the anti-*W* antibody which was also in the anti-*X* sera was not affected by seven absorptions with the *X*-positive cells, which were *W*-negative.

(2) *The inoculation of rabbits with poorly agglutinating cells*

Eight rabbits were inoculated, four with poorly agglutinating *Z* cells and four with poorly agglutinating *Y* cells. The number of rabbits which responded to the inoculation was surprisingly low; after four courses, only two rabbits produced an antibody against the cells with which they had been inoculated. These were *Y*-negative animals which had been inoculated with *Y*-positive cells. The two antisera were titrated, by direct agglutination, against (*a*) poorly agglutinating cells which had been used as the antigen injected and (*b*) strongly agglutinating *Y*-positive cells. In one serum (2156) the 'antigen cells' (54) gave a strong reaction only when the serum was used neat, while the titre after titrating with strongly agglutinating cells was 16. In the other serum (A 9) the 'antigen cells' (1517) showed a titre of 4, while the titre for strongly agglutinating cells was 64.

https://doi.org/10.1017/S0022172400000917 Published online by Cambridge University Press

DISCUSSION

In the foregoing experiments it is shown that the red cells from some rabbits agglutinate more firmly and to a higher titre in homologous iso-antisera than other cells of the same blood group. On investigation it can be demonstrated by direct measurement using radioactive iodine (Boursnell *et al.* 1955), and also indirectly by a method of comparative absorptions, that the cells which agglutinate poorly pick up less antibody from the serum than the cells which agglutinate strongly, but the cells agglutinating less well can absorb out from a serum all the antibody with which the strongly agglutinating cells react. There are several ways in which this phenomenon may be discussed, and these can be grouped under three headings. Variations in the agglutinability of a cell may be governed by the number of available antigen sites on the red cell surface, or by the disposition of the sites on the red cell surface. Thirdly, however, it may be due to cross-reactions between members of closely related antigen-antibody systems.

The third hypothesis will be examined first, not because it is the most attractive, but because it must be disproved before any other hypothesis can be seriously considered.

In the absorption experiments the fact that stands out against the theory of cross-agglutination is the ability of the poorly agglutinating cells—if enough of them are used—to absorb out the antibody completely. The paper of Landsteiner & van der Scheer (1936) serves as a good example to illustrate the behaviour of nearly related antigen-antibody systems. Though there is considerable cross-reaction between an immune serum to a particular azoprotein antigen and other such antigens sufficiently related in chemical structure, in general, absorption of a serum with a heterologous cross-reacting antigen may weaken the reaction of the homologous antigen but does not entirely remove it. However, the operative words are 'in general', and there are instances of a heterologous antigen removing the whole of the antibody. An example is the experiments in which Heidelberger & Kendall (1934) showed that a rabbit antiserum to a highly coloured *R*-salt-azo-benzidine-azo-egg albumin antigen (apparently free from uncombined egg albumin) would form a precipitate with egg albumin. Sometimes the egg albumin would not precipitate all the antibody to the dye antigen, but one antiserum was studied from which egg albumin, if added in sufficient amount, would precipitate all the antibody. However, only from one anti-dye serum could the antibody be completely removed by egg albumin. In1942 Haurowitz & Schwern were unable, after exhaustive absorptions, to remove cross-reacting antibodies with one antigen. They prepared a rabbit anti-arsanil sheep-serum globulin and absorbed samples of it exhaustively with arsanil ovalbumin, followed by sheep globulin. Each of these removed its own part of the total antibody from the serum, but left an antibody fraction which would precipitate the homologous arsanil sheep-serum globulin.

The other experiment which very strongly suggests that poorly agglutinating cells carry antigens of exactly the same specificity as the strongly agglutinating cells was that in which poorly agglutinating cells were used as antigens for injection. The resulting antiserum still agglutinated the two types of cell in the same ratio.

https://doi.org/10.1017/S0022172400000917 Published online by Cambridge University Press

That the agglutinability of the red cells of the rabbit is determined by the number of available antigen sites on the cell is a hypothesis that may perhaps best be explained by referring to Table 2, taken from a paper by van Loghem, Kresner, Coombs & Roberts (1950). The experiment was one in which human red cells carrying the *D Rh* antigen were sensitized with decreasing concentrations of incomplete anti-*D* serum. The sensitized cells were washed three times and then

Table 2. *Agglutination of Rh-positive red cells, sensitized with various dilutions of incomplete anti-D antiserum, by anti-human globulin sera*

(From van Loghem *et al.* 1950)

Anti-globulin serum no.	Dilutions of incomplete anti-*D* antiserum	Dilutions of anti-globulin serum in normal rabbit serum diluted 1:20													
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	1:8192	1:16.384
1914	1:64	++	++	++	++	++	++	++	++	++	++	++	++	++	−
	1:256	++	++	++	++	++	++	++	++	++	++	++	++	+	−
	1:512	++	++	++	++	++	++	++	++	++	++	++	++	+	−
	1:1024	++	++	++	++	++	++	++	++	++	++	±	±	−	−
	1:2048	++	++	++	+	±	±	−	−	−	−	−	−	−	−
	1:4096	+	+	±	−	−	−	−	−	−	−	−	−	−	−

titrated against an anti-human globulin serum. It will be clear from the table that there is a chequer-board of titrations of incomplete anti-*D* serum vertically and anti-globulin horizontally. As the concentration of the incomplete anti-*D* serum decreased, fewer antigen sites were available for the anti-globulin serum and the titre of the anti-globulin serum apparently fell. A direct analogy may be drawn between these experiments and the variation in agglutinability of rabbit cells. The strongly agglutinating cell offers many antigen sites to the antibody, the poorly agglutinating cell only a few. The results of the experiments with ^{131}I (Boursnell *et al.* 1953) would prove this hypothesis by direct measurement, if one accepts that the phenomenon is not due to cross-reactions between closely related antigen-antibody systems.

In human blood-grouping work it is well known that red cells carrying certain antigens in the homozygous state give a higher titre against homologous antisera than red cells carrying the same antigens in the heterozygous state. Examples of this effect of 'dosage' of antigen are shown by the *M* and *N* antigens (Landsteiner & Levine, 1927) and, from the *Rh* system, *c* (Race, Taylor, Boorman & Dodd. 1943), C^w (Callender & Race, 1946) and *e* (Mourant, 1945). This effect and some of its implications are discussed in a paper by Mourant (1947) on dominance and recessiveness in the human blood groups. Nevertheless, the expression of the concept of 'dosage' is complicated when applied to the rabbit cell. The antigens *Z* and *Y* are probably of an allelomorphic trio, the third being the antigen *W* (Heard. 1955). Among the fifty-two rabbits typed for these three antigens, two examples of strongly agglutinating cells have been *ZY* cells; the poorly agglutinating cells,

https://doi.org/10.1017/S0022172400000917 Published online by Cambridge University Press

however, have all been *ZW* or *YW* cells. In human blood-group work there are two examples in which the reactions of red cells to antisera of one blood-group system may be affected by their carriage of certain antigens of another system. Andresen (1948) has described depression of Le^b reactions produced by the presence of the A^1 antigen. More recently Ikin, Mourant & Pugh (1953) have described a serum which agglutinated saline suspensions of all A_1D positive cells and, less strongly, those of A_2D positive cells. All *O*, *B* and A_2BD positive cells were agglutinated in albumin but not in saline; but they ascribed this effect to the presence of two antibodies in the particular serum investigated.

The absorption experiments can be compared with the results of testing absorbed *Brucella* antisera with *Brucella* antigens. Miles & Wilson (1932) found that by the use of sera prepared against perfectly smooth strains it was possible, by agglutination absorption techniques, to divide the smooth *Brucella* antigens into two types, one predominant in *abortus* and the other in *melitensis* strains. They brought evidence to suggest that the distinction between *abortus* and *melitensis* strains is due, not to the presence in each of a qualitatively different antigen, but to the different quantitative distribution of two common antigens. If proper regard is paid to the relationship between the absorbing dose and the titre of the serum, monospecific sera can be prepared in which the major agglutinin of the type alone persists. By means of these sera unknown strains of the *Brucella* group can be rapidly typed by direct agglutination. Nevertheless, to quote from the third edition of Topley & Wilson, 'why it is that a serum from which the whole of the minor and part of the major agglutinins have been absorbed, will agglutinate only those organisms in which the corresponding antigen predominates is rather puzzling'. The authors suggest—and this was further substantiated by Miles (1939)—that failure of a critically absorbed serum to agglutinate the heterologous organism might be due to an insufficiency of available minor antigen sites on the organism. By reason of its paucity in *melitensis* sites *B. abortus*, when it is tested against a pure *melitensis* antiserum, may be likened to a poorly agglutinating rabbit red cell, but it behaves as a strongly agglutinating rabbit red cell when it is used with a pure *abortus* antiserum.

The conventional practice when using absorption techniques is to assume, after a serum has been absorbed with a particular antigen until the antigen ceases to show a reaction with the serum, that the antibody reacting with that antigen has been removed. In Fig. 2 one can see the results of absorbing a rabbit iso-antiserum with poorly agglutinating cells and then testing the serum with strongly and poorly agglutinating cells. The shaded area shows a zone where the poorly agglutinating cells are no longer agglutinated by the imperfectly absorbed serum but the strongly agglutinating cells are. The presence of this zone shows very clearly the possibility of making grave technical errors when trying to separate a number of red cell antibodies, all unknown, occurring simultaneously in rabbit iso-antisera. It also shows the dangers of using weak sera for grouping purposes.

Another example of the variation in agglutination of red cells is given by the behaviour of bovine red cells. Whilst working on bovine red cells, Gleeson-White,

https://doi.org/10.1017/S0022172400000917 Published online by Cambridge University Press

Heard, Mynors & Coombs (1950) found that red cells from certain oxen were not agglutinated by human infectious mononucleosis sera, whilst other bovine cells were agglutinated by these same sera; yet, all bovine red cells sensitized with these sera were fully susceptible to lysis in the presence of guinea-pig complement. These observations were also made when guinea-pig and rabbit anti-ox red cell sera were used to sensitize bovine cells. However, the sensitization of this 'in-agglutinable' type of ox red cell can be detected by treatment with trypsin. After further work Coombs, Gleeson-White & Hall (1951) put forward, and by experimental evidence supported, a hypothesis that the antigen receptors of the bovine red cell are situated at different levels on the cell wall and that the building up of anti-globulin lattices from the sensitizing antibody to the outer effective limit of the cell wall would bring about the agglutination of these cells.

That the depth of the antigen sites on the surface of the red cell may play some part in the agglutinability of rabbit cells is suggested by the observation that the titre of the serum by the indirect anti-globulin sensitization test is often as high with poorly agglutinating cells as with strongly agglutinating cells. However, the inagglutinable ox cell absorbs antibody out of a serum most efficiently, quite unlike the poorly agglutinating rabbit cell.

SUMMARY AND CONCLUSIONS

1. Rabbit cells show a marked variation in agglutination when reacting with apparently homologous iso-antisera.

2. This phenomenon has been investigated in two ways:

(*a*) Samples of apparently homologous antisera have been absorbed with (i) strongly agglutinating cells, (ii) poorly agglutinating cells. It was found that strongly agglutinating cells absorbed the antibody completely and rapidly from the serum, whilst poorly agglutinating cells, though they absorbed it completely, did so much more slowly. Also, when a serum which had been absorbed with poorly agglutinating cells until these no longer reacted with it was titrated against strongly agglutinating cells, these were still strongly agglutinated. This showed a source of serious error when investigating new sera by absorption techniques and when using weak antisera for grouping purposes.

(*b*) Poorly agglutinating cells were injected into rabbits, and the antisera so obtained were examined against strongly agglutinating cells and against the poorly agglutinating cell used as the antigen. The antibody response was poor; two out of eight rabbits developed an antibody, and strongly agglutinating cells reacted more strongly with these antisera than the poorly agglutinating cells which had been used as the antigen.

3. Three hypotheses for this variation in agglutination have been put forward and discussed:

(*a*) That the variation is due to cross reactions between numbers of closely related antigen-antibody systems. The results of the investigations 2(*a*) and 2(*b*) suggest that this is unlikely.

(*b*) That the disposition of antigen sites on the surface of the red cell is a factor

https://doi.org/10.1017/S0022172400000917 Published online by Cambridge University Press

determining the agglutinability of the cell. It is considered that this might play a subsidiary role in the phenomenon as it is shown in the rabbit.

(*c*) That rabbit red cell agglutinability is mainly determined by the number of antigen sites on the cell available to the antibody. The experimental results can be explained on this hypothesis, and it is suggested that this may be the main factor accounting for the behaviour of rabbit red cells. It is pointed out that supporting evidence for this is afforded by measurements with radio iodinated antisera.

This work formed part of a thesis for the degree of Ph.D. of the University of Cambridge and was done during the tenure of a grant from the Medical Research Council. I am sincerely grateful to Dr R. R. A. Coombs for his constant encouragement and the advice he gave me. I would also like to thank Prof. H. R. Dean for the interest he took in this work and the cordial hospitality of his laboratory.

REFERENCES

ANDRESEN, P. H. (1948). The blood group system *L*. A new blood group L_2. A case of epistasy within blood groups. *Acta path. microbiol. scand.* **25**, 728.

BOURSNELL, J. C., COOMBS, R. R. A. & RIZK, V. (1953). Studies with marked antisera. Quantitative studies with antisera marked with iodine ^{131}isotope and their corresponding red cell antigen. *Biochem. J.* **55**, 745.

BOURSNELL, J. C., HEARD, D. H. & RIZK, V. (1955). Studies with marked antisera. 2. Variation in the specific adsorption by red cells from different rabbits of one ^{131}I-marked rabbit iso-antiserum. *J. Hyg., Camb.*, **53**, 420.

CALLENDER, S. & RACE, R. R. (1946). A serological and genetical study of multiple antibodies formed in response to blood transfusion by a patient with lupus erythematosus diffuses. *Ann. Eugen., Lond.*, **13**, 102.

COOMBS, R. R. A., GLEESON-WHITE, M. H. & HALL, J. G. (1951). Factors influencing the agglutinability of red cells. 2. The agglutination of bovine red cells previously classified as 'inagglutinable' by the building of 'anti-globulin: globulin lattice' on the sensitized cells. *Brit. J. exp. Path.* **32**, 195.

GLEESON-WHITE, M. H., HEARD, D. H., MYNORS, L. S. & COOMBS, R. R. A. (1950). Factors influencing the agglutinability of red cells. The demonstration of a variation in susceptibility of agglutination exhibited by the red cells of individual oxen. *Brit. J. exp. Path.* **31**, 321.

HAUROWITZ, F. & SCHWERN, P. (1942). The valence of antibodies and the structure of the antigen-antibody precipitate. *Brit. J. exp. Path.* **23**, 146.

HEARD, D. H. (1955). The recognition of four red cell antigen-antibody systems in the rabbit. *J. Hyg., Camb.*, **53**, 398.

HEIDELBERGER, M. & KENDALL, F. E. (1934). Quantitative studies on the precipitin reaction. The role of multiple reactive groups in antigen antibody union as illustrated by an instance of cross precipitation. *J. exp. Med.* **59**, 519.

IKIN, E. W., MOURANT, A. E. & PUGH, V. W. (1953). An anti-Rh serum reacting differently with *O* and *A* red cells. *Vox Sanguinis*, **3**, 74.

KELLNER, A. & HEDAL, E. F. (1953). Experimental erythroblastosis fetalis in rabbits. 1. Characterisation of a pair of allelic blood group factors and their specific immune isoantibodies. *J. exp. Med.* **97**, 33.

KNOPFMACHER, H. P. (1942). A study of four antigenic components of rabbit erythrocytes. *J. Immunol.* **44**, 121.

LANDSTEINER, K. & LEVINE, P. (1927). Further observations on individual differences of human blood. *Proc. Soc. exp. Biol., N.Y.*, **24**, 941.

LANDSTEINER, K. & VAN DER SCHEER, J. (1936). On cross examination of immune sera to azo proteins. *J. exp. Med.* **63**, 325.

https://doi.org/10.1017/S0022172400000917 Published online by Cambridge University Press

LEVINE, P. & LANDSTEINER, K. (1929). On immune isoagglutinins in rabbits. *J. Immunol.* **17**, 559.

VAN LOGHEM, J. J., KRESNER, M., COOMBS, R. R. A. & FULTON ROBERTS, G. (1950). Observations on a prozone phenomenon encountered in using the anti-globulin sensitization test. *Lancet*, ii, 729.

MILES, A. A. (1939). The antigenic surface of smooth *Brucella abortus* and *melitensis*. *Brit. J. exp. Path.* **20**, 63.

MILES, A. A. & WILSON, G. S. (1932). The serological differentiation of smooth strains of the *brucella* group. *Brit. J. exp. Path.* **13**, 1.

MOURANT, A. E. (1945). A new rhesus antibody. *Nature, Lond.*, **155**, 542.

MOURANT, A. E. (1947). Dominance and recessiveness in the human blood groups. *Nature, Lond.*, **160**, 353.

RACE, R. R., TAYLOR, G. L., BOORMAN, K. E. & DODD, B. E. (1943). Recognition of Rh genotypes in man. *Nature, Lond.*, **152**, 563.

TOPLEY & WILSON'S *Principles of Bacteriology and Immunity* (1946). 3rd ed. p. 824. Arnold.

(*MS. received for publication* 14. II. 55)

https://doi.org/10.1017/S0022172400000917 Published online by Cambridge University Press