STUDIES WITH MARKED ANTISERA

2. VARIATION IN THE SPECIFIC ADSORPTION BY RED CELLS FROM DIFFERENT RABBITS OF ONE ^{131}I-MARKED RABBIT ISO-ANTISERUM

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(With 3 Figures in the Text)

INTRODUCTION

In a previous paper (Heard, 1955*a*) the identification and isolation of four distinct iso-antibodies in the rabbit which allowed four antigen-antibody systems to be distinguished has been described. It has also been shown (Heard, 1955*b*) that one apparently single iso-antibody caused different degrees of agglutination in red cells from different rabbits. This is unlikely to be due to cross-reactions between members of closely related antigen-antibody systems. The differences may, however, be a reflexion of the number of available antigenic sites on the red cells studied.

The successful use of radioactive iodine (^{131}I) as a quantitative marker for antisera to red cell antigens has been reported by Boursnell, Coombs & Rizk (1953). Iodine containing ^{131}I was introduced into a number of antisera without affecting the antigenic properties in any demonstrable way. It was shown that considerable differences in the amount of ^{131}I antibody specifically adsorbed by red cells in two distinct non-agglutinating systems, could most likely be related to a difference in the total number of available specific antigenic sites on the respective red cell surfaces.

It was felt that it might prove profitable to apply the radio-iodination method to the rabbit iso-antibody problem in an attempt to obtain more information about the relative number of antigenic sites on the rabbit red cell.

There is one particular advantage to be gained from comparisons in which only one antiserum is used. It has been pointed out (Boursnell *et al.* 1953) that two difficulties arise when the results obtained with different systems are compared. When saturation of the available sites is attempted, unless the molecular weights of the antibodies and their degree of iodination relative to that of whole-serum proteins are known in each case, the calculation, with these values assumed, of the number of antigenic sites, both relative and absolute, is open to correction. In this particular study, where merely the ratio of the number of antigenic sites on different homologous cells is being investigated by the use of only one ^{131}I antiserum, these difficulties are avoided.

This paper sets out the details and results of the application of the radioactive tracer technique to the problem outlined in the first paragraph.

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MATERIALS AND METHODS

(1) *Iodination of antiserum.* This was carried out as described by Boursnell *et al.* (1953), and the radioactivity determinations were also performed as in that paper.

(2) *Serological materials and methods.* (i) *Rabbit iso-antiserum.* This was a pool of samples from rabbit 1275 which had been absorbed with red cells from rabbits I and IX to remove traces of a secondary weak antibody (Heard, 1955*a*).

(ii) *Rabbit cells.* The cells from rabbits 1409, 2403 and 1511 were chosen as demonstrating a typical range of agglutinability. It seemed probable (Heard, 1955*a*) that the cells from rabbit 1511 were 'negative'—with the complete absence of the antigen under consideration. 4 ml. blood was taken by ear prick into 4 ml. 3·4 % (w/v) tri-sodium citrate solution. The cells were spun and washed with the citrate solution diluted with an equal volume of saline (0·9 % (w/v) NaCl), then twice more with saline. From the resulting packed deposit a 4 % suspension was made in normal rabbit serum (heat inactivated) to reduce the non-specific absorption of marked serum by the cells (cf. Boursnell *et al.* 1953). In some cases as indicated, the cells were washed with 3·3 % (w/v) $MgSO_4.7H_2O$ solution replacing the saline. Heard (1955*a*) has used this salt as a diluent for iso-antisera and red cells, and the specific adsorptions of ^{131}I iso-antiserum in the presence of this diluent or of saline are compared in this paper.

EXPERIMENTAL

(1) *'Titrations'.* The procedures adopted followed closely those of a normal serological titration but without the possibility of an end point. Three portions of ^{131}I rabbit antiserum 1275 were 'titrated' (using doubling dilutions in a constant volume of 0·2 ml.) against equal volumes (0·2 ml.) of 4 % suspensions, in normal rabbit serum, of red cells from rabbits 1409, 2403 and 1511 respectively. The ^{131}I serum was also diluted in heat-inactivated unmarked normal rabbit serum. The contents of each tube were mixed and incubated at 37° C. for 40 min. The tubes were then centrifuged and the cells washed three times with 2 ml. quantities of saline; the final deposits of cells were transfered to planchets for radioactivity determinations. The results are shown in Fig. 1. A comparison was also made (Fig. 2) by the above titration procedure of the effect of the replacement of normal saline by 3·3 % (w/v) $MgSO_4.7H_2O$ upon the specific adsorption of the ^{131}I rabbit iso-antiserum.

(2) *Attempted saturation of red cells and assessment of the relative numbers of antigenic sites on red cells from rabbits* 1409 *and* 2403. This was carried out essentially as in the experiments described by Boursnell *et al.* (1953), in which equal amounts of packed cell deposits were incubated for 20 min. periods a varying number of times with fresh quantities of the ^{131}I antiserum. After this treatment each sample of cells was washed three times with saline and transferred to the planchets.

0·5 ml. amounts of a 0·5 % suspension in normal rabbit serum of washed cells from rabbits 1409, 2403 and 1511 were placed in three sets of five tubes, each set corresponding to the cells from the individual rabbits. The supernatant serum was removed from the cell deposits after centrifuging, and the five cell deposits in each

set treated 1, 3, 5, 7 and 9 times respectively with 0·1ml. quantities of ^{131}I rabbit serum 1275 (diluted 1 in 4 with heat-inactivated pre-inoculation unmarked rabbit serum). The results of this experiment are shown in Fig. 3.

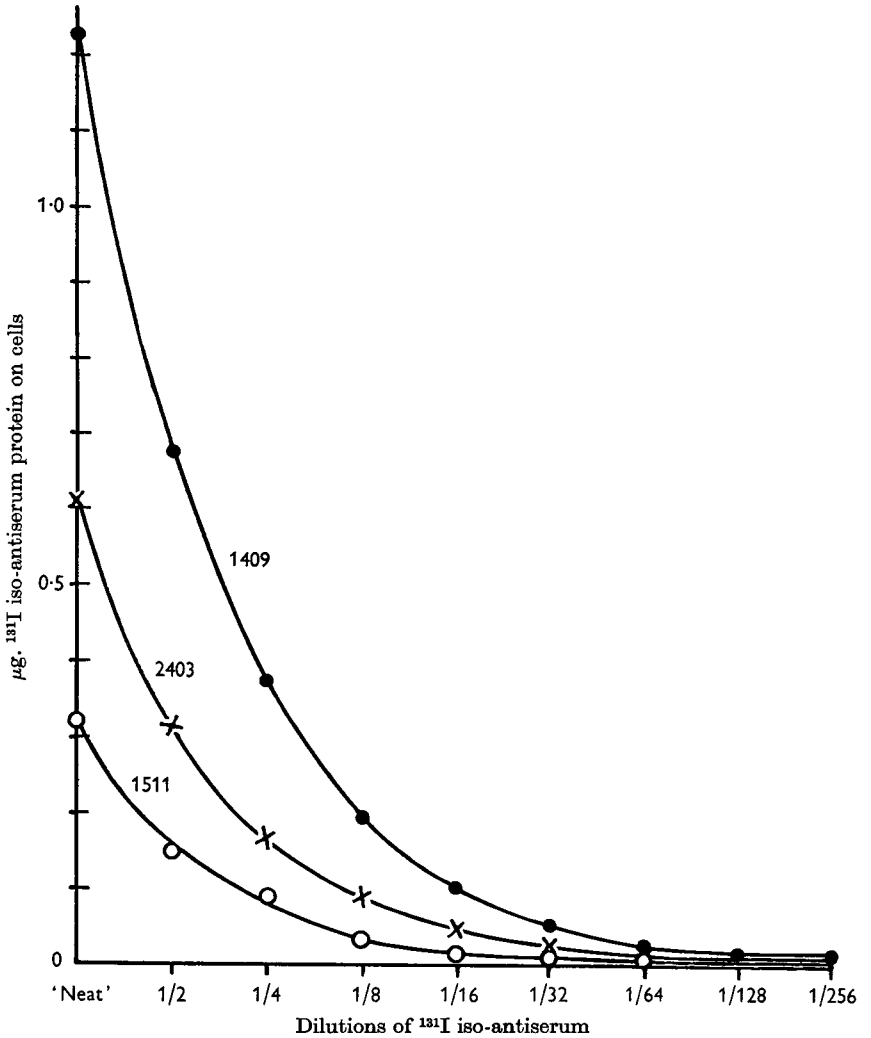

Fig. 1. 'Titration' of 0·2 ml. ^{131}I rabbit serum 1275 (absorbed prior to iodination) against 0·2 ml. 4 % suspensions of rabbit cells 1409, 2403 and 1511 in heat-inactivated normal rabbit serum ('neat', 2·3 % protein).

DISCUSSION

The results of the 'titration' and saturation experiments given in this paper suggest that, if the antibody in the rabbit serum 1275 is a single one, the variations encountered between the cells from rabbits 1409 and 2403, compared with those from the 'negative' rabbit 1511, are due to differences in the number of functional antigenic sites on the cell surface.

If it is accepted that there is only one antibody in the serum under investigation, the difficulties in calculation mentioned above do not arise when a computation is

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made of the ratio of the numbers of antigenic sites on cells from different animals by the use of portions of the same ^{131}I antiserum. Under these conditions the expression for the number of sites (S) on a single red cell

$$S = \frac{P \times N}{\text{mol. wt.} \times 10^6 \times N \times R},$$

given by Boursnell *et* al. (1953) reduces to

$$\frac{S_1}{S_2} = \frac{\frac{P_1}{N_1} - \frac{P_{\text{neg.}}}{N_{\text{neg.}}}}{\frac{P_2}{N_2} - \frac{P_{\text{neg.}}}{N_{\text{neg.}}}},$$

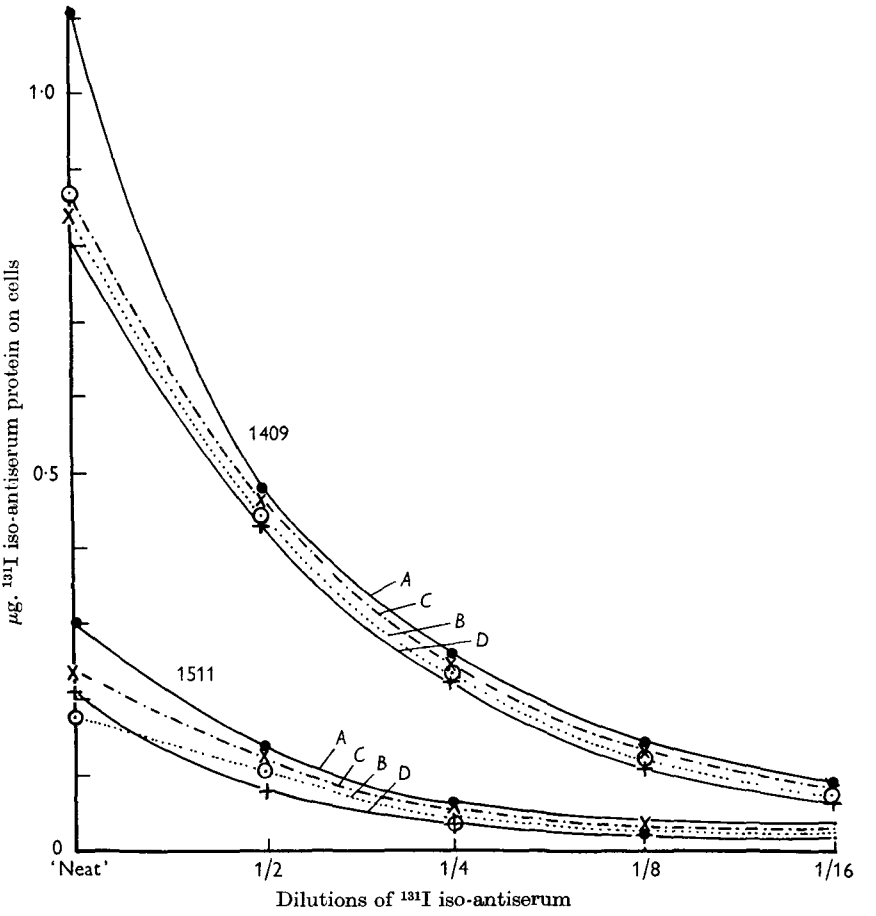

Fig. 2. Effect of saline and 3·3 % (w/v) $MgSO_4.7H_2O$ solution on absorptions of ^{131}I rabbit iso-antiserum 1275 by rabbit cells. 'Titration' of 0·2 ml. ^{131}I rabbit serum 1275 (absorbed prior to iodination) against 0·2 ml. 4 % suspensions of cells from rabbits 1409 and 1511 in heat-inactivated normal rabbit serum. ^{131}I serum dialysed against saline ('neat', 2·6 % protein). Curve *A*, cells washed in saline; curve *B*, cells washed in $MgSO_4$ solution. ^{131}I serum dialysed against $MgSO_4$ solution ('neat', 2·7 % protein); curve *C*, cells washed in saline; curve *D*, cells washed in $MgSO_4$ solution. The cells used with the ^{131}I serum dialysed against $MgSO_4$ solution were washed in the fluid before being made up in saline.

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where S_1/S_2 = the ratio of the number of sites on cells from rabbits 1409 and 2403 respectively, P_1, P_2 and $P_{neg.}$ = weight (μg.) of antibody absorbed by each type of cell at saturation, N_1, N_2 and $N_{neg.}$ = number of cells of each type used. The subscript 'neg.' refers to values for the 'negative' cells from rabbit 1511.

Calculations on the basis of this expression using the results given in Fig. 3 show that there are about six times the number of sites per cell on 1409 as there are on 2403 cells. In another similar experiment a ratio of seven was obtained for the cells from the same rabbits.

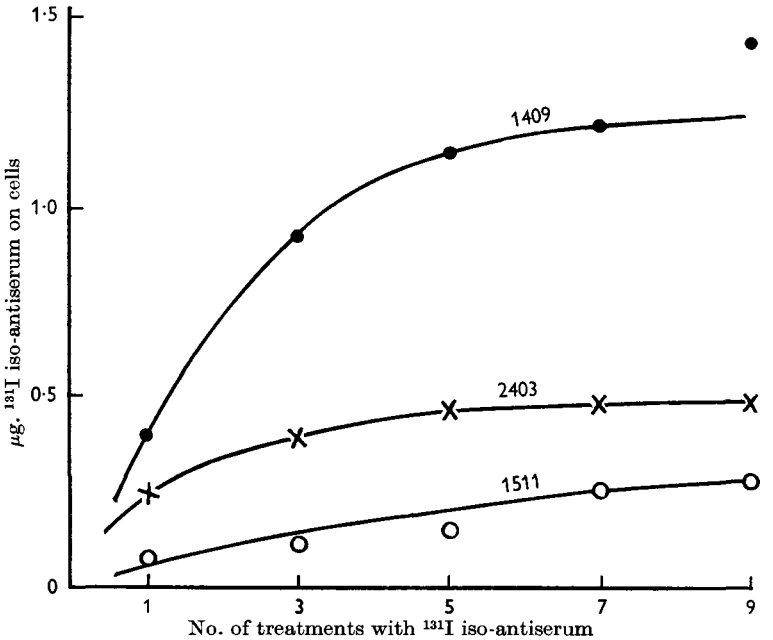

Fig. 3. Attempted saturation of the functional antigenic sites on rabbit cells 1409 and 2403. Repeated treatments of 0·5 ml. 0·5 % suspensions of the washed cells (in heat-inactivated unmarked normal rabbit serum) with 0·1 ml. quantities ^{131}I rabbit iso-antiserum 1275 (absorbed prior to iodination) (protein, 3·6 %). Number of cells used ($\times 10^7$): 1409, 3·6; 2403, 4·56; 1511, 4·4.

It is interesting to note that the $MgSO_4$ solution which has been found in some respects to be superior to a saline medium (Heard, Hinde & Mynors, 1949) makes no difference to the amount of ^{131}I antibody absorbed by the cells. This would seem to suggest that, whatever is the cause of the 'stickiness' of the red cells mentioned by Heard *et al.* (1949), it cannot primarily be non-specific absorption of protein.

SUMMARY

1. An apparently pure rabbit iso-antibody marked with ^{131}I has been shown to adsorb specifically to a greater extent on some rabbit cells than on others. This accords with previous evidence that the same iso-antibody exhibits a higher titre with the cells which are shown here to absorb more marked antibody than with those which adsorb less.

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2. Saturation of two of these red cell types by repeated application of fresh quantities of the ^{131}I iso-antibody until no more is specifically adsorbed, suggests that the number of functional antigenic sites is six or seven times greater on the one than on the other cell.

3. There is no apparent difference in the amount of ^{131}I iso-antibody adsorbed by any one type of rabbit cell in comparative titrations using 3·3 % $MgSO_4.7H_2O$ medium instead of normal saline.

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REFERENCES

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 antigens. *Biochem. J.* **55**, 745.

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

Heard, D. H. (1955*b*). Factors influencing the agglutinability of red cells. Variation of the red cells of the rabbit in susceptibility to agglutination by homologous iso-antisera. *J. Hyg., Camb.*, **53**, 408.

Heard, D. H., Hinde, I. T. & Mynors, L. S. (1949). An experimental study of haemotitic disease of the newborn due to iso-immunization of pregnancy. I. An attempt to produce the disease in the rabbit. *J. Hyg., Camb.*, **47**, 119.

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