The sera of all humans contain the isoagglutinins anti-A and anti-B, except when the corresponding agglutinogens are present in the red cells. Thus the sera of group O persons contain both anti-A and anti-B and the sera of group A persons anti-B alone. There are rare negative exceptions to this general principle, some of which are probably genetically determined (Race and Sanger, 1958). The factors determining the development of the isoagglutinins are not fully understood. The infant is born with its mother’s agglutinins, but commences to form its own in the first few months of life (Yliruokanen, 1958). These ‘natural’ isoagglutinins are not associated with isohaemolysins.

Thomsen and Kettel (1929) determined the titres of the anti-A and anti-B agglutinins in the blood of Danish subjects and found that the anti-A titres were generally higher than the anti-B titres. The anti-A in group O sera was of higher titre than the anti-A in group B sera, but no difference was found with anti-B in group O and group A sera.

Cutbush, Falconer and Mollison (1950) found that about 20% of 125 group O sera contained \( \alpha \)-haemolysins. These haemolysins arise following injection of human serum (Aubert, Boorman and Dodd, 1942), purified blood group specific substances from animal tissues (Witebsky, Klendshoj and McNeil, 1944) or from human sources (Loutit and Morgan, 1946), serum containing antitoxins and antibodies against bacteria (Davidsohn, 1938), prophylactic bacterial vaccines (Cutbush et al., 1950) and in cases of heterospecific ABO pregnancies (Smith, 1945). Coombs and his coworkers (Winstanley, Konugres and Coombs, 1957; Konugres and Coombs, 1958) have demonstrated that sera containing haemolysins agglutinate red cells from pigs with the A antigen and that the haemolysins, but not the ‘natural’ anti-A agglutinins, are removed by absorption of the sera with such pig red cells. They postulate that the haemolysins and the ‘immune’ agglutinins may act on an antigen called A' present in human red cells and in A pig red cells.

The work to be reported in this paper had three objects. The first was to compare the titres of anti-A and anti-B in sera from group B and from group A.
subjects with the titres in sera from group O subjects. The second was to
determine whether titration of anti-A and anti-B in a viscous medium (polyvinyl-
pyrrolidone) increases the titre. It has been reported that the titre of immune
sera is enhanced when the titration is performed using serum instead of saline
as the diluent (Boorman, Dodd and Morgan, 1945). The third purpose of the
investigation was to determine, using freshly collected group AB serum, the
incidence of immune haemolysins in the sera of group O, group A and group B
Australian blood donors.

METHODS

On each day the agglutinin titres were determined in an equal number (usually four) of
group O, group A and group B sera. The anti-A titre was measured against both A1 and A2
red cells and the anti-B titre against B cells. The A1 and A2 cells were obtained daily from
the same subjects and the B cells from either of two subjects whose cells were shown to give
the same titre with a given serum.

Red cell suspensions: These were prepared in freshly collected group AB serum. Blood
was collected into physiological saline solution from the fingers of the subjects and the red
cells washed twice with saline before being added to AB serum so as to give a two per cent
cell suspension.

Sera: Sera of appropriate groups were selected at random from samples obtained from
adult blood donors. The sera were not activated before use and were free of haemoglobin.

Titration of sera: Dilutions of the sera were prepared in "3 × 3/8" test tubes and
doubling dilutions were made with a Pasteur pipette for a total of 12 tubes. One drop of
each dilution was added to one drop of the cell suspensions in similar tubes. Two titrations
were made of each serum against the same cells and to each tube of the second 2 drops of
10% polyvinylpyrrolidone (Polyvidone) were added. This material was used by Ward (1953)
for detecting the presence of incomplete anti-D agglutinins.

The cell-serum mixtures were placed at 37°C for 1 hour and the tubes then centrifuged
for 1 minute at 500 rpm. The first tubes in each titration were examined for the presence
of free haemoglobin in the supernatant fluid and when this was found the titre of the haemo-
lysin was recorded at the dilution of the serum in the last tube showing evidence of haemo-
lysis. The contents of the tubes without Polyvidone were examined by transferring the cells
to a microscope slide with a Pasteur pipette. The end-point of the titrations was recorded
as the last dilution of the serum, prior to the addition of the red cell suspension, in which
microscopic agglutination was detected. After being centrifuged the tubes containing Polyvi-
done were rotated between the hands (as described by Ward, 1953) to disperse completely the
button of cells at the bottom of the tube. The cells were then examined with the microscope
and the end-point recorded as before.

RESULTS

The mean titres and the distribution of the titres are shown in Table 1 and
the mean titres are compared in Fig. 1. The same number (47) of group O,
group A and group B sera were examined. It is clear from these results that
(1) the mean titres of both anti-A and anti-B are higher in group O sera than
in group B and group A sera respectively; (2) the mean titres of anti-B in both
group O and group A sera are less than the mean titres of anti-A against A1
cells in group O and group B sera respectively; (3) the mean titres of anti-A
against A2 cells are approximately one-fifth of those against A1 cells.

In Fig. 2 the titres of anti-A against A1 and A2 cells have been plotted for
the 47 group O and 47 group B sera. It is evident that there is good correlation
between the titres; when the titre against A1 cells is high it is also relatively
Table 1. Distribution of end points in titrations of iso-agglutinins

<table>
<thead>
<tr>
<th>Titre</th>
<th>Group B serum (anti-A)</th>
<th>Group O serum (anti-A and anti-B)</th>
<th>Group A serum (anti-B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>v. A_1 cells</td>
<td>v. A_2 cells</td>
<td>v. A_1 cells</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>5</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>32</td>
<td>10</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>64</td>
<td>8</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>128</td>
<td>11</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>256</td>
<td>4</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>512</td>
<td>5</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>1024</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2048</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

| Mean | 169.4 | 34.1 | 242.9 | 47.2 | 195.1 | 67.3 |

Fig. 1. The mean titres of anti-A and anti-B in group O, group A and group B sera.
Fig. 2. The relation between the titres of anti-A against A1 cells and against A2 cells.

Fig. 3. The titration of iso-hemoagglutinin and iso-haemolysin in a viscous medium.
high against A2 cells and vice versa. One serum is of particular interest because its titre against A1 cells was 8 but no agglutination was obtained with the A2 cells used. Agglutination was, however, obtained against the same cells in the titration with Polyvidone. (Fig. 3).

The incidence of isohaemolysins is shown in Table 2, from which the following points can be seen. (1) Anti-A haemolysins were much more common than anti-B haemolysins. (2) Nearly half the group O sera (23 out of 47) contained anti-A haemolysins compared with 27.6% of the group B sera. (3) Anti-A haemolysins were more frequently found against A1 cells than against A2 cells. (4) When haemolysins were present the mean agglutinin titre of the sera was in general higher than when haemolysins were absent. (5) The haemolysin titres were low and not significantly different in any of the groups.

Table 2. The incidence and titre of iso-haemolysins

<table>
<thead>
<tr>
<th></th>
<th>Group B serum (anti-A)</th>
<th>Group O serum (anti-A)</th>
<th>Group A serum (anti-B)</th>
<th>Group O serum (anti-B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>v. A1 cells</td>
<td>47</td>
<td>47</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>v. A2 cells</td>
<td>47</td>
<td>47</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Number of sera examined</td>
<td>13</td>
<td>23</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Percentage</td>
<td>27.6</td>
<td>48.9</td>
<td>8.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Mean titre of saline agglutinins in sera with haemolysins</td>
<td>425.8</td>
<td>317.2</td>
<td>72.0</td>
<td>576.0</td>
</tr>
<tr>
<td>Mean titre of haemolysins</td>
<td>4.0</td>
<td>2.7</td>
<td>2.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Mean titre of saline agglutinins in sera without haemolysins</td>
<td>71.4</td>
<td>30.6</td>
<td>171.7</td>
<td>178.2</td>
</tr>
</tbody>
</table>

It is also of interest that both group O sera with anti-B haemolysins contained anti-A haemolysins. Of the 141 sera examined, 97 were from males and the incidence of haemolysins in these sera was 35%. Only 6, or 14.7%, of the 34 females showed evidence of haemolysins.

The titrations with polyvidone gave equivocal results when compared with the standard titrations of the same sera. In a few instances the titres were increased by as many as four or five tubes by the addition of polyvidone. However, when haemolysins were present the increase in titre was usually nil and never more than one tube. This suggests that enhancement of the titre does not necessarily indicate the presence of immune agglutinins.

**DISCUSSION**

Two interesting features of the above results, which in general confirm the observations of other workers, are firstly the predominant role of anti-A compared with anti-B, and secondly the high titres and frequent haemolysins in group O sera. The factors responsible for the development of anti-A and anti-B
are not clearly understood. Some workers believe that their presence or absence is genetically determined. Others suggest that they are the result of immunization in early life against the A and B antigens. They point out that agglutinins are absent in newborn infants unless they are also present in the maternal serum, and that the infant's own agglutinins develop during the first few months of life. Proponents of the genetic theory would postulate that the genes controlling the presence or absence of anti-A are more effective than those controlling the presence of anti-B. On the other hand, if the immunological theory is favoured the findings would suggest either that A is a more powerful antigen than B, or that it is more widespread in food, bacteria and other substances ingested or inhaled by man. The greater frequencies of anti-A haemolysins lend some support to the immunological theory as haemolysins are unquestionably the result of immunization.

The other interesting feature, the importance of group O in relation to the titre of the isoagglutinins, is also difficult to explain. The findings suggest that the development of one agglutinin is enhanced by the simultaneous development of the other. On the basis of an immunological origin this theory is supported by the well-known fact that specific absorption of one agglutinin from group O sera usually results in nonspecific removal of the other. Furthermore, immunization with A or B substances usually stimulates both anti-A and anti-B in group O sera. On a genetic basis it could be postulated that the presence and expression of the gene determining the development of an agglutinin are enhanced by the operation of the gene determining the development of the other agglutinin.

The anti-A titrations are thought to involve two agglutinins, anti-A1 (which acts only on A1 cells) and anti-A (which acts on all A cells but alone on A2 cells). Obviously anti-A1 is a more powerful agglutinin than anti-A because its titres were higher in both group O and group B sera.

The variable incidence of haemolysins in the different groups is interesting. Immunization as a result of pregnancy would be expected to be more common amongst group O women (de Burgh, Sanger and Walsh, 1947; Walsh and Kooptzoff, 1954) but this has not been a factor in the present series where haemolysins were less frequent in the female than in the male subjects. One can only speculate that the group O subjects are rendered more susceptible by unknown factors during intra-uterine or extra-uterine life to subsequent immunization by A and B antigens in food, bacteria, vaccines and prophylactic antisera.

**SUMMARY**

The titres of anti-A and anti-B isoagglutinins have been measured in the sera of 47 group O, 47 group A and 47 group B Australian blood donors. It was found that the titres of both agglutinins were higher in group O subjects than in group A and group B subjects, and that the anti-A against A1 cells was higher than the anti-B. The incidence of haemolysins varied in the different groups, with the highest in group O subjects (49%) and the lowest in group B subjects (9%). Anti-A haemolysins were more frequent than anti-B haemolysins.

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REFERENCES


* Director of The National Blood Transfusion Centre of Australia.