

# SOME STUDIES ON THE NATURALLY OCCURRING LEUCOCYTE AGGLUTININS<sup>1, 2</sup>

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The purpose of this report is to describe some investigations which attempt to define the nature and incidence of naturally occurring leucocyte agglutinins.

Agglutinins have been demonstrated in the sera of patients with acquired hemolytic anemia (1) and thrombocytopenic purpura (2, 3) and these have been distinguished from naturally occurring anti-red cell (4) and anti-platelet (5) agglutinins. Leucocyte agglutinins have been demonstrated in various clinical conditions including lupus erythematosus (6), aleukemic leukemia (6), pancytopenia (6, 7), agranulocytosis due to pyrimidin (8) and chronic neutropenia (9). Some have implicated these agglutinins as the causative factors for the leukopenia in these diseases.

Goudsmit and van Loghem (6) tested the sera of 100 healthy donors against type O white cells. They found weakly positive clumping reactions in 10 instances. Dausset, Nenna, and Brecy (10) studied the sera of 300 normal donors for the presence of agglutinins to compatible leucocyte suspensions and failed to demonstrate any. Dausset (11) cited some unpublished experiments in collaboration with van Loghem in which combinations of 50 normal sera and 50 corresponding varieties of white cells were tested for leucocyte agglutination. They observed frequent but inconsistent agglutinations in the combinations which were incompatible for the ABO blood groups. Moeschlin and Schmid (12) tested blood samples of 30 persons in 380 leucocyte serum combinations without regard for A, B and O blood groups. They noted leucocyte agglutination in 60 cases of

the 380 combinations. Ninety-seven per cent of these agglutinations occurred in leucocyte serum combinations which were incompatible with respect to the A, B, and O, blood groups and they concluded that this agglutination was caused by the blood group specific iso-agglutinins. This inference was made in spite of the fact that leucocyte agglutination was absent in two-thirds of the combinations in which it would have been expected on the basis of intensive erythrocyte agglutination. They thought that it was improbable that a low serum titer of group specific erythrocyte agglutinins could be responsible for the absence of leucocytic agglutination.

## METHODS

Plasma was handled with sterile technique and was stored at  $-20^{\circ}\text{C}$  until used. Sequestrene was used as an anticoagulant throughout. All glassware was silicized and the metal parts were coated with arquad each time the equipment was used. The silicone was stripped from the glassware after each usage by soaking in 10 per cent Na OH solution. Blood was collected for the preparation of white cells in the following manner: A needle was inserted in the antecubal vein and blood was allowed to flow up to the mark of a 15-ml. centrifuge tube containing 1 ml. of 1 per cent sequestrene and 1.25 ml. of Dextran having an intrinsic viscosity greater than .41. The centrifuge tube was covered with parafilm and inverted gently three times. The blood was allowed to settle for about 30 minutes and the supernatant was removed. The white cell counts were usually between 10,000 and 15,000 per cu. mm. This plasma contained platelets and about equal numbers of red cells and white cells. The plasma samples and leucocyte suspensions were adjusted to pH 6.9 by the addition of 1/9 N citric acid. Methyl orange was used as an indicator and about 1 drop of acid was required for each .4 ml. of plasma and about .3 ml. of acid for each 7 ml. of leucocyte suspension.

When a diluent was required in preparing serial dilutions, a non-reacting type A plasma of low titer was used after it had been absorbed with type B cells.

*Test for leucocyte agglutinins.* This test was performed by mixing .15 ml. of white cell suspension with .40 ml. of test plasma and one drop of 30 per cent bovine albumin in a  $10 \times 75$  mm. serological test tube. This was incubated in a water bath at  $37^{\circ}\text{C}$  and read after two

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TABLE I

*Protocol and results of a positive test for the presence of a leucocyte agglutinin inhibitor in saliva*

| TEST PLASMA<br>ML. | SALIVA<br>ML. | CONTROL PLASMA<br>ML. | CELLS<br>ML. | RESULT<br>AGGLUTINATION |
|--------------------|---------------|-----------------------|--------------|-------------------------|
| .40                | —             | —                     | .15          | ++++                    |
| .394               | .006          | —                     | .15          | 0                       |
| .388               | .012          | —                     | .15          | 0                       |
| .375               | .025          | —                     | .15          | 0                       |
| .35                | .05           | —                     | .15          | 0                       |
| .30                | .10           | —                     | .15          | 0                       |
| .20                | .20           | —                     | .15          | 0                       |
| —                  | —             | .40                   | .15          | 0                       |
| .394               | —             | .006                  | .15          | ++++                    |
| .388               | —             | .012                  | .15          | ++++                    |
| .375               | —             | .025                  | .15          | ++++                    |
| .35                | —             | .05                   | .15          | +++                     |
| .30                | —             | .10                   | .15          | ++                      |
| .20                | —             | .20                   | .15          | +                       |

hours. Each test for leucocyte agglutination was performed on at least two and in some cases three different occasions. The leucocytes were resuspended after incubation by shaking the test tube quickly about six times. A drop was poured on a glass slide and allowed to flow out in a thin layer by tilting the slide. The slide was studied for white cell clumping under the low power of the microscope either with reduced light or with phase microscopy.

*Erythrophagocytic index.* After the result of the test for leucocyte agglutinins was read microscopically, there was a considerable portion of the test material left in the serological test tube. This was allowed to resettle for two hours, and dry smears were made of the sediment on cover slips which were stained with Wright's stain. Five hundred white cells were counted and the number of leucocytes which had ingested red cells was noted. The percentage which contained red cells was designated the erythrophagocytic index (13). An index of two or above was arbitrarily designated as significant.

*Test for inhibition by specific polysaccharide of blood group A.* The naturally occurring leucocyte agglutinins were tested for inhibition by adding one drop of specific polysaccharide of blood group A reagent<sup>3</sup> to .4 ml. of the type O or type B plasma which was to be tested. This was incubated for 30 minutes at 25° C before being added to the leucocyte suspension as in the test for leucocyte agglutinins. A positive control test for leucocyte agglutinins contained in the type O or type B plasma was always run. A second control consisted of one drop of specific polysaccharide of blood group A reagent being incubated with the type O or type B plasma before addition of a 2 per cent suspension of type A red cells in saline.

*Test for the potency of the specific polysaccharide of blood group A reagent.* Equal volumes of this substance were added to the serial dilutions of anti A blood grouping plasma and were incubated for 30 minutes at 25° C before the type A test cells were added. The specific polysaccharide reagent was found to inhibit the anti-A blood grouping plasma at a titer of 1:8.

*Test for salivary antigens.* Saliva was tested for the presence of A and B substances as follows: The saliva in a test tube was placed in a boiling water bath for 20 minutes. It was then centrifuged and the clear supernatant liquid was diluted serially with saline. One-tenth ml. of this material was added to .1 ml. of anti A or anti B typing sera and allowed to stand at room temperature for 30 minutes. Then, .1 ml. of a 2 per cent suspension of A or B cells was added. After 30 minutes the tubes were centrifuged and examined for agglutination. A control tube contained no saliva and another control contained saliva of a known secretor. In the first control there was a 4+ agglutination and the same was found in all dilutions of the unknown when they were non-secretors. Secretors were defined as those whose saliva inhibited agglutination in a dilution of 1:8. The typing sera was adjusted with saline so that a titer of 1:128 produced a

trace of clumping when tested against the appropriate red cells.

*Test for salivary inhibition.* The saliva of the white cell donor which was tested for its ability to inhibit the leucocyte agglutinin was heated for 30 minutes at 100° C to destroy any enzymes present. This saliva was then serially diluted with the plasma containing the agglutinin before being added to the leucocyte suspension and albumin as in the test for leucocyte agglutinins. The effect of dilution was controlled by substituting a non-reacting plasma for the saliva. Table I illustrates an example of complete inhibition of an agglutinin by a white cell donor's saliva.

*Test for hemolysins.* Hemolysins were tested for in the following manner: The serum to be tested was inactivated at 56° C for thirty minutes after which .5 ml. portions of serial 2 fold dilutions in .85 per cent sodium chloride were added to each of nine tubes. Normal saline was added to the tenth tube which served as a control. One-tenth ml. of fresh, normal, unheated AB serum (diluted with an equal part of saline) and .5 ml. of a 3 per cent saline suspension of thrice washed test cells were added to each of the ten tubes, which were then shaken. The mixtures were placed in a water bath at 37° C for thirty minutes, centrifuged for one minute at 1,500 RPM and examined for the presence of hemolysis.

*Absorption of the C antibody of group O plasma.* Plasma from a group O donor was absorbed with group B cells. The cells used for absorption were washed three times with normal saline solution and then packed. One volume of plasma was mixed with one volume of these cells and placed in a water bath at 37° C for one-half hour, then allowed to stand at room temperature for one-half hour, then centrifuged and the plasma re-

<sup>3</sup> Kindly furnished for use by Sharpe and Dohme Co.

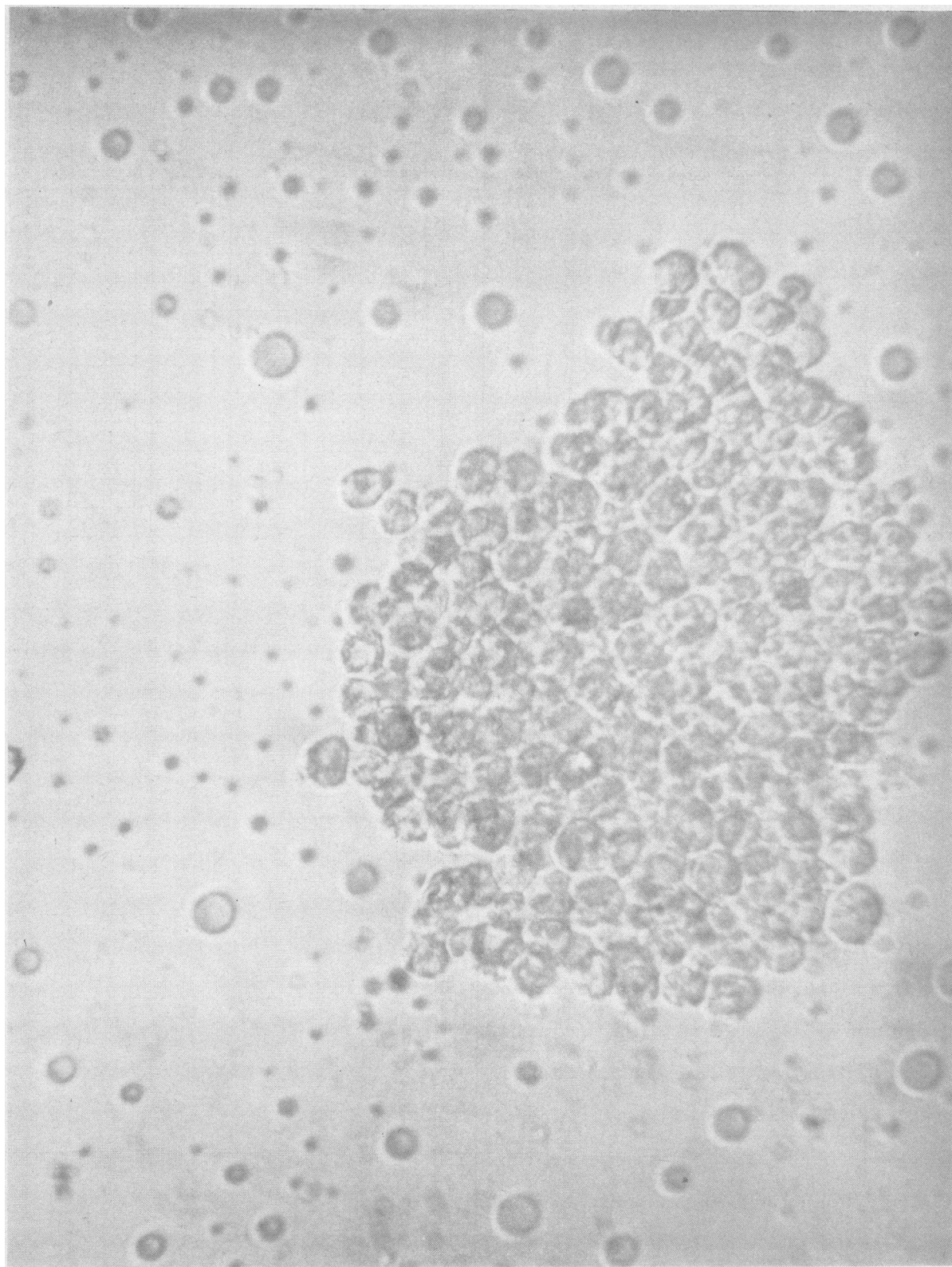


FIG. 1. PHOTOMICROGRAPH UNDER HIGH DRY MAGNIFICATION SHOWING A CLUMP COMPOSED ENTIRELY OF LEUCOCYTES

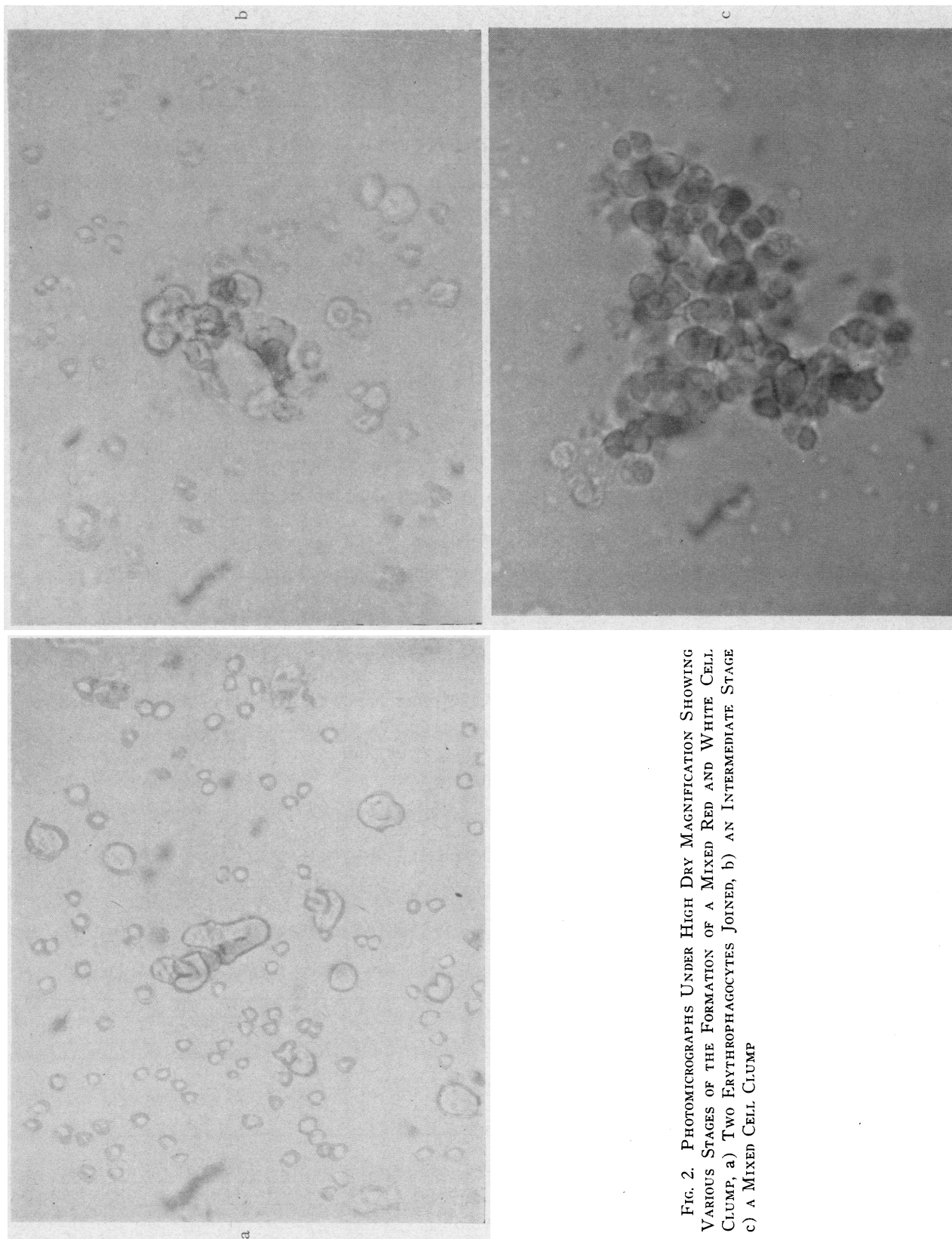


FIG. 2. PHOTOMICROGRAPHS UNDER HIGH DRY MAGNIFICATION SHOWING VARIOUS STAGES OF THE FORMATION OF A MIXED RED AND WHITE CELL CLUMP, a) TWO ERYTHROPHAGOCYTES JOINED, b) AN INTERMEDIATE STAGE c) A MIXED CELL CLUMP

TABLE II

*The results of tests for leucocyte agglutination (A) and erythrophagocytosis (E), (as measured by the erythrophagocytic index) repeated for four successive days are compared*

| REPRODUCIBILITY<br>ON SUCCESSIVE DAYS |                       |                                    |     |      |    |      |    |      |    |      |
|---------------------------------------|-----------------------|------------------------------------|-----|------|----|------|----|------|----|------|
| PLASMA<br>DONOR<br>TYPE               | CELL<br>DONOR<br>TYPE | PLASMA<br>DONOR<br>ANTI-B<br>TITER | DAY |      |    |      |    |      |    |      |
|                                       |                       |                                    | 1   |      | 2  |      | 3  |      | 4  |      |
|                                       |                       |                                    | A   | E    | A  | E    | A  | E    | A  | E    |
| O-                                    | B +                   | 1:256                              | 2+  | 26   | 2+ | 13.6 | 3+ | 0    | 2+ | 10.4 |
| O-                                    | B +                   | 1:64                               | 2+  | 42.8 | 3+ | 25.2 | 2+ | 10.4 | 2+ | 24.4 |
| O+                                    | B +                   | 1:256                              | 2+  | 15.2 | 2+ | 8.8  | 1+ | 0    | 1+ | 6.0  |
| A+                                    | B +                   | 1:128                              | 3+  | 0    | 2+ | 0    | 1+ | 0    | -  | 0    |
| A+                                    | B +                   | 1:256                              | 4+  | 0    | 2+ | 0    | -  | 0    | -  | 0    |

moved. This plasma was then tested for leucocyte agglutinins and erythrophagocytosis against white cell suspensions from group A donors.

Controls of unabsorbed plasma from the same group O donor were tested simultaneously.

*Test for the ability of plasma to cause erythrophagocytosis in the presence of incompatible red cells.* Red cells which were incompatible with respect to the ABO group of the plasma being tested were washed three times in large quantities of physiological saline. One-half ml. of a 2 per cent suspension of these red cells was centrifuged and the supernatant was discarded. One-half ml. of a leucocyte suspension in plasma collected in the usual manner was added to the sedimented red cells. These were resuspended by gentle agitation and incubated for two hours at 37° C. This preparation was examined for clumping; and, after resettling for one hour, smears of the sediment were made. These smears were stained with Wright's stain and examined for erythrophagocytosis.

#### RESULTS

To date, a total of 750 tests for leucocyte agglutination have been performed, and the erythrophagocytic index has been determined on 450 of these. It was noted in this series that positive agglutination reactions involving leucocytes and plasma which were compatible for the ABO blood groups resulted in clumps composed entirely of leucocytes (Figure 1). These clumps were predominantly composed of granulocytes but the presence or percentage of mononuclear cells involved was difficult to determine. These agglutination reactions were uniformly weak, difficult to reproduce and therefore difficult to study.

Positive agglutination reactions between leucocytes and plasma which were incompatible for the ABO blood groups resulted in clumps composed of a mixture of red and white cells (Figure 2c). These reactions were somewhat stronger, at times reaching a titer of 1:4. The average agglutinin, however, is not present on dilution and is variable from day to day. Table II shows the results of repeating the agglutination study on a group of these weak agglutinins daily for four days. On the other hand, the results on any one day are reproducible as shown in Table III. Erythrophagocytic indices were determined for each of these tests.

Unless otherwise noted, the following observations concern the agglutination of the mixed cell type.

TABLE III

*The results of tests for leucocyte agglutination (A) and erythrophagocytosis (E), (as measured by the erythrophagocytic index) repeated five times on the same day are compared*

| REPRODUCIBILITY<br>ON SAME DAY |                       |              |      |    |      |    |      |    |      |    |      |
|--------------------------------|-----------------------|--------------|------|----|------|----|------|----|------|----|------|
| PLASMA<br>DONOR<br>TYPE        | CELL<br>DONOR<br>TYPE | NO. OF TESTS |      |    |      |    |      |    |      |    |      |
|                                |                       | 1            |      | 2  |      | 3  |      | 4  |      | 5  |      |
|                                |                       | A            | E    | A  | E    | A  | E    | A  | E    | A  | E    |
| O +                            | B +                   | 4+           | 50.4 | 4+ | 39.6 | 4+ | 50.8 | 4+ | 49.6 | 4+ | 36.8 |
| A +                            | B +                   | -            | 0    | -  | 0    | -  | 0    | -  | 0    | -  | 0    |
| O +                            | A +                   | 2+           | 26.8 | 2+ | 25.6 | 2+ | 26.4 | 2+ | 26.4 | 2+ | *    |
| A +                            | A +                   | -            | 0    | -  | 0    | -  | 0    | -  | 0    | -  | 0    |
| O +                            | A +                   | 2+           | 26.4 | 2+ | 19.6 | 2+ | 23.2 | 2+ | 23.3 | 2+ | 19.5 |

\* Indicates that the test was not performed.



*Physico-chemical nature of the agglutinin causing the mixed cell clumping*

Some of the properties of the agglutinating substance were studied by determining its titer in several of the combinations which were incompatible for the ABO red cell types. The effect of various physico-chemical factors on these titers was then observed.

The agglutinating substance was found to be moderately thermostable resisting heating to 56° C for 30 minutes. Heating above this temperature tended to cause clotting of the plasma, however, the occasional one did not clot on heating at 65° C for 10 minutes. In these the agglutinating substance was destroyed. There was no change in the avidity of the plasma over a temperature range of 4° C to 37° C or a pH range of 6.8 to 9.0. The number of white cells between 2,500 and 20,000 cu. mm. did not influence the reaction. The ratio

of plasma to white cell suspension did not affect the result if it varied between equal parts of each and 2½ of plasma to 1 part of cell suspension.

The entire group of tests for leucocyte agglutination (160 combinations of plasma and leucocyte donor) were repeated with and without albumin added and were read at one and two hours. There was no difference between incubation at one and two hours. None became positive after one hour that were not positive at that time. In 17 instances a positive reaction was present with albumin that was negative without it. Of these 17 tests smears were studied for erythrophagocytosis in 9 instances in which there was an incompatibility between the plasma and white cell donor for the ABO blood groups. In all of these instances a significant erythrophagocytic index was found in the presence of albumin and no erythrophagocytosis was produced without it.

*Incidence and distribution*

The plasma of 24 donors was tested against the leucocytes of 7 donors on at least two and in a few instances three occasions. In all 160 combinations were tested. Erythrophagocytic indices were determined at least once for each combination. A positive test was defined as one which showed agglutination on at least two occasions. There were 30 positive tests out of the 160 combinations (Table IV). In 84 of the 160 tests (52.5 per cent) for leucocyte agglutination the plasma samples and leucocyte donors were compatible with respect to A, B and O red cell types. In 76 of the 160 tests (47.5 per cent) the combinations were incompatible.

In 6 of the 30 tests (20 per cent) the plasma samples and leucocyte donors were compatible with respect to the ABO red cell types. In 24 of the 30 positive tests (80 per cent) the combinations were incompatible (Figure 3).

*Erythrophagocytosis*

When stained smears were made of the leucocytes used in the test for leucocyte agglutinins, it was noted that erythrophagocytosis was present in some.

In the 6 positive tests in which the plasma donors and leucocyte donors were compatible with respect to the A, B, and O red cell types none

TABLE IV  
THE DISTRIBUTION OF NATURAL  
LEUCOCYTE AGGLUTININS\*  
CELL DONORS

|    | TYPE | I  | II | III | IV | V  | VI | VII |
|----|------|----|----|-----|----|----|----|-----|
|    |      | O+ | O+ | A+  | A+ | A+ | B+ | B+  |
| 1  | O+   | 0  | 0  | 0   | 0  | 0  | 0  | 0   |
| 2  | O+   | 0  | 0  | 0   | 0  | 0  | +  | +   |
| 3  | O-   | 0  | 0  | +   | +  | +  | +  | 0   |
| 4  | O+   | 0  | 0  | 0   | 0  | 0  | +  | 0   |
| 5  | O+   | 0  | 0  | 0   | 0  | +  | 0  | 0   |
| 6  | O+   | 0  | 0  | +   | 0  | 0  | 0  | 0   |
| 7  | O+   | 0  | 0  | 0   | 0  | +  | 0  | 0   |
| 8  | O+   | 0  | 0  | +   | +  | +  | +  | 0   |
| 9  | O+   |    |    | +   | +  | +  |    |     |
| 10 | O+   |    |    | +   | +  | +  | +  |     |
| 11 | A+   | 0  | 0  | 0   | 0  | 0  | 0  | 0   |
| 12 | A+   | 0  | 0  | 0   | 0  | 0  | 0  | 0   |
| 13 | A+   | 0  | 0  | 0   | 0  | 0  | 0  | 0   |
| 14 | A+   | +  | 0  | 0   | 0  | 0  | 0  | 0   |
| 15 | A+   | 0  | 0  | 0   | 0  | 0  | 0  | 0   |
| 16 | A+   | 0  | 0  | 0   | 0  | 0  | 0  | 0   |
| 17 | A+   |    | 0  | 0   | 0  | 0  | 0  | 0   |
| 18 | A+   | +  | 0  | 0   | 0  | 0  | 0  | 0   |
| 19 | A+   | 0  | 0  | 0   | 0  | 0  | 0  | 0   |
| 20 | B+   | 0  | 0  | 0   | 0  | 0  | 0  | 0   |
| 21 | B+   | 0  | 0  | 0   | 0  | 0  | 0  | 0   |
| 22 | B+   | 0  | 0  | +   | +  | +  | 0  | 0   |
| 23 | AB+  | 0  | 0  | 0   | 0  | 0  | +  | 0   |
| 24 | AB+  | 0  | 0  | +   | 0  | 0  | +  | 0   |

\* The results of the tests for leucocyte agglutination are shown. Agglutination is indicated by +; 0 denotes no agglutination; a blank space means the test was not performed.

showed erythrophagocytosis. Of the 24 positive tests which were incompatible 22 (92 per cent) showed a significant erythrophagocytic index. None of the 66 tests which were incompatible with respect to the A, B, and O red cell types but did not show clumping had a significant erythrophagocytic index. A significant erythrophagocytic index was not observed in the absence of agglutination (Figure 3).

*Inhibition of agglutination and erythrophagocytosis by specific polysaccharide of blood group A*

Nine positive leucocyte agglutination tests involving incompatible combinations due to the presence of the anti A agglutinin in the plasma were repeated in the presence of the specific polysaccharide of blood group A. All the tests were inhibited by this reagent. None of the erythrophagocytic indices was positive.

*Inhibition of agglutination and erythrophagocytosis by saliva*

The saliva of four out of six of the panel of cell donors so tested demonstrated the ability to inhibit leucocyte agglutination and erythrophagocytosis involving the donor's own cells. These four

persons were secretors of A or B substance. The two whose saliva did not produce inhibition were non-secretors of the A or B substance. A group of 10 non-secretors of the A and B substance was studied. The saliva of these individuals was tested for its ability to inhibit an agglutination involving the donors' white cells. None of those tested inhibited the reaction.

*Hemolysins and Hemagglutinins*

The sera of 7 of 8 type O plasma donors which produced leucocyte agglutination and erythrophagocytosis were tested for the presence of hemolysins. In only one of those tested was an hemolysin demonstrated.

The anti A and anti B titers of the plasma donors are shown in Table V.

*The C antibody of group O plasma*

Five combinations of group O plasma and white cell suspensions which were positive for leucocyte agglutination and showed significant erythrophagocytosis were re-examined after absorption of the C antibody. In no instance was there less leucocyte agglutination or erythrophagocytosis after this absorption had been carried out.

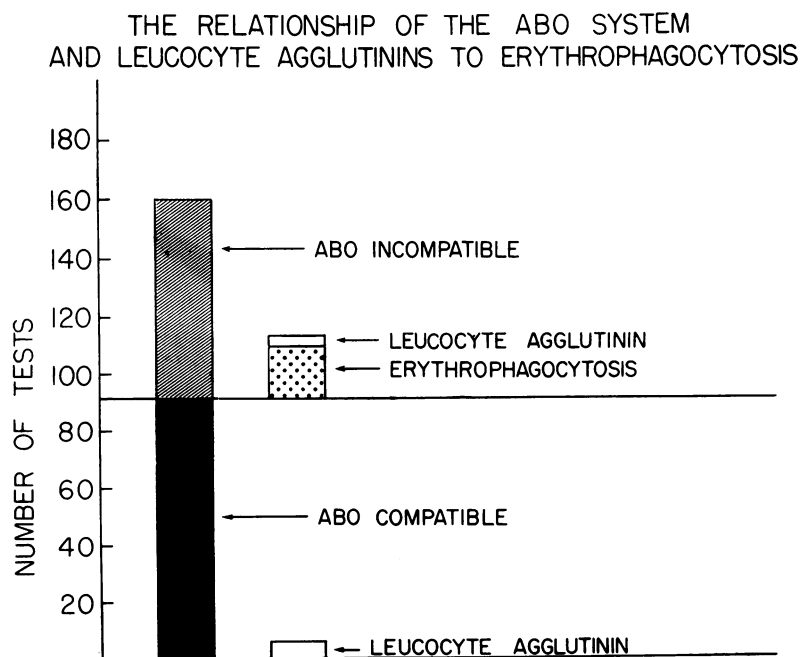


FIGURE 3

TABLE V

RELATIONSHIP OF HEMAGGLUTINATION  
ERYTHROPHAGOCYTOSIS AND LEUKAGGLUTINATION\*

|               |    | LEUCOCYTE DONORS |      |      |      |    |    |    |     |   |
|---------------|----|------------------|------|------|------|----|----|----|-----|---|
|               |    | ANTI             | ANTI |      | III  | IV | V  | VI | VII |   |
|               |    | A                | B    | E.P. | A+   | A+ | A+ | B+ | B+  |   |
| PLASMA DONORS | 8  | O+               | 64   | 32   | 53.8 | +  | +  | +  | +   | 0 |
|               | 3  | O+               | 256  | 128  | 20   | +  | +  | +  | +   | 0 |
|               | 22 | B+               | 256  |      | 22.4 | +  | +  | +  | 0   | 0 |
|               | 2  | O+               | 256  | 128  | 4.8  | 0  | 0  | 0  | +   | + |
|               | 5  | O+               | 256  | 128  | 10.6 | 0  | 0  | +  | 0   | 0 |
|               | 6  | O+               | 128  | 128  | 4.2  | +  | 0  | 0  | 0   | 0 |
|               | 7  | O+               | 64   | 128  | 7.4  | 0  | 0  | +  | 0   | 0 |
|               | 1  | O+               | 64   | 64   | 0    | 0  | 0  | 0  | 0   | 0 |
|               | 12 | A+               |      | 512  | 0    | 0  | 0  | 0  | 0   | 0 |
|               | 13 | A+               |      | 32   | 0    | 0  | 0  | 0  | 0   | 0 |
| 14            | A+ |                  | 128  | 0    | 0    | 0  | 0  | 0  | 0   |   |
| 15            | A+ |                  | 256  | 0    | 0    | 0  | 0  | 0  | 0   |   |
| 18            | A+ |                  | 64   | 12.4 | 0    | 0  | 0  | 0  | 0   |   |
| 20            | B+ | 64               |      | 0    | 0    | 0  | 0  | 0  | 0   |   |
| 21            | B+ | 64               |      | 0    | 0    | 0  | 0  | 0  | 0   |   |

\* The results of the tests for hemagglutination; erythrophagocytosis (erythrophagocytic index) and leucocyte agglutinins for some of the plasma-leucocyte combinations are shown. The numbers used to designate the plasma donors refer to the plasma donors in Table IV. E.P. indicates the erythrophagocytic index.

*The ability of the test plasma to cause erythrophagocytosis in the presence of incompatible red cells*

This test was performed on 15 of the 24 plasmas tested for leucocyte agglutination. Eight of those investigated developed a significant erythrophagocytic index. These results are illustrated in Table V.

## DISCUSSION

Various methods have been used to prepare white cell suspensions relatively free of red cells and platelets. Some have prepared them from the buffy coat after centrifugation (14). Others have used flotation techniques (15), or methods to increase sedimentation of the red cells by rouleaux formation (16). Moeschlin and Schmid (12) used heparin as an anti-coagulant and Dausset, Nenna, and Brecy (10) used defibrinated blood. In our hands centrifugation, heparin and attempts at defibrinating with glass beads all caused injury to the white cell as evidenced by a non-specific

stickiness. Sequestrene appeared to be the ideal anticoagulant in preventing non-specific clumping of the white cells. Fibrinogen was discarded as a sedimenting agent for red cells because of its tendency to precipitate out of solution on incubation at 37° C. Dextran was easily available and did not seem to cause clumping.

The method described here for preparing the leucocyte suspension is simple and produces white cells which do not clump spontaneously (Figure 4). They are irregular in outline, undergo active amoeboid motion, are capable of erythrophagocytosis, in general appear to be viable. The main disadvantage of the method is that sequestrene binds the divalent cations and in this way inhibits the action of complement (17). Plasma was chosen as a diluent because saline was found to cause non-specific clumping of the white cells. It is possible that this was due to impurities in the sodium chloride. The tests were performed at pH 6.9 because others (18, 19), had found that





FIG. 4. PHOTOMICROGRAPH UNDER LOW POWER SHOWING THE RESULTS OF A NEGATIVE TEST FOR A LEUCOCYTE AGGLUTININ

certain hemolysins are more active at this acidity. Later in the study it was discovered that pH had little effect on the test; to maintain uniformity, however, all tests were performed at pH 6.9.

False positive clumping caused by injury to the leucocyte suspension produced a stringy irregular type of clump. The clump caused by an agglutinin was more tightly packed and symmetrical. Oc-

asionally, however, they were difficult to differentiate morphologically. Although each leucocyte suspension was examined for signs of non-specific clumping, one could not be sure that the cells in an individual test had become injured and were clumped non-specifically. The absence of day to day reproducibility and difficulty in differentiating specific and non-specific clumping must be

considered in the interpretation of any studies of leucocyte agglutination. These have been referred to by other authors (8, 10), but have not been emphasized. Because of this, rather rigid criteria were adopted and only those which were positive on two occasions were counted. This eliminated 27 positive tests which were not present on repeat. Three of these were incompatible with respect to the A, B, and O red cell types and 24 were compatible. Undoubtedly, some leucocyte agglutinins were in this group.

The finding of erythrophagocytosis in 22 of the 24 tests which contained a mixture of red and white cells led to some speculation as to its cause. The inhibition of both the agglutination and the erythrophagocytosis by the saliva of appropriate secretors indicted the incompatibility of the plasma and cells. As the antigens  $Le^a$  and  $Le^b$  are present in saliva in addition to the antigens of the ABO blood group similar studies were carried out for inhibition by the specific polysaccharide of blood group A. This substance inhibited both leucocyte agglutination and erythrophagocytosis but no correlation was found between these two phenomena and the titer of the red cell agglutinins.

The majority of the tests which were positive for leucocyte agglutinins and erythrophagocytosis involved combinations of group O plasma and group A white cell suspensions. Unger and Wiener (20) have presented evidence that the C antibody in the plasma of a group O mother may cause erythroblastosis fetalis in a group A fetus. Absorption of the C antibody of the group O plasma, however, failed to reduce its tendency to cause agglutination of the leucocytes or erythrophagocytosis.

Bonnin and Schwartz (21) had demonstrated that an hemolysin and complement were required in addition to an hemagglutinin for erythrophagocytosis to occur due to the presence of an incompatibility for the A, B, and O red cell types. Complementary action in this system seemed doubtful due to the presence of sequestrene. Though it was not possible to test this hypothesis directly by heat inactivation as the white cell suspensions were badly damaged by heating to 56° C for 30 minutes, the fact that only one positive plasma donor out of those tested showed lysis made the usual mechanism seem even less likely.

The fact that leucocyte suspensions of the

plasma donors in their own plasma caused erythrophagocytosis in certain instances when mixed with incompatible red cells ruled out the participation of a leucocyte agglutinin in the reaction as a factor. The correlation between the ability of these plasmas to cause erythrophagocytosis and to cause mixed cell clumping raised the question that the former may be related to the latter.

In order to investigate this possibility the process of mixed cell clump formation was examined under the microscope. In addition cinephotomicrographic studies were made and the following observations were noted: first, the red cells became spherocytes and clumped. Next, after about 15 minutes, the white cells became sticky and coming in contact with spherocytes became attached to them. The white cell then engulfed the red cell by surrounding it with pseudopodia. This was a complicated process and took 15 to 30 minutes. Eventually the red cell was taken into the white cell. Subsequently, two erythrophagocytes joined (Figure 2a). Cinephotomicrography revealed that this occurred in a very specific manner. When two erythrophagocytes made contact at the non-red cell pole of the leucocyte they did not stick together. The chance touching of a white cell pseudopod to the red cell pole of the erythrophagocyte, however, caused sticking of the two. It appeared that the red cell was attractive to another erythrophagocyte, even though it was inside a white cell.

Large mixed red and white cell clumps were formed by a continuation of the process of erythrophagocytes clumping together (Figures 2a, b, c).

It would appear that these mixed cell clumps were caused by a tendency to erythrophagocytosis alone, and that no white cell agglutinin was present. The reason why one plasma causes erythrophagocytosis to a greater degree than another is unexplained and this is being investigated further.

#### SUMMARY AND CONCLUSIONS

The plasmas of 24 normal donors were tested against the white cells of 7 normal donors. In all, 160 combinations of plasma and white cells were tested and 30 showed agglutination reactions. Two types of cell clumps were noted, the "pure" white cell clump and the mixed red and white cell clump. Six of the positive tests were of the "pure" white cell variety and these occurred in plasma white cell combinations which were compatible

for the ABO blood groups. In twenty-four of the positive tests the clumps formed were of the mixed cell type; these occurred with combinations which were incompatible. The mixed cell clump appears to be the result of a strong tendency on the part of certain plasmas to cause erythrophagocytosis rather than the action of a leucocyte agglutinin. There is no relationship between the leucocyte agglutinins and the ABO blood groups. In that naturally occurring leucocyte agglutinins do exist it would be well in studying a patient's plasma or serum for abnormal leucocyte agglutinins to test it against a panel of leucocytes which are compatible for the ABO blood groups.

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