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## ON INDIVIDUAL DIFFERENCES IN HUMAN BLOOD.\*

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The existence of individual differences in human blood was shown long ago by means of normal isoagglutinins (1). Soon thereafter variations were found also in the blood of animals (goats) through the use of immune sera prepared by injecting goats with blood of different individuals of the same species (2). These observations were later extended to other animals.

Curiously enough the reactions with normal human isoagglutinins do not occur in a, so to speak, haphazard manner but they separate the human bloods into four sharply defined groups designated as O, A, B, and AB. (1, 3).\*\* It seems superfluous to describe the well known properties of these four groups.

In contradistinction to the simple scheme encountered with human blood, are the findings of Todd and White (5) who studied the serum of cattle immunized against cattle plague with the blood of infected animals. Taking advantage of the isohemolytic properties of such sera they discovered a remarkable variety in cattle blood; and similar conditions have been observed in the blood of chickens (Landsteiner and Miller (6)).

Attempts have been made also to discover further differences in human blood in addition to the group distinction. Evidence along this line has been furnished by von Dungern and Hirschfeld (7) in experiments with absorbed normal animal sera. It is difficult however to apply this method to a systematic study and the work was not carried on further. Following the technique of von Dungern and Hirschfeld we found some differences (see also Landsteiner and

\* See the preliminary reports in *Proc. Soc. Exp. Biol. and Med.*, 1927, xxiv, 600, 941.

\*\*In the present paper the nomenclature adopted by the American Association of Immunologists is used (4).

Witt (8)) aside from the conspicuous ones characterizing the groups, or those ascribable to the subdivision of group A, or due to the agglutinable factor found by Schiff (9) in blood cells of group O (*cf.* Witebsky and Okabe (10), Hirszfeld (11)). Our experiments were not regularly successful and frequently variations of smaller order could not be confirmed on repetition of the tests.

Another way of showing distinctions among individual bloods but also somewhat difficult of application, was found in the reactions of "cold agglutinins" of normal human sera (12-14).

The present paper concerns itself with a method of differentiating human bloods which yielded clear-cut and reliable results. It is based upon the use of immune agglutinins.

Using this technique Hooker and Anderson (15) found that immune sera produced in rabbits by injection of blood of group O still contained agglutinins for O blood after absorption with cells of any other group. The authors were inclined to explain this effect on the assumption of a property common to group O bloods.

#### EXPERIMENTAL.

Our first observations were made in experiments with stock anti-human blood immune sera from rabbits. Out of forty-one sera four were found that, after exhaustion with one sample of human blood, still contained agglutinins acting on a majority of bloods of all four groups while other bloods were not agglutinated. These tests showed the existence of an agglutinable property unrelated to the isoagglutinogens A and B, and differing from the latter in that there was not found a corresponding isoagglutinin in human serum. Naturally endeavors were made to produce immune sera endowed with the peculiar property described, by injecting rabbits with bloods possessing the new quality which may be designated as M. This was found to be rather difficult because only a few of the rabbits produce potent sera specific for M. However, on immunizing a sufficient number of animals, several such sera were obtained.

Some of the immune sera exhibited a different effect. When they were absorbed with blood of the type M+, the supernatant fluid reacted intensely and selectively on certain blood specimens, thus revealing a second agglutinable property (N).



The production of antibodies for N by injecting positively reacting bloods succeeded easily and some such sera were found among our supply of anti-human blood immune sera.

As was to be expected, anti-N agglutinins were not found in normal human sera. Also in normal animal sera we have not yet detected agglutinins for M or N.

An immune serum for a third agglutinable factor P was prepared by injecting blood (of colored individuals) selectively acted upon by absorbed normal rabbit and beef serum (according to the method of von Dungern and Hirschfeld).

In order to prepare specifically reacting agglutinin solutions, the inactivated immune sera in a dilution of 1:15 to 1:30 were treated with half the volume of packed, washed blood cells lacking the respective agglutinogens. A second treatment with the same or a smaller quantity of blood was required ordinarily to remove completely the agglutinins acting on human blood in general. The mixtures were allowed to stand for 1 hour at room temperature and were centrifuged. The fluids for N were prepared at first in this manner; subsequently, as will be explained below, the mixture of blood and serum was kept for  $\frac{1}{2}$  to 1 hour at 37–40°C.

The details of the procedure have to be determined in preliminary experiments and the absorbing blood must be selected with regard to the properties of the serum, *e.g.*, the presence of group agglutinins. Before setting up the main experiments the fluids were controlled by testing them with known bloods.

The tests were made by adding to 3 or more drops of the agglutinating fluids 1 drop of 2.5 per cent suspension of washed blood. The readings were made after the tests had stood for 2 hours at room temperature, or 1 hour at 37°C., if the fluids had been prepared at this temperature. The strength of the reaction is indicated as follows: F. tr. = faint trace; tr. = trace;  $\frac{1}{2}\pm$ , +,  $+\pm$ , etc. + signifies clumps visible without magnification or with a hand lens (magnification 6 $\times$ ) or clumps of medium size seen in the microscopic field (magnification 100 $\times$ ); ++ signifies large clumps seen with the naked eye and +++ complete agglutination.

For the production of immune sera freshly drawn and citrated blood (mostly of group O), after washing, was injected into rabbits at weekly intervals. The first injection of 3 cc. was given intravenously; the following injections of 4 cc. each, intraperitoneally. The sera were tested by absorption 6 days after the third and each subsequent injection. The animals were bled (mostly after four or five injections) the day following the tests when the sera had a sufficient content of the desired antibodies, *i.e.*, when they gave powerful specific reactions after absorption. For the preparation of anti-M immune sera it seems preferable to inject bloods of the M+ N- type.

TABLE I.

The immune sera diluted 1:20 were absorbed with suitable blood lacking the particular agglutinin, as described. The N agglutinins were prepared by absorption at room temperature (see page 769). Readings were made after 2 hours at room temperature.

| Blood No. ....                                 | 815 | 816 | 817 | 819 | 821 | 822 | 823 | 824 | 825 | 826 | 828 | 829 | 830 | 831 | 832 | 833 | 834 | 835 | 836 | 837 | 838 | 839 | 840 | 841 |
|------------------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Group.....                                     | O   | O   | O   | B   | O   | O   | B   | O   | B   | O   | O   | B   | O   | A   | O   | A   | O   | O   | O   | O   | A   | O   | A   | O   |
| Immune serum<br>1; agglutinins<br>for M.....   | ++  | ++  | ++  | ++  | ++  | 0   | ++  | 0   | ++  | ++  | ++  | 0   | 0   | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | 0   |
| Immune serum<br>18; agglutin-<br>ins for N.... | 0   | tr. | 0   | +   | ++  | ++  | 0   | ++  | ++  | +   | ++  | ++  | ++  | +   | +   | 0   | ++  | 0   | +   | ++  | +   | +   | +   | ++  |
| Immune serum<br>5; agglutinins<br>for P.....   | +   | +   | ++  | ++  | +   | +   | +   | +   | ++  | +   | +   | +   | 0   | ++  | +   | +   | 0   | +   | +   | +   | +   | +   | 0   | 0   |

It was of considerable importance to have at one's disposal a number of individuals whose blood could be examined repeatedly. The work was facilitated also by keeping particular specimens in a mixture recommended by Rous and Turner (5 volumes of 5.4 per cent glucose solution, and 2 volumes of a 3.8 per cent sodium citrate solution, for 3 volumes of blood) (16). In this solution the erythrocytes were still agglutinable when the blood was kept sterile in the refrigerator for several weeks.

TABLE II.  
*Frequency of M in the Four Blood Groups.*

| Group .....          |                 | O    |      | A    |      | B    |      | AB   |      | Total Number |      |
|----------------------|-----------------|------|------|------|------|------|------|------|------|--------------|------|
| Reactions for M..... |                 | +    | -    | +    | -    | +    | -    | +    | -    | +            | -    |
| Men                  | Number.....     | 299  | 64   | 285  | 48   | 114  | 21   | 34   | 15   | 732          | 148  |
|                      | Percentage..... | 82.4 | 17.6 | 85.6 | 14.4 | 84.4 | 15.6 | 69.4 | 30.6 | 83.2         | 16.8 |
| Women                | Number.....     | 115  | 40   | 80   | 17   | 32   | 10   | 13   | 3    | 240          | 70   |
|                      | Percentage..... | 74.2 | 25.8 | 82.5 | 17.5 | 76.2 | 23.8 | 81.2 | 18.8 | 77.4         | 22.6 |
| Total Number.....    |                 | 414  | 104  | 365  | 65   | 146  | 31   | 47   | 18   | 972          | 218  |
| Percentage.....      |                 | 79.9 | 20.1 | 84.9 | 15.1 | 82.5 | 17.5 | 72.3 | 27.7 | 81.7         | 18.3 |

TABLE III.  
*Frequency of N in the Four Blood Groups. Absorptions and Tests at 37°C.*

| Group.....           | O   |    | A   |    | B  |    | AB |   | Total No. |      |
|----------------------|-----|----|-----|----|----|----|----|---|-----------|------|
| Reactions for N..... | +   | -  | +   | -  | +  | -  | +  | - | +         | -    |
| Total No. ....       | 162 | 48 | 143 | 46 | 28 | 10 | 6  | 3 | 339       | 107  |
| Percentage.....      |     |    |     |    |    |    |    |   | 76.0      | 24.0 |

It is seen from the table that positive reactions are much more frequent than negative ones. The percentage figures for the four groups do not deviate greatly from the total average except for group AB, but here the number of individuals examined is too low to warrant any conclusion. A similar remark may apply to the figures for the sexes.

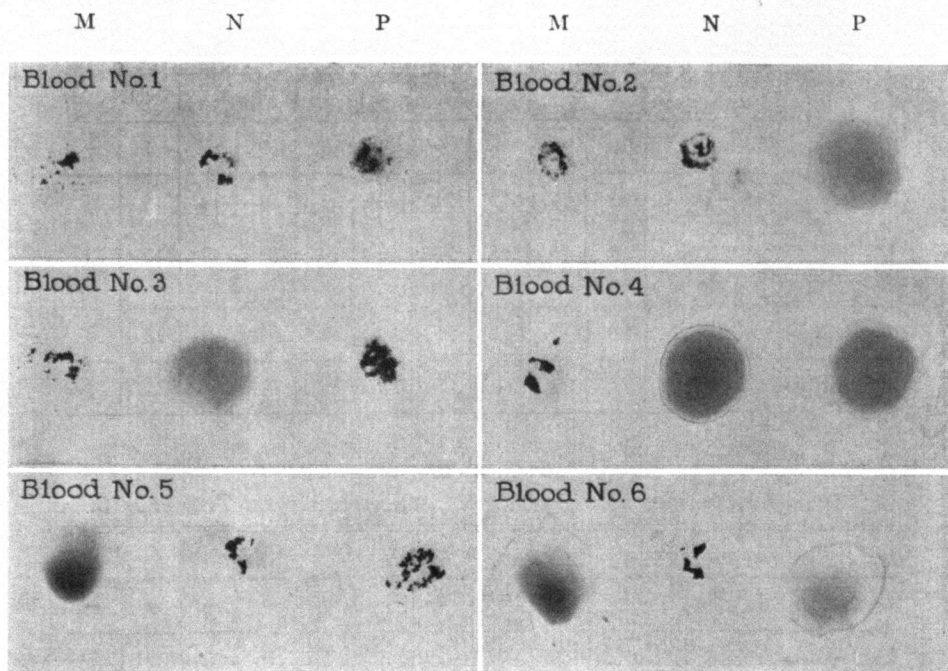


FIG. 1. Six selected bloods of group O tested with agglutinins for M, N, and P; natural size.

The distribution of the property N among the four blood groups is summarized in Table III.

For P our present results do not permit of a similar statistical survey but in general they indicate that there is no characteristic group distribution.

From the presence or absence of three agglutinogens, M, N, or P, there would follow eight possible combinations. Of these six actually

have been found in groups O and A. In groups B and AB some of the rarer types have not yet been found, most likely because of the comparatively smaller number of specimens completely examined.

The six combinations observed are illustrated in the photographs (Figs. 1 and 2). The tests reproduced were made with blood from

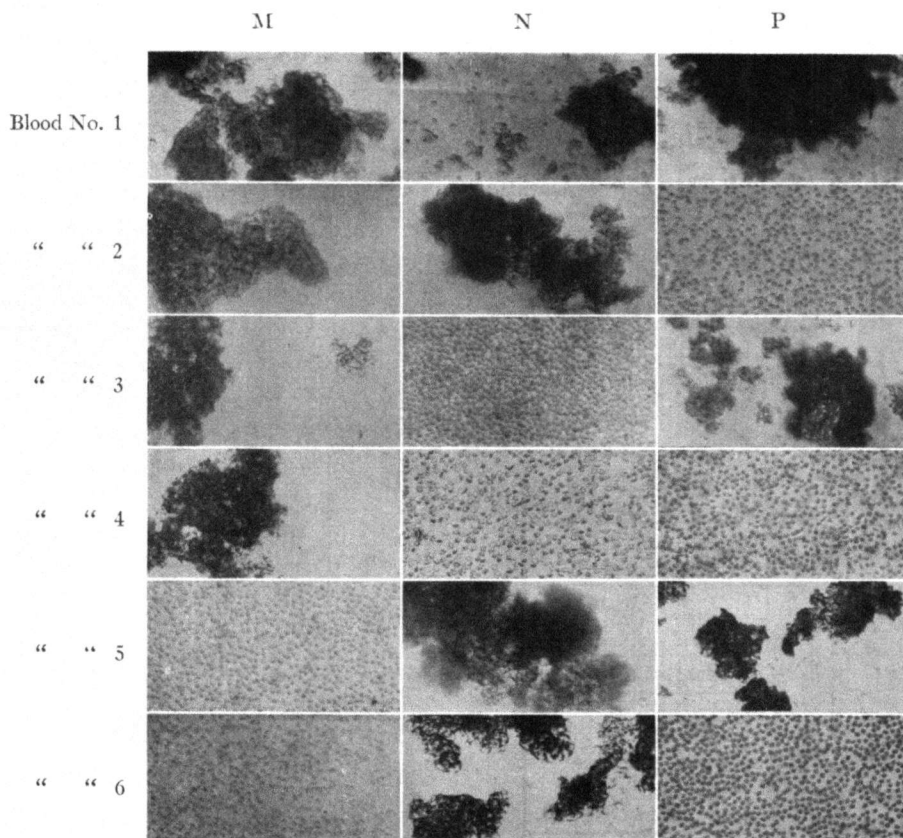


FIG. 2. The same tests as shown in Fig. 1, magnified 1:250.

six selected individuals all belonging to group O. The absorptions and tests for N were carried out at room temperature.

The two combinations not observed are those in which both properties M and N are absent. From the incidence of M— and N— bloods



TABLE IV.

Several immune sera absorbed with one M — blood (549). Sera 8, 21, and 22 diluted 1:15; Sera 1 and 20 diluted 1:20.

| Agglutinins for<br>M: serum No. | Group O |     |     |        |     |     |     | Group A |     |        |        |     |     |        | Group B |     | Group AB |     |    |        |     |     |
|---------------------------------|---------|-----|-----|--------|-----|-----|-----|---------|-----|--------|--------|-----|-----|--------|---------|-----|----------|-----|----|--------|-----|-----|
|                                 | 201     | 205 | 235 | 244    | 252 | 262 | 266 | 279     | 199 | 220    | 242    | 300 | 349 | 366    | 368     | 370 | 391      | 392 | 23 | 560    | 272 | 522 |
| 1                               | +       | +   | +   | f. tr. | +   | +   | 0   | +       | 0   | 0      | 0      | +   | 0   | 0      | +       | +   | +        | +   | +  | 0      | 0   | +   |
| 8                               | +       | +   | 0   | 0      | +   | +   | 0   | +       | 0   | 0      | 0      | +   | 0   | 0      | +       | +   | +        | +   | +  | 0      | 0   | +   |
| 20                              | +       | +   | 0   | 0      | +   | +   | 0   | +       | 0   | 0      | 0      | +   | 0   | 0      | +       | +   | +        | +   | +  | 0      | 0   | +   |
| 21                              | +       | +   | +   | f. tr. | +   | +   | 0   | +       | tr. | f. tr. | f. tr. | +   | 0   | f. tr. | +       | +   | +        | +   | +  | f. tr. | 0   | +   |
| 22                              | +       | +   | tr. | f. tr. | +   | +   | 0   | +       | 0   | 0      | f. tr. | +   | 0   | 0      | +       | +   | +        | +   | +  | f. tr. | 0   | +   |

TABLE V.

One Anti-M Serum Absorbed with Various M — Bloods.

| Agglutinins<br>for M prepared<br>from serum 8<br>by absorption<br>with various<br>bloods | Group O        |  |  |  |  |  | Group A        |                |                |                |                |                | Group B        |                |     |                | Group AB       |                |                |     |     |     |     |     |
|------------------------------------------------------------------------------------------|----------------|--|--|--|--|--|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-----|----------------|----------------|----------------|----------------|-----|-----|-----|-----|-----|
|                                                                                          |                |  |  |  |  |  | A <sup>2</sup> | A <sup>1</sup> | A <sup>2</sup> | A <sup>1</sup> | A <sup>1</sup> | A <sup>1</sup> | A <sup>1</sup> | A <sup>2</sup> |     | A <sup>1</sup> | A <sup>2</sup> | A <sup>1</sup> | A <sup>2</sup> |     |     |     |     |     |
|                                                                                          |                |  |  |  |  |  | Subgroup       | Group          | Blood No.      |                |                |                |                |                |     |                |                |                |                |     |     |     |     |     |
| 306                                                                                      | O              |  |  |  |  |  | f. tr.         | +              | +              | 359            | 369            | 382            | 506            | 512            | 566 | 575            | 198            | 236            | 291            | 292 | 531 | 532 | 535 | 557 |
| 359                                                                                      | A <sup>1</sup> |  |  |  |  |  | +              | +              | +              | +              | +              | +              | +              | +              | +   | +              | +              | +              | +              | +   | +   | +   | +   | +   |
| 566                                                                                      | A <sup>1</sup> |  |  |  |  |  | +              | +              | +              | +              | +              | +              | +              | +              | +   | +              | +              | +              | +              | +   | +   | +   | +   | +   |
| 220                                                                                      | A <sup>2</sup> |  |  |  |  |  | +              | +              | +              | +              | +              | +              | +              | +              | +   | +              | +              | +              | +              | +   | +   | +   | +   | +   |
| 369                                                                                      | A <sup>2</sup> |  |  |  |  |  | +              | +              | +              | +              | +              | +              | +              | +              | +   | +              | +              | +              | +              | +   | +   | +   | +   | +   |

one would expect an incidence of about 4 per cent bloods lacking both properties. This discrepancy evidences a negative correlation between the two agglutinable properties M and N, a conclusion supported by the fact that invariably M— bloods reacted intensely with the N reagent.

To form a correct judgment of the significance of the phenomena it was of importance to examine in how far they are influenced by variations in the reagents. In the first place a comparison was made of several anti-M immune sera in order to establish whether we were dealing with a definite agglutinable property or whether the results change contingent upon the special serum employed. The following experiments in which five anti-M immune sera were absorbed with the same blood specimen furnish proof for the first alternative (Table IV).

It is evident from the table that the strongly positive reacting bloods are the same regardless of the serum employed. In no case is the reaction of a blood intensely positive with one anti-M serum and negative with another. Identical results have since been obtained with several additional immune sera. Whether there is any disproportion in the strength of the reactions when M+ bloods are tested with a number of immune sera we are unable to decide as yet. With the sera 8 and 20 the reactions are almost uniformly strong or entirely negative. The remaining sera showed slight or weak reactions with some bloods which, doubtless, according to the other tests, lack the property M. These will be discussed presently.

In a second series of tests (Table V) one of the sera, No. 8, was absorbed with five different bloods, one of group O and four of group A, two of which belonged to subgroup AA<sup>1</sup> and two to AA<sup>2\*</sup>.

This experiment agrees with the former, for, no matter which blood was used for the preparation of the fluid, the strong reactions occurred with the same blood specimens. Hence it follows that all these reactions involve one sort of agglutinin and its corresponding agglutinin.

The fluid prepared by absorption with blood of group O gives weak or moderate agglutination effects with all the bloods of group A or AB, evidently due to the presence in the immune serum of an agglutinin for A. These reactions are removed by absorption with blood AA<sup>1</sup> while after treatment of the immune serum with blood AA<sup>2</sup> there is still some agglutination with bloods AA<sup>1</sup> (or AA<sup>1</sup>B).

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\* For the nomenclature of the subgroups see (8, 14).

Similar interfering reactions were encountered not infrequently throughout these studies. In general they were brought about by the presence in the sera of normal or immune antibodies for the group factors A and B, of antibodies acting by preference on bloods of group O (Schiff (9)) and by the coexistence in the same immune serum of more than one of the new agglutinins; besides there may be other agglutinogens not yet analyzed. Suitable absorption experiments serve to eliminate such additional antibodies.

TABLE VI, *a*.

Serum 20 diluted 1:15 was absorbed once for 2 hours at room temperature with half its volume of blood lacking the factor M. Some of the fluid obtained was removed and the remainder was divided into two equal parts. One of these two fractions was further absorbed three times at room temperature with half its volume of the M- blood, and the other was simultaneously treated in the same manner but with washed sheep blood; after each absorption a small amount of fluid was withdrawn for the tests. The last absorption was made at 0°C. and the fluid was obtained by centrifuging in a jacket containing ice and water.

The fluids of the first, fourth, and fifth absorptions were titrated in progressively doubled dilutions, using 3 drops of the liquid and 1 drop of 2.5 per cent suspension of M+ and M- blood. Readings were made after the tests were kept 2 hours at room temperature.

| Fluid after    | Absorbed with blood | Test with blood | Fluid diluted 1: |        |        |        |     |
|----------------|---------------------|-----------------|------------------|--------|--------|--------|-----|
|                |                     |                 | 15               | 30     | 60     | 120    | 240 |
| 1st absorption | Human M-            | M+              | +++              | ++     | +      | f. tr. | 0   |
|                |                     | M-              | 0                | 0      |        |        |     |
| 4th "          | Human M-            | M+              | +±               | +      | f. tr. | 0      | 0   |
|                |                     | M-              | 0                | 0      |        |        |     |
|                | Sheep               | M+              | ++±              | ++     | +      | 0      | 0   |
|                |                     | M-              | 0                | 0      |        |        |     |
| 5th "          | Human M-            | M+              | +                | f. tr. | 0      | 0      | 0   |
|                |                     | M-              | 0                | 0      |        |        |     |
|                | Sheep               | M+              | ++±              | ++     | +      | tr.    | 0   |
|                |                     | M-              | 0                | 0      |        |        |     |

The question whether the properties M+ and M- are of a qualitative nature was approached by means of repeated absorptions of the immune sera with M- blood. In the experiment recorded in Table VI, *a*, and Table VI, *b*, it was found that the antibody for M could

be gradually absorbed from serum 20, the greatest effect being obtained at low temperature while in serum 72, even after several absorptions, there was only an indifferent diminution of the specific agglutinin, not more marked with human than with sheep blood which was used as a control. Consequently the property M is characterized as a particular agglutininogen according to serological terminology.

Some anti-M sera were found whose specificity could be recognized by different degrees of agglutination when the exhausted sera were tested with the two sorts

TABLE VI, *b*.

An experiment similar to the preceding was made with anti-M immune serum 72. This serum diluted 1:30 required two absorptions with human M— blood for removing the common agglutinins. After withdrawing some of the fluid it was divided in two portions and the experiment was continued as above with human blood and sheep blood. The last absorption was carried out at 0°C. The fluids of the second, fourth, and fifth absorptions were tested as before.

| Fluid after    | Absorbed with blood | Tested with blood | Fluid diluted 1: |      |     |        |     |
|----------------|---------------------|-------------------|------------------|------|-----|--------|-----|
|                |                     |                   | 30               | 60   | 120 | 240    | 480 |
| 2nd absorption | Human M—            | M+                | +++              | +++  | +   | +      | 0   |
|                |                     | M—                | 0                | 0    |     |        |     |
| 4th “          | Human M—            | M+                | +++              | ++   | +   | +      | 0   |
|                |                     | M—                | 0                | 0    |     |        |     |
|                | Sheep               | M+                | +++              | +++± | +±  | +      | 0   |
|                |                     | M—                | 0                | 0    |     |        |     |
| 5th “          | Human M—            | M+                | ++               | +±   | ±   | f. tr. | 0   |
|                |                     | M—                | 0                | 0    |     |        |     |
|                | Sheep               | M+                | ++               | +±   | ±   | 0      | 0   |
|                |                     | M—                | 0                |      |     |        |     |

of corpuscles; they were not good for further work because the species agglutinins could not be removed without a simultaneous loss of the specific action.

The action of several (six) anti-N immune sera on a series of bloods and the effect of the exhaustion at room temperature of two immune sera with various bloods were studied in an analogous manner as

described for the property M. Since the strong reactions occurred always with the same bloods the experiments warrant the assumption that here, too, a definite serological factor comes into play. This factor may be subject to some variation as will be discussed presently.

A difficulty was encountered, owing to the fact that on treating anti-N immune sera several times with N- bloods, there was a rather rapid diminution of the agglutinins for N. As a consequence it was not easy to estimate the adequate degree of absorption, although fluids of marked specificity could be prepared repeatedly (see Table I).

TABLE VII.

Each of four anti-N immune sera diluted 1:20 were absorbed three times with one-half volume of pooled blood of four individuals of group A lacking N. One set of the absorptions was performed at room temperature and the other at 37° (water bath). In the latter case the fluids were separated by centrifuging for about 1 minute at high speed in a jacket of warm water (about 50°): At the end of the centrifuging the temperature of the water was 37° or but little below. The fluids were tested with six selected bloods of group A, two of which reacted negatively, two moderately, and two intensely.

| Absorptions and tests at room temperature |                |           |        |        |       |     |     |
|-------------------------------------------|----------------|-----------|--------|--------|-------|-----|-----|
| Anti-N<br>immune<br>serum                 | Fluid after    | Blood No. |        |        |       |     |     |
|                                           |                | 806       | 1010   | 546    | 931   | 851 | 953 |
| 18                                        | 1st absorption | tr.       | ±      | +++    | +++   | +++ | +++ |
|                                           | 2nd "          | 0         | 0      | ±      | tr.   | ++  | ++  |
|                                           | 3rd "          | 0         | 0      | 0      | 0     | ±   | ±   |
| 22                                        | 1st "          | 0         | 0      | ++     | +++   | +++ | +++ |
|                                           | 2nd "          | 0         | 0      | ±      | ±     | +++ | ++  |
|                                           | 3rd "          | 0         | 0      | f. tr. | 0     | +   | ±   |
| 26                                        | 1st "          | f. tr.    | f. tr. | ++     | +++   | +++ | +++ |
|                                           | 2nd "          | 0         | 0      | ++     | ±     | ++  | +++ |
|                                           | 3rd "          | 0         | 0      | ±      | +     | ++  | ++  |
| 61                                        | 1st "          | tr.       | 0      | +++    | +++   | +++ | +++ |
|                                           | 2nd "          | 0         | 0      | ±      | +     | +++ | +++ |
|                                           | 3rd "          | 0         | 0      | tr.    | f.tr. | +   | +   |

TABLE VII—*Concluded.*

| Absorptions and tests at 37° |                |           |        |      |      |      |      |
|------------------------------|----------------|-----------|--------|------|------|------|------|
| Anti-N<br>immune<br>serum    | Fluid after    | Blood No. |        |      |      |      |      |
|                              |                | 806       | 1010   | 546  | 931  | 851  | 953  |
| 18                           | 1st absorption | ++        | +±     | +++± | ++++ | +++± | +++± |
|                              | 2nd “          | f. tr.    | 0      | +++± | +++± | ++++ | ++++ |
|                              | 3rd “          | 0         | 0      | ++   | +±   | +++± | +++± |
| 22                           | 1st “          | ±         | +      | ++   | ++++ | ++++ | ++++ |
|                              | 2nd “          | 0         | 0      | ++   | ++   | ++++ | +++± |
|                              | 3rd “          | 0         | 0      | +±   | +    | +++± | +++± |
| 26                           | 1st “          | ++        | +±     | +++± | +++± | ++++ | +++± |
|                              | 2nd “          | +±        | +      | +++± | ++++ | ++++ | ++++ |
|                              | 3rd “          | 0         | 0      | +++± | +++± | ++++ | +++± |
| 61                           | 1st “          | ++        | ++     | +++  | +++  | +++  | +++  |
|                              | 2nd “          | +         | ±      | +++± | +++± | +++  | +++  |
|                              | 3rd “          | tr.       | f. tr. | +±   | ++   | +++  | +++  |

The technic was improved by carrying out the absorptions and also the tests at 37° or 40° C.\* Under these conditions the N antibodies are diminished by repeated absorption with N- bloods at a slow rate (Table VII) and the results are generally satisfactory. On random selection of anti-N sera and absorbing bloods, also weak or moderate reactions are apt to occur with bloods that react negatively when other immune sera or absorbing bloods are chosen. This may be due to quantitative or qualitative variations in the agglutinin N aside from other reactions as discussed above for the property M (p. 766).

The agglutinable property designated as P has not been studied extensively. Doubtless the reactions as presented in Table I are different from those for M and N and are independent of the group agglutinogens A and B; furthermore we found a characteristic distribution of P in white and colored individuals.\*\* But it has not been

\* With this method, incidentally, a considerable proportion of stored antihuman blood immune sera were found to contain smaller or larger fractions of N agglutinins.

\*\* See *Proc. Soc. Exp. Biol. and Med.*, 1927, xxiv, 941.

established that parallel results can be obtained with several immune sera which would define a single quality.

A serum of an individual in group B containing an abnormal isoagglutinin was recently described by Ottenberg and Johnson (17). Having had the opportunity to examine this serum, we found in conformity with the authors named, agglutination reactions (of moderate to weak intensity) with numerous bloods of groups O and B. By absorbing this serum with certain bloods A, it was possible to show that the abnormal isoagglutinin acted also on the majority of group A bloods.

These isoagglutination reactions do not coincide with the reactions for M, N, or P.

The observations described open the possibility of making an individual diagnosis of human blood for forensic purposes in cases in which this could not be done hitherto. In preliminary experiments the properties M and N could be demonstrated in blood kept in a dry state (on glass) for several weeks. The method consisted in absorbing specifically reacting fluids with stromata prepared from small amounts (50 mg.) of the dried blood.

*Immunization Experiments.*—To determine whether the formation of the antibodies described, depends solely on the individuality of the animal used or also upon the antigen injected, the following experiment was carried out: 12 rabbits were injected with blood of the type M+ N— and 6 with blood of the type M— N+, all belonging to group O. Out of the 12 animals after four injections of M+ N— blood there were 1 strong, 1 weak anti-M immune sera, and 5 of rather moderate strength. Two of the latter became strongly active after one or two additional injections. In this series there were 2 or 3 which had a weak action for N. (After a further injection 2 other animals receiving M+ N— blood developed antibodies for N, 1 of moderate and 1 of weak activity.)

Of the 6 animals receiving M— N+ blood all developed immune sera specific for N; 4 reacted strongly and 2 moderately; none of these contained antibodies for M.

The experiment shows that potent antibodies were obtained when the homologous agglutigen was injected but that antibodies of weak activity were also produced by animals which had not received the corresponding antigen. These observations are not exceptional



and similar cases were reported, *e.g.*, by Weil and Felix (18) and Furth (19) with bacilli of the typhoid group (*cf.* Halber and Hirschfeld (20)).

*Tests with Blood of Anthropoid Apes.*—The presence in the blood of apes of agglutinogens indistinguishable from the human group fac-

TABLE VIII.

*Tests for M in Blood of Primates.\**

| Anti-M<br>immune<br>serum<br>absorbed<br>with<br>human<br>blood | Chimpanzees |    |     |    |    |    |   |    |    |    | Ourang |   | Gibbons |   |   |   |   | Man |     |
|-----------------------------------------------------------------|-------------|----|-----|----|----|----|---|----|----|----|--------|---|---------|---|---|---|---|-----|-----|
|                                                                 | 1           | 2  | 3   | 4  | 5  | 6  | 7 | 8  | 9  | 10 | 1      | 2 | 1       | 2 | 3 | 4 | 5 | M-  | M+  |
| M-                                                              | ++          | ++ | ++± | ++ | ++ | ++ | + | ++ | ++ | ++ | ++     | + | 0       | 0 | 0 | 0 | 0 | 0   | ++± |
| M+                                                              | 0           | 0  | 0   | 0  | 0  | 0  | 0 | 0  | 0  | 0  | ++     | + | 0       | 0 | 0 | 0 | 0 | 0   | 0   |

\* The blood of the first 6 chimpanzees, Ourang 1, and Gibbon 1 were examined at the same time.

TABLE IX.

*Tests for N in Blood of Primates. Absorptions and Tests Were Made at 37°C. Serum 18 Was Tested after 2 Absorptions (18a) and 3 absorptions (18b).*

| Absorbed<br>with<br>blood | Immune<br>anti-N<br>serum No. | Chimpanzees |        |     |     | Ourang | Man |        |
|---------------------------|-------------------------------|-------------|--------|-----|-----|--------|-----|--------|
|                           |                               | 3           | 4      | 5   | 10  |        | N+  | N-     |
| N-                        | 12                            | tr.         | tr.    | tr. | tr. | ++     | ++± | 0      |
|                           | 18a                           | ++±         | ++     | ++± | ++± | ++     | +++ | f. tr. |
|                           | 18b                           | +           | +      | ±   | ±   | ±      | +++ | 0      |
|                           | 22                            | +           | +      | 0   | +   | +      | ++± | 0      |
| N+                        | 12                            | 0           | 0      | 0   | 0   | ±      | 0   | 0      |
|                           | 18a                           | 0           | ±      | ±   | 0   | ++     | tr. | 0      |
|                           | 18b                           | 0           | f. tr. | tr. | 0   | ±      | 0   | 0      |
|                           | 22                            | 0           | f. tr. | 0   | 0   | +      | 0   | 0      |

tors A and B has been shown previously (Landsteiner and Miller (21), *cf.* von Dungern and Hirschfeld (7)). Some experiments were made to establish whether also the new agglutinable properties are to be found in the blood of anthropoids. To account for the existence of agglutinins in the blood of apes that cannot be removed by human

cells,\* absorptions had to be made with bloods both lacking and possessing the factor in question; a positive reaction was indicated when agglutination took place in the first but not in the second instance.

The tests for P were negative in the blood of chimpanzees and 1 ourang. As to the quality M, it appeared to exist in the erythrocytes of each of 10 chimpanzees, but not in the blood of 5 gibbons (1 *Hylobates lar*, 3 *Hylobates leuciscus*, 1 *Symphalangus syndactylus*) and 2 ourangs (Table VIII). The reactions for N in the blood of chimpanzees were distinctly positive with one of the immune sera, but moderate or faint with two other sera tested (Table IX).

That the properties M and N in the blood of chimpanzees and man are related though not entirely identical is seen from the results with the various anti-N immune sera and from the observation that one anti-M serum very active for human blood acted on chimpanzee blood positively but markedly less so than the other sera.

Of the lower mammals and birds there were examined for the property M: 2 macacus rhesus, 2 vervets, 1 baboon, 1 sapajou, 1 lemur; 1 horse, 4 cattle, 1 sheep, 2 pigs, 1 dog, 1 cat, 25 rabbits, 2 guinea pigs, 2 rats, 1 mouse; 1 duck, 1 chicken, and 1 pigeon. For N only rabbits, 23 in number, were examined (absorptions at room temperature). The tests gave negative results.

#### DISCUSSION.

In the present studies a method is described which led to the detection of well defined individual differences in human blood in addition to those characterizing the blood groups. On repeated examination of the same individuals the properties were constant. The reactions observed indicate the existence of distinct agglutinable properties. This is substantiated by the fact that for M there are no transitions between positive and negative reactions, since fluids with a titre of 1:64 for M+ blood did not react on M- blood; also blood negative for M has practically no affinity to the antibody of certain anti-M immune sera as shown by absorption experiments at room temperature.

With the two agglutinable properties N and P, an appreciable ab-

\* See the tests with ourang blood.

sorption effect is brought about at room temperature also by blood negative in the agglutination test. The phenomenon can possibly be explained on the assumption that the antibodies for the particular agglutinin and those for human blood in general are not entirely segregated but are partly in some sort of combination. This view is supported by the fact, already mentioned, that certain anti-M sera behave similarly while others stand repeated absorptions. There are other cases which may call for an analogous explanation. It has been shown, f. i., that from some normal human sera of group O, corpuscles A or B absorb not only the homologous but also a part of the heterologous isoagglutinins (8). Similarly agglutinin  $\alpha^1$  of human sera O and B can be removed by bloods of group A which lack A<sup>1</sup>, particularly when the absorptions are made at low temperature.

The ultimate significance of the factors determined by serological reactions is still a matter for discussion and it is not at all certain whether to each factor there corresponds a special compound that might be isolated chemically (14). But there is evidence from a study of families that the agglutinable factors M and N are constitutional properties that are inherited as Mendelian characters.\* As to their antigenic nature it is true that the immunization depends largely upon the individual response of the animal but, even so, the experiments indicate that the antibodies can be formed as a result of specific antigenic action.

The division of human blood into only four well defined blood groups was not in harmony with the manifold individual variations that become evident from the experiences on transplantation of normal tissues and tumors. Thus there was some reason to presume that the serological differences of cells and the transplantation specificity are phenomena of a different nature. This gap seems to be bridged by some previous findings (7, 14) and the present studies.

The six types aforementioned, if present, as is likely, in each of the four blood groups and in the subgroups of groups A and AB, differentiate 36 varieties of human blood. This number does not include the variations in the strength of the reactions which may also be determined constitutionally and it is improbable that we succeeded

\* See *Proc. Soc. Exp. Biol. and Med.*, 1927, xxiv, 941, and unpublished results.

in detecting all differences which can be demonstrated by means of antibodies derived from rabbits. Possibly other animals when injected with human blood would furnish sera with new specific qualities. As stated already, with a reagent of a different sort, namely an abnormal isoagglutinin in a particular group B serum (Ottensberg and Johnson (17)), reactions were obtained which did not run parallel with those shown by the rabbit immune sera. To be sure this reagent is not available for general use, but still it further doubles the number of human blood varieties that can be differentiated.

Summing up all the known observations on the subject one is led to the opinion that almost every individual human blood may have its characteristic serological features (see Todd and White (5)) as already conjectured by von Dungern and Hirschfeld, although at present there is no actual method which would permit of an individual diagnosis of human blood. Conceivably this end could be achieved by the use of immune isolysins.

The results of studies concerning the heredity of the agglutinable properties and their distribution in populations of different racial composition are reserved for subsequent communications.

The findings dealt with have thus far no direct bearing on the selection of donors in transfusions because of the absence of corresponding agglutinins in normal human sera for the new agglutinogens.

#### SUMMARY.

A clear-cut differentiation of human blood, aside from the blood groups, could be made by means of special agglutinating immune sera. The observations point to the existence of several agglutinable factors for which no agglutinins are demonstrable in normal human sera. In view of the latter circumstance the results reported do not imply any change in the scheme of the four blood groups.

The body of serological evidence leads to the inference of a high degree of biochemical differentiation among individuals.

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