

ISOAGGLUTINATION OF HUMAN CORPUSCLES*

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THE occurrence of isoagglutinins in human serum has been observed and studied more or less extensively by a number of investigators notably Halban,¹ Landsteiner,² Grünbaum,³ Donath,⁴ Descatello and Sturli,⁵ Herter,⁶ Ascoli,⁷ Le Monaco and Panici,⁸ Eisenberg,⁹ Martin,¹⁰ and others.¹¹

It may be regarded as well established that the serum of the majority of healthy individuals above six years of age contains isoagglutinin; that there are several distinct isoagglutinins; that there is marked difference in the agglutinability of the corpuscles of different individuals; that autoagglutinins are very seldom if ever demonstrable *in vitro*; and that there has not been demonstrated any essential difference between isoagglutination in health and disease; hence the phenomenon at present has no diagnostic significance.

Landsteiner and, following him, Descatello and Sturli point out that individuals may be separated into three main groups by means of isoagglutination, namely: (1) those whose corpuscles are not agglutinated by the sera of Groups II and III, but whose sera agglutinate the corpuscles of these groups; (2) those whose corpuscles are agglutinated by the sera of Group III, and whose sera agglutinate the corpuscles of Group III; (3) those whose corpuscles are aggluti-

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¹ *Wien. klin. Wchnschr.*, 1900, 13, p. 545 (with Landsteiner), *Münch. med. Wchnschr.*, 1902, 49, p. 473.

² *Centralbl. j. Bakt., Orig.*, 1900, 27, p. 361; *Wien. klin. Wchnschr.*, 1901, 14, p. 1132; (with Leiner), *Centralbl. j. Bakt., Orig.*, 1905, 38, p. 548; (with Reich), *ibid.*, 1905, 39, p. 712.

³ *Brit. Med. Jour.*, 1900, p. 1089.

⁴ *Wien. klin. Wchnschr.*, 1900, 13, p. 497.

⁵ *Münch. med. Wchnschr.*, 1902, 49, p. 1090.

⁶ *Medical Record*, 1902, 61, p. 118.

⁷ *Münch. med. Wchnschr.*, 1901, 48, p. 1239.

⁸ *Münch. med. Wchnschr.*, 1902, 49, p. 1066.

⁹ *Wien. klin. Wchnschr.*, 1901, 14, p. 1020.

¹⁰ *Centralbl. j. Bakt., Orig.*, 1905, 39, p. 704.

¹¹ *Klein Wien. klin. Wchnschr.*, 1902, 49, p. 716; Schenck, *Münch. med. Wchnschr.*, 1905, 52, p. 1623; Paci, *Centralbl. j. Path.*, 1902, 13, p. 356.

nated by the sera of Group III, and whose sera agglutinate the corpuscles of Group II.

The corpuscles of Groups I and II are not agglutinated by the sera of the respective group. The corpuscles of Group III are not infrequently agglutinated by the sera of that group except the strictly homologous serum.

This general grouping, to which there are but few exceptions, is illustrated in Table 1 which gives the rearranged results of an experi-

TABLE 1.
ISOAGGLUTINATION IN TWENTY NORMAL MEN.
Corpuscles.

| | | I | | | | | | | | II | | | | | | | | | | III | | |
|-----|-------|-------|---|---|----|----|----|----|----|----|---|---|---|---|---|---|----|----|----|-----|----|----|
| | | | 4 | 5 | 12 | 13 | 14 | 16 | 19 | 2 | 1 | 3 | 6 | 7 | 8 | 9 | 10 | 11 | 18 | 20 | 15 | 17 |
| ERA | I | 4... | o | o | o | o | o | o | o | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | | 5... | o | o | o | o | o | o | o | o | + | + | + | + | + | + | + | + | + | + | + | + |
| | | 12... | o | o | o | o | o | o | o | o | + | + | + | + | + | + | + | + | + | + | + | + |
| | | 13... | o | o | o | o | o | o | o | o | + | + | + | + | + | + | + | + | + | + | + | + |
| | | 14... | o | o | o | o | o | o | o | o | + | + | + | + | + | + | + | + | + | + | + | + |
| | | 16... | o | o | o | o | o | o | o | o | + | + | + | + | + | + | + | + | + | + | + | + |
| | | 19... | o | o | o | o | o | o | o | o | + | + | + | + | + | + | + | + | + | + | + | + |
| | II | 2... | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | + | + |
| | | 1... | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | + | + |
| | | 3... | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | + | + |
| | | 6... | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | + | + |
| | | 7... | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | + | + |
| | | 8... | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | + | + |
| | | 9... | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | + | + |
| | | 10... | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | + | + |
| | | 11... | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | + | + |
| | | 18... | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | + | + |
| | 20... | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | + | + | |
| | III | 15... | o | o | o | o | o | o | o | + | + | + | + | + | + | + | + | + | + | + | o | o |
| | | 17... | o | o | o | o | o | o | o | + | + | + | + | + | + | + | + | + | + | + | o | o |

ment in which 0.1 c.c. of the serum of each of 20 normal, young adult men was mixed with 1 c.c. of a 2.5 per cent suspension of the blood of each of the 20 individuals. The readings were made after the tubes had remained in the incubator for two hours and in the ice-box over night. The exceptions to Landsteiner's grouping are given in heavy type.

This experiment has been repeated twice at intervals of one to two months with practically the same result as that given in the table. In the second test Serum 2 is recorded as not containing any agglutinin, but in the third a small amount of agglutinin for corpuscles of Group III was demonstrated. In the third test the amount of

agglutinin in Serum 13 was much smaller than on the two previous occasions, and it did not under the conditions of the experiment agglutinate all the corpuscles in Group II. The only striking and constant exception to the general scheme is the complete failure on all occasions to demonstrate any agglutinin in Serum 17. The absence of agglutinin in the sera of persons whose corpuscles fall in Group III is not at all unusual as will be made clear shortly.

In order to study still further this question of variation with respect to isoagglutination five normal persons representing the three different groups were selected and their blood tested at frequent intervals throughout a period covering several months. The results obtained have been constant with only one exception—the serum of one of the two members of Group III gradually lost its agglutinin.

Of the 76 persons whose blood has been investigated in such a manner that grouping is possible, 36 belong to Group I, 26 to Group II, and 14 to Group III. Of the 14 belonging to Group III at least six were without any agglutinin, hence we may say that about 90 per cent of individuals possess isoagglutinin (Table 2).

TABLE 2.
ISOAGGLUTINATIVE GROUPING OF SEVENTY-SIX INDIVIDUALS.

| | Group I | Group II | Group III |
|--------------------------------------|---------|----------|-----------|
| In Health..... | 16 | 14 | 5 |
| Scarlet Fever..... | 4 | 0 | 1 |
| Pneumonia..... | 4 | 3 | 4 |
| Typhoid Fever..... | 4 | 4 | 3 |
| Advanced Pulmonary Tuberculosis..... | 8 | 5 | 1 |
| | 36 | 26 | 14 |

As Table 2 shows, there is no striking alteration in the agglutinative grouping of the blood of persons suffering with various diseases, more particularly pneumonia, scarlet fever, typhoid fever, and advanced pulmonary tuberculosis. Whether any special change occurs in the original agglutinative properties of the serum or in the agglutinability of the corpuscles in the course of any of these or of other diseases has not been investigated. Of a family of five, the mother and three children belonged to Group I, the remaining child to Group II.

We may distinguish three main agglutinins, namely the agglutinin in the sera of Group I for the corpuscles of Group II and III; the agglutinin of Group II for the corpuscles of Group III; and the

agglutinin of Group III for corpuscles of Group II. That it concerns distinct bodies with special affinities for the corpuscles upon which they act is shown readily by means of absorption experiments in which definite quantities of serum are treated with an excess of corpuscles; after removal of the corpuscles by centrifugalization the agglutinative action of the serum so treated is determined in the usual way. The results are:—Corpuscles of Group I do not absorb any agglutinin; corpuscles II do not absorb agglutinin II for Group III, but they do absorb agglutinin I for both Groups II and III; and corpuscles III do not absorb agglutinin III for Group II but they do absorb agglutinin I for both Groups III and II. Of corpuscles one may say then that some have no agglutinophile receptors (Group I); that others have at least two distinct agglutinophile receptors (Groups II and III); and that corpuscles are immune to agglutinins in their own serum, if for no other reason than because of lack of suitable receptors. In addition to the three main agglutinins others no doubt occur, if only occasionally. In Group III there often is interagglutination, but I have not examined into its mechanism.

As I have pointed out elsewhere human serum may contain opsonin for human corpuscles,¹ and the question naturally arises as to what is the relation between human iso-opsonin and isoagglutinin. So far as my observations go they appear to be distinct substances, since the same serum may contain opsonin for certain corpuscles without necessarily containing agglutinin for those corpuscles, and vice versa (Tables 3 and 4). Tables 3 and 4 also show that there is marked variation in the susceptibility of the corpuscles of different individuals to iso-opsonin. I have also found that the hemo-opsonin for human

TABLE 3.
ISOAGGLUTININ AND ISO-OPSONIN IN SCARLET FEVER.
CORPUSCLES.

| | | 1 | 2 | 3 | 4 | 5 |
|------|--------|---|---|---|---|---|
| SERA | 1..... | o | o | o | o | + |
| | 2..... | o | o | o | o | + |
| | 3..... | o | o | o | o | + |
| | 4..... | o | o | o | o | + |
| | 5..... | o | o | o | o | o |

The heavy type means phagocytosis.

o=no agglutination.

+ = agglutination.

¹ *Jour. Infect. Dis.*, 1906, 3, p. 721.

TABLE 4.
ISOAGGLUTININ AND ISO-OPSONIN IN TYPHOID FEVER AND PNEUMONIA.
CORPUSCLES.

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|------|-------|---|---|---|---|---|---|---|---|
| SERA | 1.... | o | o | o | o | + | + | + | + |
| | 2.... | o | o | o | o | + | + | + | + |
| | 3.... | o | o | o | o | + | + | + | + |
| | 4.... | o | o | o | o | + | + | + | + |
| | 5.... | o | o | o | o | + | + | + | + |
| | 6.... | o | o | o | o | + | + | + | + |
| | 7.... | o | o | o | o | + | + | + | + |
| | 8.... | o | o | o | o | + | + | + | + |

1, 3, and 7 are typhoid-fever cases; 2, 8, 5, and 6 are pneumonia cases; 4 is serum disease. For explanation of signs see note under Table 3.

corpuscles in the serum of normal or diseased human beings is not absorbed by corpuscles that resist its opsonic action. On the contrary corpuscles that are subjected to phagocytosis, when treated with opsonic serum, remove opsonin from the serum. In this respect there is complete analogy with the relation between non-agglutinable and agglutinable corpuscles to isoagglutinin. The experiments are not yet extensive enough, however, to determine anything definite as regards the kinds of opsonin in human serum.

It has not been possible to demonstrate the presence in human serum or citrated plasma of any definite and pronounced anti-isoagglutinin. The admixture of increasing quantities of sera from individuals of one group to sera of members of either of the other groups does not in any way hinder in any decisive manner the clumping of the proper corpuscles. Agglutination takes place in mixtures of defibrinated blood, containing isoagglutinins and agglutinable corpuscles. Again, agglutination takes place in a mixture of the freshly drawn blood of an individual of Group I with an equal quantity of that of a member of Group III, coagulation being prevented by adding as much again as the total quantity of blood of citrate solution (1 per cent of citrate of sodium in m/8 NaCl solution) or by means of hirudin solution. On account of the great mass of blood corpuscles in such mixtures the occurrence of agglutination may be recognizable under the microscope only.

The prolonged treatment of erythrocytes with serum free from suitable isoagglutinin in no way hinders or delays the agglutination of the corpuscles by active serum.

Human isoagglutinins are stable bodies—heating serum to 60°

C. for 30 minutes does not destroy them and they persist in serum kept in the ice-box for many months, even when contaminated with bacteria. Serum filtered through porcelain retains its original agglutinating power practically unchanged.

The amount of agglutinin varies considerably in sera from different persons (Table 5). Several comparative demonstrations by means of

TABLE 5.
THE SMALLEST AMOUNT OF VARIOUS SERA CAUSING DISTINCT ISOAGGLUTINATION.
5 per Cent Suspension of Corpuscles + Serum in NaCl Solution—each 0.5 c.c.

| SERA (SOURCE AND GROUPING) | GROUPING OF CORPUSCLES | | | |
|------------------------------------|------------------------|-------|-------|-------|
| | 2 | 2 | 3 | 3 |
| Normal..... 1 | .0015 | .006 | .012 | .006 |
| Normal..... 2 | ... | ... | .006 | .003 |
| Normal..... 2 | ... | ... | .025 | .012 |
| Normal..... 3 | .0015 | .0015 | ... | ... |
| Pneumonia..... 1 | .0015 | .0015 | .006 | .006 |
| Typhoid fever..... 1 | .003 | .006 | .006 | .025 |
| Typhoid fever..... 1 | .006 | .006 | .025 | .025 |
| Typhoid fever..... 3 | .012 | .006 | ... | ... |
| Scarlet fever..... 1 | .006 | .006 | .025 | .025 |
| Chronic pulmonary tuberculosis.. 1 | .006 | .003 | .006 | .003 |
| Chronic pulmonary tuberculosis.. 2 | ... | ... | .006 | .012 |
| Chronic pulmonary tuberculosis.. 3 | .012 | .006 | ... | ... |
| Serum disease..... 1 | .0003 | .0003 | .0003 | .0003 |
| Serum disease..... 2 | ... | ... | .003 | .003 |

progressive dilutions of the sera have failed so far to reveal any larger amount of isoagglutinins in the blood of patients with scarlet fever, pneumonia, typhoid fever, and tuberculosis than that of normal persons, but further observations are necessary before final conclusions are justifiable. In the sera of two cases of serum disease, one of which belonged to Group I, the other to Group II, the amount of isoagglutinin was the greatest of any serum yet examined; .0003 c.c. of the serum of the case in Group I being strongly agglutinative. Table 5 also shows that the different corpuscles of the same group vary in their agglutinability by the same serum; furthermore that sera of Group I may contain different amounts of agglutinin for Groups II and III.

I have not been able to find any isoagglutinins in the serum of rabbits, guinea-pigs, dogs, horses, and cattle. In each instance 0.1 c.c. of 10 or 20 different sera were tested upon 1 c.c. of a 2.5 per cent suspension of washed corpuscles from as many animals.

From the practical point of view isoagglutination of human corpuscles is of immediate interest in connection with the determination

of the opsonic index and with transfusion of blood. All who have worked with the opsonic index are familiar with the fact that occasionally the mixtures show marked clumping of the erythrocytes which naturally interferes more or less with the desired accuracy. In order to avoid this annoyance the washed blood (leucocytes) should be secured from an individual of Group I, i. e., from one whose corpuscles are not agglutinable.

By means of a delicate and highly refined technique Crile¹ recently has practiced direct transfusion of blood from normal to diseased human beings, and with marked apparent success so that it is not unlikely that the practice of transfusion may increase. The common occurrence of isoagglutinins in human serum suggests that under certain special conditions homologous transfusion might prove dangerous by leading to erythrocytic agglutination within the vessels of the subject transfused. It has not been feasible to conduct animal experiments upon this point because of failure to demonstrate isoagglutinins in the serum of suitable animals. It may be pointed out, however, that the possible danger here indicated can be avoided by the selection of a donor whose corpuscles are not agglutinated by the serum of the recipient, and whose serum does not agglutinate the corpuscles of the latter; that is to say, donor and recipient should belong to the same group and preferably to Group I or II. The actual relation *in vitro* of the sera of prospective donors and the recipient to the respective corpuscles are readily determinable in a short time in the manner outlined in the foregoing.

¹ *Jour. Am. Med. Assoc.*, 1906, 47, p. 482.