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STUDIES IN ISOAGGLUTINATION.

III. TONICITY IN ISOHEMAGGLUTINATION.*

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In the introduction to the first paper in the present series,[1] an outline was given of the main facts concerning the isoagglutination of human bloods, and the division of human bloods into four sharply defined varieties or groups, according to the way in which they agglutinate each other. For the present purpose these groups may be briefly summarized as follows: In group I the serum agglutinates the cells of all other groups, but the cells are not agglutinable. In group II the serum agglutinates the cells of groups III and IV, and the cells are agglutinable by the sera of groups I and III. In group III the serum agglutinates the cells of groups II and IV, and the cells are agglutinable by sera of groups I and II. In group IV the serum is non-agglutinative, and the cells are agglutinable by sera of all other groups.

Three years ago Gay attempted to show that a relation existed between the resistance of human red blood cells to laking by hypotonic solutions and their isoagglutinative grouping. In an examination of twelve bloods he found that corpuscles not agglutinated by any sera (i. e., those belonging to group I) are more susceptible to laking by saline solutions of low percentage content than are

* Received for publication, March 17, 1911. This study is one of a projected series on *isoagglutination*, which in turn constitutes a section of a comprehensive plan of research on the composition of protoplasm, as well as the structural and dynamic relationships of cell constituents and products. These investigations are now in progress in the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, and under the auspices of the George Crocker Special Research Fund.

[1] Ottenberg, *Jour. Exper. Med.*, 1911, xiii, 425.

other corpuscles, and that definite tonicity groups exist corresponding to agglutination groups. He suggested that, as sera are assumed to be of the same tonicity as the contents of the red cells,[2] the differences in tonicity might be the cause of the agglutinations.

The hypertonicity of sera belonging to the first group would explain their power of agglutinating cells of the other groups. The hypertonicity of the serum of one of the agglutinable groups would explain its agglutination of the cells of the other group. But, as Gay himself points out, it would not explain the reverse interaction between the groups, which also occurs.

Agglutination groupings are permanent for the individual. They are hereditary. Isoagglutinative serum is active at a considerable dilution—generally up to about 1 in 32—with ordinary physiological saline solution. The resistance of human red cells is known to vary widely in disease and under special conditions, such as follow the ingestion of salts (Sutherland and McCay). These important facts led us to doubt whether so simple an explanation as Gay offers can account for the phenomena. We therefore determined to reëxamine the subject and repeat Gay's experiments.

The technique which we followed was practically identical with that of Gay. With a clean, dry, hollow needle, fifteen to twenty cubic centimeters of blood were obtained from an arm vein and defibrinated by a few glass beads in a perfectly clean bottle. (Gay used a rod instead of beads, and obtained a somewhat larger amount of blood.) After the cells had sedimented during six hours in an ice box, all the supernatant clear serum was pipetted off and the sedimented cells were used for the tonicity tests. Bloods of which the serum showed any trace of laking were discarded.

It is not necessary to describe here again the well-known method of making the agglutination tests and of arranging the bloods in their correct groups.

The resistance of the red blood cells was tested by Hamburger's method, as used by Gay. A series of salt solutions ranging in concentration from 0.38 per cent. to 0.57 per cent. of sodium chlorid was prepared. In the first experiments, the difference between the

[2] This assumption is not entirely warranted, as the work of Theobald Smith and others has shown.

successive concentrations was 0.03 per cent., as in Gay's work. Later it was made 0.02 per cent. All precautions were taken to make the solutions accurate and constant. Chemically pure sodium chlorid dried to constant weight was used, and fresh solutions were prepared for each new group of cases tested. Evaporation was carefully prevented.

In testing each blood for tonicity, a series of small test tubes was arranged, each tube containing five cubic centimeters of one of the graded salt solutions. The sedimented unwashed red cells were shaken, to insure uniform distribution, and 0.1 cubic centimeter of the suspension was added to each tube. The tubes were shaken and allowed to stand at room temperature for a short time. Readings were made then, and again after the tubes had been in an ice box for twelve hours.

The amount of hemolysis was estimated for each tube of the series by comparing its color with the colors of a standard series made up separately with each blood. This scale was prepared by laking 0.4 cubic centimeter of the sedimented red cells in twenty cubic centimeters of distilled water (the same ratio of blood as that in the tests). This was designated 100 per cent. hemolysis and dilutions of this were then made to represent 80, 70, 60, 40, 30, 20, 10, 5, and 2.5 per cent. hemolysis. Each of these solutions was put in a tube of the same caliber as that of the tubes used in the tests.

In order to facilitate our discussion, we reproduce Gay's[3] tables.

GAY'S RESULTS.

Non-Agglutinable Cells. Class I.

NaCl solution (%).	0.39	0.42	0.45	0.48	0.51	
	Amount of hemolysis (percentage).					
Blood No. 18	100	75	40	5	5	Aug. 26
	100	100	60	35	10	Sept. 24
" 22	100	90	75	50	2.5	Aug. 18
	100	40	15	5	2.5	Aug. 26
" 34	80	80	35	15	10	
" 35	95	50	15	10	5	
" 38	95	35	10	10	2.5	

[3] Gay, *Jour. Med. Research*, 1907–8, xvii, 330.

Agglutinable Cells. Class II.

NaCl solution (%).	0.39	0.42	0.45	0.48	0.51	
	Amount of hemolysis (percentage).					
Blood No. 17	90	65	25	15	5	Aug. 18
	90	50	15	5	2.5	Aug. 26
	60	65	15	5	5	Sept. 24
" 26	85	35	10	5	5	Aug. 18
	40	10	2.5	2.5	2.5	Aug. 26
	85	35	5	5	5	Sept. 24
" 30	70	70	10	5	2.5	
" 33	60	50	10	5	2.5	
" 37	70	45	25	5	0	

Agglutinable Cells. Class III.

NaCl solution (%).	0.39	0.42	0.45	0.48	0.51	
	Amount of hemolysis (percentage).					
Blood No. 23	65	25	5	5	5	Aug. 18
	80	15	5	5	2.5	Aug. 26
	50	40	10	5	0	Sept. 24
" 25	60	15	20	5	5	

Gay's conclusions from these results are as follows: "(1) That human corpuscles which are not agglutinated by foreign human sera are uniformly more susceptible to laking by salt solution of low percentage than are corpuscles which are agglutinated by foreign isosera. (2) That definite groups as regards resistance to salt solutions are present, which groups coincide with the groups classified according to agglutination. (3) That the resistance of a given blood does not change apparently within a month or more, and probably corresponds to the relatively unchanging relations manifested in isoagglutination."

Gay's tables do not entirely justify his conclusions, as an examination of the data will show. Thus the first two of five bloods belonging to class II exhibited, on several occasions, only the most minute differences from the last three of the five in class I. The differences between the same blood on several dates are, in some cases (e. g., No. 26), as great as the differences between bloods in the different classes.[4]

Our own work consisted of the examination of the blood of twenty-two persons. In our first group of six cases (table I), the

[4] Relations must be ascertained from general comparisons of the results for one blood with the results for another blood. The irregularities are so numerous that a comparison of different bloods as tested by any particular salt solution involves contradictions. As a rule, readings of more than 70 per cent. hemolysis can not be accurate, because the intensity of the colors makes such readings extremely uncertain. These remarks apply to our own, as well as to Gay's results.

TABLE I.

Sodium chlorid solution (per cent.).	0.42	0.45	0.48	0.51	0.54	0.57
	Amount of hemolysis (per cent.).					
Group I	25	5	0	0	0	0
	20	2.5	0	0	0	0
	40	5	0	0	0	0
Group II	60	10	2.5	0	0	0
	60	7	2.5	0	0	0
Group III	50	15	2.5	0	0	0

three classes showed a definite grouping as regards corpuscular tonicity. The blood in class I, however, proved to be the most resistant, that is, it was the least hemolyzable by hypotonic saline solution. This, strangely enough, is exactly the reverse of Gay's finding. The two groups of agglutinable cells showed little difference in saline concentration from each other, those of class II being least resistant.

Our second series (table II) consisted of the bloods of six other individuals. Here, the blood of the first group (only one individual in this group) is most easily hemolyzed, and the agglutinable cells evidence most resistance.

TABLE II.

Sodium chlorid solution (per cent.).	0.40	0.42	0.44	0.46	0.48
	Amount of hemolysis (per cent.)				
Group I	80	50	20	10	2.5
Group II	50	40	20	15	5
	50	20	10	5	2.5
	80	60	20	5	0
	60	10	10	2.5	0
Group III	60	25	15	2.5	2.5

In our third group of ten cases (table III), the first or non-agglutinable group showed instances of both low and high tonicity. Here, the second and third groups showed high tonicity, the reverse of Gay's findings.

TABLE III.

Sodium chlorid solution (per cent.).	0.38	0.40	0.42	0.44	0.46
	Amount of hemolysis (per cent.).				
Group I	60	40	20	10	0
	50	40	15	5	0
	40	30	10	0	0
	80	60	20	5	0
	80	60	40	10	5
	80	70	40	10	5
Group II	70	60	30	15	5
	80	40	40	10	5
	90	80	60	20	10
Group III	90	80	60	40	20

Correlating our results, we see that instances of high and of low tonicity occur with about equal frequency in the groups of agglutinable and non-agglutinable cells. We therefore conclude that there is no support for the opinion that isoagglutination of human blood is due simply to variations of molecular concentration.

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