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STUDIES IN ISOAGGLUTINATION.

II. THE OCCURRENCE OF GROUPED ISOAGGLUTINATION IN THE LOWER ANIMALS.*

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In the previous paper of this series,[1] the isoagglutinins which occur in nearly all human bloods, and according to which all human beings can be divided into four sharply separated groups, were discussed. For some time it has seemed to us that a constant and hereditary characteristic like this grouping must have some very fundamental significance, and is not likely to be limited to one species of animal.

Throughout the literature it is stated that normal isoagglutinins occur only in human beings. Von Dungern has recently shown that immune isoagglutinins can be developed in dogs by treating them with each other's blood, and that two isoagglutinable substances occur in dog bloods. He has also shown, with the method of absorption of agglutinins from animal sera by human red blood cells, that the serum of many animals contains agglutinins which have the same selective activity on human cells as that shown by human isoagglutinins. Likewise he has demonstrated by the absorption method with human sera and the red cells of other animals, that the cells of many other animals contain agglutinable substances

* 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.

identical in their susceptibilities with the isoagglutinable substances of human cells.

Grouped isoagglutination in animals seems to have escaped observation.

EXPERIMENTAL OBSERVATIONS.

We have investigated the occurrence of isoagglutination in thirty-two rabbits and in eleven steers.

Blood of Rabbits.—The rabbits were taken in groups of about ten, and the serum of each rabbit was tested against the red blood cells of all the other rabbits in the group. Suspensions (3 or 5 per cent.) of washed red blood cells in 0.9 per cent. salt solution were used; and in general one volume of cell suspension was mixed with three volumes of serum. The mixtures were made in capillary pipettes of three to five millimeters in diameter, using the modified Wright technique as described by Epstein and Ottenberg.[2]

In the first set of examinations of rabbits, the tubes were sealed with paraffin and kept at room temperature for twenty-four hours. In the remaining series, the tubes were put in a thermostat for one hour at 37° C., and then in an ice box for twenty-four hours. The results were about the same.

TABLE I.

Sera.

Cells			I			II			III			IV	Kind of rabbit.
			3	6	12	13	14	1	9	10	11	7	
	I	3	−	−	−	−	−	−	−	−	−	−	White
		6	−	−	−	−	−	−	−	−	−	−	White
		12	−	−	−	−	−	−	−	−	−	−	Hare
	II	13	+	++	+	−	−	−	−	−	−	−	Black
		14	+	++	+	−	−	+?	−	−	−	−	White
		1	−	+	−	−	−	−	−	−	−	−	White
	III	9	++	+	++	+	++	++	−	−	−	−	Black
		10	++	+	+	+?	+	+	−	−	−	−	White
		11	++	++	++	−	+++	+	−	−	−	−	Hare
	IV	7	−	−	−	−	−	−	−	−	−	−	White

In those tubes in which the more marked agglutinations occurred, the phenomenon could be observed after about twenty to thirty

[2] Ottenberg and Epstein, *Arch. Int. Med.*, 1909, iii, 467

minutes. In the majority of cases, agglutination was seen only when the tubes were reëxamined after twenty-four hours. Most of the tests were examined microscopically also, but only agglutination which could be seen with the naked eye was recorded. In general, the agglutination results were not as striking as those with human blood. Tables I and II give the data arranged in groups.

TABLE II.

Sera.

Cells			I			II	III			IV				Kind of rabbit
			25	31	37	22	35	21	29	24	26	28	40	
	I	25	−	−	−	−	−	−	−	−	−	−	−	White
		31	−	−	−	−	−	−	−	−	−	−	−	White
		37	−	−	−	−	−	−	−	−	−	−	−	Gray
	II	22	+	++	++	−	−	−	−	−	−	−	−	White
	III	35	+	++	+?	+?	−	−	−	+?	−	−	−	Gray
		21	+	++	+++	++	+?	−	+?	+?	−	−	−	White
		29	+?	++	++	+	−	−	−	−	−	+?	−	White
	IV	24	−	−	−	−	−	−	−	−	−	−	−	White
		26	−	−?	−	−	−	−	−	−	−	−	−	White
		28	−	−	−	−	−	−	−	−	−	−	−	White
		40	−	−	−	−	−	−	−	−	−	−	−	White

Each series of observations was repeated and substantially the same results were obtained.

It is seen that the bloods divide themselves naturally into four groups. In group I the serum is agglutinative toward all agglutinable cells, but the cells are non-agglutinable. In group II the serum agglutinates cells of group III, but the cells are agglutinable only by the serum of group I. In group III the serum is generally non-agglutinative; the cells are agglutinable by the sera of groups I and II. In group IV the serum is not agglutinative, and the cells are not agglutinable (like the embryonic state of human blood). There was no relation of the grouping to the race or color of the animals. It was probably an accident that all the animals of group IV were white.

It is clear that the facts can be explained by the assumption that there are two agglutinins (which we may designate x and y) and two agglutinable substances (X and Y). Group I possesses agglu-

tinins x and y, but no agglutinable substance. Group II possesses agglutinin y and agglutinable substance X. Group III possesses agglutinable substances X and Y, but no agglutinin (with possibly occasional exceptions). Group IV possesses neither agglutinable substance nor agglutinin.

Studies of the effects of dilutions were made with a number of the sera. In general they produced decided agglutination up to a dilution of 1 in 4 (when a quarter of the total volume of the mixture was 5 per cent. cell suspension), but perceptible agglutination was observed with several of them up to a dilution of 1 in 10. It is probable that because of their feeble action these agglutinins have been overlooked.

Blood of Steers.—The blood of fifteen steers was defibrinated. Four which showed some laking were rejected. The other eleven were tested for isoagglutination with the same technique that was used in the rabbit experiments. The resultant agglutinations were much heavier than those obtained with rabbit blood, in fact they were quite as striking as human isoagglutinations.

TABLE III.

Sera.

			I		II						III		
			6	11	2	3	5	8	10	12	7	9	13
Cells	I	6	−	−	−	−	−	−	−	−	−	−	−
		11	−	−	−	−	−	−	−	−	−	−	−
	II	2	++	++	−	−	−	−	−	−	−	−	−
		3	++	++	−	−	−	−	−	−	−	−	−
		5	+	+	−	−	−	−	−	−	−	−	−
		8	+	++	−	−	−	−	−	−	−	−	−
		10	+++	++	−	−	−	−	−	−	−	−	−
		12	++	+++	−	−	−	−	−	−	−	−	−
	III	7	−	−	−	−	−	−	−	−	−	−	−
		9	−	−	−	−	−	−	−	−	−	−	−
		13	−	−	−	−	−	−	−	−	−	−	−

When the results are arranged in tabular form, the bloods of the steers are seen to fall into three groups (table III). Group I is agglutinative, but not agglutinable. Group II is agglutinable, but not agglutinative. Group III is neither agglutinable nor agglutinative. The grouping of these eleven bloods can be explained by assum-

ing that there is one isoagglutinin and one isoagglutinable substance. Bloods of group I possess agglutinin, but no agglutinable substance. Bloods of group II contain agglutinable substance, but no agglutinin. Bloods of group III contain neither agglutinable substance nor agglutinin. It is possible that examination of a larger number of animals may reveal the existence of a second agglutinin and a second agglutinable substance.

CONCLUSION.

Grouped isoagglutination is not limited to man, but is much more widespread than has been hitherto suspected. It occurs in the bloods of steers and rabbits. It seems probable that it will be found to occur in the bloods of other animals. Just how many of the isoagglutinins and the isoagglutinable substances in different species are respectively identical is still to be determined. The work is being continued with other animals.