

AUTOHEMAGGLUTINATION EXPERIMENTALLY  
INDUCED BY THE REPEATED WITH  
DRAWAL OF BLOOD.

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PLATE 22.

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There is much evidence in the literature to show that moderate losses of blood act to increase the titer of antibodies developed in response to an injected antigen.<sup>1</sup> Little attention, though, has been given to whether the production of antibodies normal to the organism is likewise stimulated. The point has considerable clinical interest, especially in connection with the remarkable resistance to infection manifested by many patients with severe anemia. In the work now reported one phase of the problem has been taken up; namely, the influence of repeated bleedings on the normal isohemagglutinins.

*Experiment 1.*—Interagglutination tests were carried out with the cells and sera of twelve normal rabbits. The cells had been twice washed and made to 5 per cent suspensions with salt solution. The sera were obtained from the clot at room temperature (22°C). Mixtures of each serum with each cell suspension in equal parts were kept at 22°C. for 15 minutes and examined with the microscope. Agglutination was found in a moderate proportion of the 144 mixtures. Autoagglutination was not noted.

On the basis of the findings, the animals were separated into two groups, each possessing about the same proportion of material for isoagglutination; that is to say, susceptible cells and agglutinating sera. One group of six rabbits was set aside as controls, and the remaining six rabbits were bled 10 cc. from the heart every 3 to 6 days during a period of 2 months. All were kept under the same conditions. From time to time the interagglutination tests were repeated, sometimes by the method just described, sometimes by mixing the whole citrated bloods in the proportions of 9 to 1 and 1 to 9, according to the method of Rous and Turner.<sup>2</sup>

<sup>1</sup> See Lippmann, *Z. exp. Path. u. Therap.*, 1914, xvi, 124, for large bibliography.

<sup>2</sup> Rous, P., and Turner, J. R., *J. Am. Med. Assn.*, 1915, lxiv, 1980.

*Experiment 2.*—Fourteen rabbits were used in two groups arranged on the same basis as in Experiment 1, but with only six individuals in the control group. The other eight animals were bled as already described during a period of 26 days. Interagglutination tests were carried out between the control animals and between these and the bled animals, but not between the individuals of this latter sort. Citrated bloods were used in the tests, which were repeated at intervals of a week or more. Tests with sera and washed cells were also made when this seemed advisable. In whole citrated bloods autoagglutination is easily seen. None was discoverable prior to the bleedings.

#### *No Induced Isoagglutinins.*

A number of the rabbits possessed isoagglutinins to start with. Some were bled, and some kept as controls. The bleedings had no demonstrable effect to alter the isoantibodies or to cause an appearance of new ones. It is true that weak isoagglutinins sometimes developed in individuals possessing none to begin with, but they were found to practically an equal degree in the controls and were probably in the nature of intercurrent serum changes such as Ottenberg and Thalhimer<sup>3</sup> have reported.

#### *Clumping in the Shed Blood.*

In five out of fourteen of the bled rabbits there developed a tendency of the red cells to clump together into masses in the shed blood. The clumping was never general, bringing together all the cells, as in the case of rabbits repeatedly transfused,<sup>4</sup> but the cell masses lay scattered here and there amid free cells. The phenomenon was not found in any of the twelve controls, nor has it since been observed in a large series of other normals. In instances of anemia, on the other hand, resulting from malnutrition, it has sometimes been met with.

The clumping phenomenon was definitely associated with the anemia following in some instances on the bleedings. Many medium sized rabbits withstand excellently the loss of 10 cc. of blood every 3 to 5 days during a long period. Their hemoglobin percentage and the appearance of the corpuscles remain practically unaffected. The clumping was never noted in these animals. In other rabbits the

<sup>3</sup> Ottenberg, R., and Thalhimer, W., *J. Med. Research*, 1915-16, xxxiii, 213.

<sup>4</sup> Rous, P., and Robertson, O. H., *J. Exp. Med.*, 1918, xxvii, 509.

bleedings rapidly brought about a moderate anemia with pale corpuscles, microcytes, and poikilocytes in circulation. In such instances the clumping occurred, though it was by no means a regular accompaniment of the condition. The number of bleedings and total loss of blood prior to appearance of the clumping were sometimes surprisingly small. Clumping was well marked in a 1,500 gm. rabbit 3 days after the last of two bleedings of 10 cc. each with an interval of 3 days between. In another animal of 1,350 gm., also bled twice, but at an interval of 7 days, the phenomenon was visible 3 days after the last bleeding. In both cases the blood loss was very poorly borne.

The clumping was plainly apparent in whole citrated bloods<sup>5</sup> allowed to stand for 15 minutes or more at room temperature and examined microscopically after dilution with salt solution. When at all marked it could be seen in thick slide preparations of the blood, as such, providing the cells were not numerous enough to interfere with the observations (Fig. 1). Under these circumstances it appeared within about a minute after the blood was shed and before any clotting had occurred. Each clump consisted of from 3 or 4 to 40 or 50 corpuscles massed helter-skelter.

#### *Cause of the Clumping.*

The question whether the phenomenon had its cause in a change in the cells, or plasma, or in both, was largely answered by the routine tests of Experiments 1 and 2. These demonstrated that the cells of the bled rabbits had undergone no alteration as regards agglutinability or inagglutinability by normal sera of known behavior. Furthermore, the clumping, like that due to the autoagglutinin present in normal plasma<sup>6</sup> and the principle present in the plasma of transfused rabbits,<sup>4</sup> did not occur at body heat. The cells remained free in citrated blood at 38°C., and the massing together which was visible at room temperature disappeared when the specimen was warmed. Corpuscles separated from the citrated plasma while warm, then washed in warm salt solution and brought together in it at room temperature,

<sup>5</sup> 10 parts of blood to 1 part of a watery 10 per cent solution of sodium citrate.

<sup>6</sup> Landsteiner, K., *Munch. med. Woch.*, 1903, i, 1812.

failed entirely to clump. But when a little of the thick cell suspension was dropped into the original citrated plasma, the cells massed together at once. All these facts showed that the clumping was due to an element in the serum and that this element has much in common with the normal and induced autoagglutinins.

*Distinguishing Traits of the Agglutinin.*

The agglutinin of the bled rabbits was able to cause clumping in the whole blood as such, or in the whole citrated blood, at room temperature (22°C.), whereas the normal agglutinin is effective at 22° only when a large amount of serum is allowed to act on a few cells.<sup>5</sup> The agglutinating principle of the bled rabbits, obtained in the free state, as in serum separated from the cells by defibrination and centrifugation at 38°C., was so strong in all cases as to bring about clumping at 22°C. in mixtures of the serum with an equal part of a 5 per cent suspension of the animal's own washed cells, and in some instances when there was a further dilution with one part of salt solution. The serum of five normal rabbits similarly separated and tested yielded not the least trace of agglutination. These results with a constant amount of antigen (the 5 per cent cell suspension) rule out the possibility that clumping in the anemic rabbits was due merely to the action of the normal autoagglutinin on an antigen (the red corpuscles) diminished in quantity by the bleedings.

Attempts to obtain the agglutinating factor in salt solution led to a singular finding. Normal autoagglutinins become much more effective as cooling proceeds from room temperature to 0°C. and are best demonstrated in the cold. The agglutinin of the bled rabbits, on the other hand, while effective at room temperature, may be relatively little enhanced in activity by further cooling, and at 0°C. may be surpassed in its action by the normal antibody.

*Experiment 3.*—A few cubic centimeters of blood were obtained from each of two normal rabbits and two which had been repeatedly bled and were the possessors of an agglutinin which caused clumping at room temperature. The sera were separated from the cells by defibrination in the warm, and centrifugation in a water jacket at 38°C. Those of the bled animals caused clumping at room temperature when mixed with an equal volume of a 5 per cent suspension of the corresponding cells twice washed in the warm. In similar mixtures of normal

sera no clumping occurred. Now 1.2 cc. of each serum was mixed with 0.1 cc. of a 50 per cent suspension of the corresponding cells, and the tubes were plunged in melting ice. The results are given in Table I.

It will be observed that the agglutinin of the normal rabbit No. 2, while practically ineffective at room temperature (22°C.), caused a more complete clumping at 0°C. than did the agglutinins of the bled rabbits, which were so active at 22°C. A second experiment along these lines gave similar results. The data do not enable one to say whether two distinct sets of antibodies are here concerned, but they

TABLE I.

Rabbit.	Room temperature (22°C.). Microscopic observations after 15 min.				0°C. Macroscopic observations.	
	Whole blood.	3 parts serum+ 1 part 5 per cent red cells.	1 part serum+ 1 part 5 per cent red cells.	1 part serum+ 1 part salt so- lution+ 1 part 5 per cent red cells.	1.2 cc. serum + 0.1 cc. 50 per cent red cells.	
					After 8 min.	After 66 min.
Normal.						
1	0	0	0		+	Sedimentation incomplete; sedi- ment finely granular.
2	0	Tr.	0		++++	Complete sedimentation into a sin- gle, solid mass.
Bled.						
3	+ -	+	Tr.		+++	Sedimented completely into fairly large masses.
4	+	+	Tr.		++++	Complete sedimentation into a few large masses.

show clearly that the effects of an autohemagglutinin at one temperature cannot safely be taken as the indicator of the effects at another. The study of such antibodies assumes in consequence great complexity.

*Agglutination and Rouleau Formation Are Not Connected.*

The observations of several authors have led them to conclude that rouleau formation is intimately connected in its cause with agglutination. Our findings in transfused rabbits<sup>4</sup> would seem also to point to this, since the appearance of new agglutinins in the blood is

always preceded by exaggerated rouleau formation. But the present results with rabbits repeatedly bled prove that the association is not obligatory. Here a partial or complete loss of the tendency to rouleau formation was regularly noted to accompany the development of the autoagglutinin (Fig. 1).

#### SUMMARY.

The repeated withdrawal of moderate quantities of blood does not lead to a development of new isoagglutinins in rabbits, or to noteworthy changes in normal ones already present. On the other hand, clumping of the animals' own cells in specimens of the whole blood is a frequent result. It is found in animals rendered anemic by the bleedings, not in those that rapidly repair their losses and remain in good condition. A similar clumping is sometimes to be seen in the blood of rabbits rendered anemic by malnutrition.

The clumping is due to a true autoagglutinin, which differs from the normal autoagglutinin in its far greater strength, as also, at least in some instances, by a peculiar variation in its activity with changes of temperature.

In the rabbits which developed isoagglutinins after bleeding, the tendency of the cells to form rouleaux was far less than the normal. It follows that rouleau formation is not essentially connected with autoagglutination, as has been assumed in the past.

In the light of the present findings a systematic search for auto-hemagglutinins in the blood of anemic patients would seem of interest. They have been noted in sick human beings (Ascoli, Klein), but not in recent years. The reason for this may well be that present day blood examinations are not of a sort to bring about their discovery. Thick films of fresh blood are seldom used for clinical purposes, and it is in thick preparations that clumping is most readily observed.

#### EXPLANATION OF PLATE 22.

Fig. 1. Autoagglutination in the blood of a rabbit rendered anemic by bleeding. Fresh slide-and-cover-glass preparation.

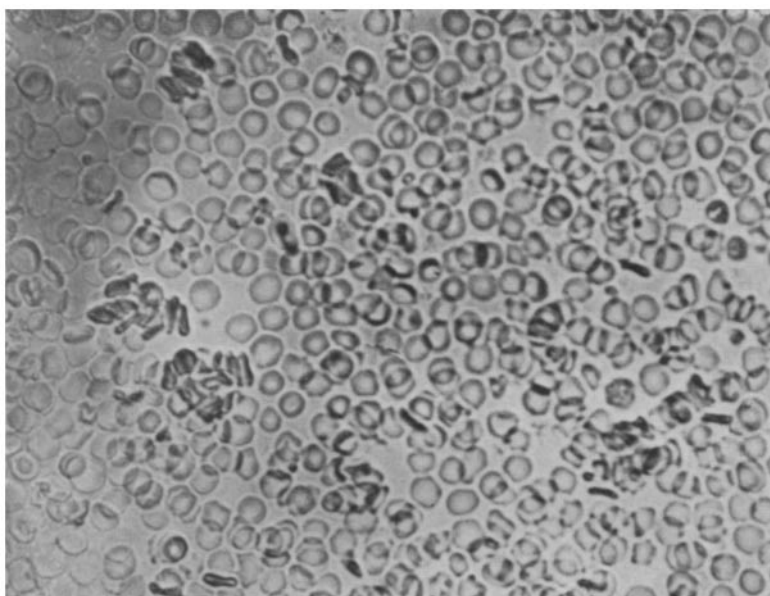


Fig. 1.

(Robertson and Rous: Autohemagglutination.)