LII. AN INVESTIGATION INTO THE PHYSICO-CHEMICAL MECHANISM OF HAEMOLYSIS BY SPECIFIC HAEMOLYSINS. (Preliminary Communication.)

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The mechanism of haemolysis by specific haemolysins has not been much investigated from the physico-chemical standpoint. It is supposed that the haemolysin affects the permeability of the envelope. Baumgarten has noticed that the first stage of haemolysis, as produced by a biological poison, is the swelling of the corpuscles, just as occurs when the serum is made hypotonic. Bang [1910] considers that haemolysis by means of Cobra poison is due to change in the composition of the lipoid membrane of the erythrocyte rendering it more permeable to salts. Through this membrane the extracellular salts and water soak gradually, until in the end the corpuscle ruptures. The behaviour of the nucleated erythrocytes of amphibians towards specific haemolysins tends to shew that their permeability is alone affected during the process [Landau 1903].

So far as I am aware, no work has been done on the behaviour of erythrocytes loaded with amboceptor towards distilled water before the complement has been allowed to act upon them. The present paper gives the results of investigations on the determination of the resisting power of amboceptor-loaded human erythrocytes to haemolysis when under the influence of distilled water. The haemolytic antisera were obtained from fowls and rabbits that had been immunised by means of injections of washed human corpuscles.

It was originally expected that amboceptor-loaded corpuscles would be less resistant than normal ones in their behaviour towards distilled water. Contrary to what was expected, I noticed a remarkable increase of the resisting power of the erythrocytes.

The method adopted for determining the resisting power of the erythrocytes is that previously described by me [1909, 1911] for the determination

of the specific resistance of erythrocytes to haemolysis. A series of experiments was performed and these are described as follows:—

In the first series, the erythrocytes were suspended in 0·85 % NaCl and allowed to haemolyse with two parts of distilled water. The mixture was allowed to stand for ten minutes and the dissolved haemoglobin was estimated in the manner previously described. This gave the resistance of the normal erythrocytes to haemolysis.

In the second series, the erythrocytes were mixed with two parts of a 10 % dilution of complement-free antihuman fowl's haemolytic serum with 0·85 % NaCl and kept in the incubator for one to three hours and subsequently treated in the same way as above with distilled water after having been thoroughly washed in 0·85 % NaCl, and the resistance of the amboceptor-loaded erythrocytes determined in the same way as above.

In the third series, the erythrocytes were treated with complement-free antihuman rabbit's haemolytic serum and their resistance to haemolysis determined.

As a control test, a few experiments were made to determine the resistance of human erythrocytes after having been treated with normal fowl's serum in the same way as above.

In this way the resistance of the erythrocytes under different conditions was determined and the following tables worked out. The resistance of the erythrocytes is expressed in terms of the relative haemoglobin-value of the resistant erythrocytes which is the ratio between the amount of haemoglobin in the resistant corpuscles and the amount in the total suspension of erythrocytes used.

Tables shewing the resistance of erythrocytes to haemolysis under different conditions:—

TABLE I.

	Resistance of normal erythrocytes to haemolysis	Resistance of erythrocytes loaded with amboceptor obtained from fowl
1	0·345	0·500
2	0·379	0·456
3	0·242	0·407
4	0·333	0·480
5	0·346	0·452
6	0·269	0·531
7	0·333	0·466
8	0·350	0·444
9	0·301	0·421
10	0·288	0·459
11	0·288	0·368

TABLE II.

	Resistance of normal erythrocytes to haemolysis	Resistance of erythrocytes loaded with amboceptor obtained from rabbit
1	0.195	0.600
2	0.188	0.378
3	0.296	0.592
4	0.240	0.480
5	0.132	0.220

TABLE III.

	Resistance of normal erythrocytes to haemolysis	Resistance of erythrocytes treated with normal fowl's serum
1	0.288	0.243
2	0.288	0.298
3	0.282	0.259
4	0.240	0.282

It will be seen from the above tables that in every case the addition of the amboceptor increased the specific resistance of erythrocytes, while the addition of normal fowl's serum did not bring about any difference in their resisting power.

The problem that we are now to solve is how it is that the erythrocytes become more resistant when they become fixed to the amboceptor but lose all their resisting power as soon as the complement acts and what is the biological significance of this remarkable phenomenon. An explanation may be offered, which however is hypothetical. In the first process of the action of haemolysin during which the amboceptor combines with the erythrocytes, we may assume that this combination is purely of the nature of *adsorption* and not true chemical combination. In this process the dimensions of the pores between the molecules of the outer wall of the erythrocytes become small due to the amboceptor molecules filling the pores of the original erythrocytes. As diffusion depends, specially, upon the dimensions of the pores of the membrane through which it takes place, less haemoglobin passes out through the red corpuscles loaded with amboceptor than the unloaded ones when they are treated with distilled water. In the case of the colloidal complex of amboceptor and erythrocyte molecules, the bodies brought into close contact with each other do not react with one another in the chemical sense. Chemical reaction which, according to Bayliss [1912], is the third and last stage of the heterogeneous reactions of colloidal complexes, takes place between the molecules of the amboceptor and of the

erythrocytes only through the agency of the complement, which probably acts more or less like a ferment. When this takes place, there is a condensation of the molecules of the colloidal complex in each erythrocyte and as a result of this, the dimensions of the pores of the membranes of the red corpuscles increase to such an extent that haemoglobin diffuses out of the corpuscles and haemolysis results.

This theory will easily explain the inhibitory influence exerted by hypertonic saline solutions on the action of specific haemolysins as has been observed by Sutherland and McCay [1911] and others. Such saline solutions would tend to reduce the size of the erythrocytes by exosmosis of water from them and as a result of this the molecules of the erythrocytes come closer to each other and haemolysis is prevented. In other words the widening of the pores of the erythrocytes brought about by the action of the complement on the amboceptor-loaded erythrocytes is counteracted by the presence of the hypertonic saline.

To test the accuracy of the theory the following experiments were performed:—

A suspension of sheep's corpuscles in normal saline was mixed with amboceptor and complement and kept in the incubator. The process of haemolysis was stopped in from five to ten minutes before it was complete, the corpuscles quickly centrifuged and the supernatant fluid replaced by N/2 NaCl, after thoroughly washing the corpuscles several times with the same. A portion of the suspension of the corpuscles in N/2 NaCl was again centrifuged and the N/2 solution was pipetted off and in its place an excess of 0.85% NaCl solution was substituted—a marked haemolysis resulted. Thus we have the following results:—

(1) Amboceptor-loaded corpuscles partially acted upon by complement, thoroughly washed in N/2 NaCl—no haemolysis.

(2) Amboceptor-loaded corpuscles partially acted upon by complement, thoroughly washed in N/2 NaCl and then the N/2 NaCl replaced by 0.85% NaCl—distinct haemolysis.

In other words amboceptor-loaded corpuscles which have been partially acted upon by the complement are haemolysed when brought in contact with normal saline. The dimensions of the pores of such corpuscles when suspended in N/2 NaCl are much less than when suspended in normal saline, i.e. there is marked widening of these pores when they are suspended in normal saline and haemoglobin diffuses out. Evidently, therefore, the pores of the amboceptor-loaded corpuscles that have been acted upon partially by the complement are much wider than those of the normal ones. The

complement must have therefore brought about a condensation of the molecules of the amboceptor-loaded corpuscles, as explained before, thereby leading to widening of their pores to such an extent as to allow the haemoglobin molecules to pass out of them, even when they are suspended in normal saline.

An attempt was made to determine the volume of the corpuscles before and after combination with amboceptor. After rejecting different methods, the following method was devised for determining the size of the corpuscles. Human and sheep's corpuscles were used for the purpose.

The corpuscles were first suspended in 0.85% NaCl and the suspension was sucked into two large-bore haematocrit tubes of exactly the same diameter and the corpuscles in both were centrifuged at a high speed for about ten minutes, after which their volume was noted. The clear supernatant fluid from one of the tubes was now pipetted off and a dilution of haemolytic amboceptor in 0.85% NaCl (one part of amboceptor and nine parts of 0.85% NaCl) introduced in its place. The amboceptor was then thoroughly mixed with the corpuscles and the mixture allowed to stand for ten minutes. The other tube which was used as a control was then shaken up and the corpuscles again mixed with the supernatant fluid. The two tubes were again centrifuged for ten minutes and the volume of the corpuscles noted. If the volume of the corpuscles in the control tube was found to be exactly equal to what was noted in it in the first experiment, then there was no error due to any change in the speed of the centrifuging tube. The volume of the amboceptor-loaded corpuscles was then noted. If the volume of the corpuscles in the control tube was different in the second experiment from what was noted in the first, then the volume of the amboceptor-loaded corpuscles was accordingly changed. In this way, absolutely accurate results were obtained.

Thus, if V be the volume of the corpuscles in the control tube after the first centrifuging and V' after the second, then $V - V'$ is the difference due to changes in the speed of the centrifuging machine.

If now v be the volume of the corpuscles before being loaded with amboceptor, and v' after being loaded with amboceptor, it is evident that $v - v'$ is not actually the change in the volume of the corpuscles. The exact change is, evidently,

$$(v - v') + \frac{v}{V}(V - V').$$

In this way, the exact change in the volume of the corpuscles due to combination with amboceptor was found and the following results obtained.

Human corpuscles.

Volume before action of amboceptor	Volume after action of amboceptor
(1) 2.75 cm.	(1) 2.75 cm.
(2) 2.75	(2) 2.75
(3) 2.50	(3) 2.50
(4) 1.50	(4) 1.50
(5) 1.45	(5) 1.50

Sheep's corpuscles.

Volume before action of amboceptor	Volume after action of amboceptor
(1) 3.30 cm.	(1) 3.30 cm.
(2) 5.38	(2) 5.20
(3) 4.50	(3) 4.20
(4) 6.23	(4) 6.22

It will be seen from the above tables that there was no reduction in the volume of the corpuscles after the action of the amboceptor and therefore the increase in their resistance to haemolysis was not due to any diminution in their volume after the action of the amboceptor.

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