RESEARCH PAPERS

THE EFFECTS OF ANAPHYLACTIC AND PEPTONE SHOCK ON THE COAGULABILITY OF RABBIT AND GUINEA-PIG BLOOD

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IN the dog, in both anaphylactic and peptone shock, there is an increase in the clotting time of the blood. This was first shown for peptone shock by Schmidt-Mülheim[1], and for anaphylactic shock by Biedl and Kraus[2]: and has been amply confirmed by other workers. Howell[3] first suggested that the anticoagulant might be heparin, and some later work by Quick[4] on peptone shock lent support to Howell's view. Waters, Markowitz and Jacques[5]—following the demonstration by Chargaff and Olson[6] that protamine combined with heparin—found that the protamine titre of the blood of an anaphylactic dog was very high, and hence inferred the presence of abnormal amounts of heparin in it. The presence of heparin in such blood was finally established by its isolation in crystalline form by Jaques and Waters[7], who also showed that its source was the liver.

In the guinea-pig and rabbit in anaphylactic or peptone shock the situation is not so clear. Not only have no attempts been made to demonstrate the liberation of heparin in these animals, but there is even dispute as to whether the clotting time of guinea-pig blood is in fact increased in anaphylactic shock. Thus, while Friedberger[8] and Dale[9] reported a prolongation of clotting time, Eagle, Johnson and Ravdin[10] found no change. Dragstedt[11], in a review of anaphylaxis, stated that there is usually no change, although sometimes the blood may become incoagulable. In the rabbit in anaphylactic shock, Auer[12], Scott[13], and Eagle, Johnson and Ravdin[10] noted a delay in blood coagulation. The last-named workers suggested that this was due to an increase in antithrombic activity. They did not, however, suggest that this increase was to be accounted for by heparin—nor, indeed, was there at that time any satisfactory test by which they could have determined the point.

The present work on rabbits and guinea-pigs was undertaken to clarify this confused situation and to determine if heparin is liberated by anaphylactic and peptone shock in these animals, as it is known to be liberated by the same conditions in the dog.

METHODS AND RESULTS

(*a*) *Anaphylactic shock in rabbits*

Rabbits were sensitised by injecting 2 ml. of horse serum on 3 occasions at intervals of 4 days. The first injection was given intravenously, and the second and third intraperitoneally. Anaphylactic shock was induced

4 weeks from the beginning of sensitisation, by the intravenous injection of 6 ml. of the antigen.

Initial experiments indicated that the clotting power of the blood was greatly reduced. It remained to determine if heparin was concerned in this change. For this purpose blood samples from shocked animals were treated with protamine sulphate, following control experiments which indicated that protamine had itself no action on the clotting time of normal blood.

For the control experiments sets of 9 small test tubes were set up in a water bath at 37° C. To the first 5 were added, in 0·9 per cent. saline solution, 0·025, 0·05, 0·10, 0·20 and 0·40 mg. of protamine sulphate respectively. To the sixth and seventh were added 10 units of heparin, the seventh tube having in addition 0·1 mg. of protamine sulphate, which was the amount found to neutralise 10 units of heparin *in vitro*. The eighth and ninth tubes were controls. The volume in each tube was made up to 0·4 ml. with normal saline solution.

In each of 4 experiments approximately 12 ml. of blood was collected by heart puncture from normal rabbits. 1 ml. was added immediately, and at random, to each of the tubes, and the clotting times determined. The results are shown in Table I. These indicate that protamine sulphate has no action on the formation of the normal clot.

TABLE I

CLOTTING TIMES OF NORMAL RABBIT BLOOD AFTER TREATMENT WITH HEPARIN AND PROTAMINE SULPHATE

Normal rabbit No.	Protamine sulphate added to each tube					Heparin 10 units	Heparin 10 units + protamine 0·1 mg.	Control	Control
	0·025 mg.	0·05 mg.	0·10 mg.	0·20 mg.	0·40 mg.				
	minutes							minutes	
1	3½	4½	4	5½	6	>60	5	4	5
2	2	2	2	2½	2	>60	2	2	2
3	3½	3½	4	4½	5½	>60	5	3	5
4	2	2	2	2	3	>60	3	2	2

Similar observations were then made with blood withdrawn from sensitised rabbits 10 minutes after administration of the shocking dose of antigen. The first 5 tubes were set up as in the experiments just described, but the sixth, seventh and eighth tubes were all controls. The

TABLE II

CLOTTING TIMES OF THE BLOOD OF ANAPHYLACTIC RABBITS

Shocked rabbit No.	Protamine sulphate added to each tube					Control	Control	Control	Remarks
	0·025 mg.	0·05 mg.	0·10 mg.	0·20 mg.	0·40 mg.				
			minutes				minutes		
1	—	>60	>60	>60	>60	>60	>60	>60	Slight clot formation
2	31	26	30	27	30	30	25	22	Formation of semi-solid clots only
3	—	30	35	30	36	38	31	29	
4	25	26	21	21	27	29	20	24	
5	28	26	60	37	60	27	31	40	
6	>60	>60	>60	>60	>60	>60	>60	>60	Slight clot formation

blood from shocked animals, unlike that from normal rabbits, did not produce hard and solid clots, and the end-points were consequently more difficult to determine. The times reported, therefore, refer in the main, to the formation of semi-solid clots. The results of these observations are given in Table II. It seems reasonable to infer from them that the lowered coagulability of the blood of rabbits in anaphylactic shock is not due to heparin, since the presence of protamine sulphate, which inactivates heparin, does not affect the clotting time.

(*b*) *Anaphylactic and peptone shock in guinea-pigs*

Anaphylactic shock was produced by the intravenous injection of horse serum or 1 per cent. egg white solution, following a sensitising period of at least 14 days. Peptone shock was produced by a single intravenous injection of 1·25 g./kg. of peptone-oxoid, which had been treated with permutit in order to remove most of the free histamine.

Clotting times were obtained in the following way. The heart was exposed at death, which usually occurred within 3 to 5 minutes of the injection, and a glass melting point tube, 150 mm. × 1·0 mm., was inserted into the left ventricle. The tube quickly filled with blood, since the heart continued to beat for a short time after respiration had ceased. A small piece of the tube was then gently broken off one end, every 30 seconds, until thin fibrin threads appeared. The blood was then considered to have clotted. We have found previously that this method, though without the high degree of accuracy of some techniques, has given good results when determining clotting times following the administration of heparin. The results of this series of experiments are given in Table III, the first row of this gives the mean clotting time for a group of 36 normal animals, the others the mean clotting times in various experimental circumstances.

TABLE III

CLOTTING TIMES OF GUINEA-PIG BLOOD IN CONDITIONS OF ANAPHYLACTIC AND PEPTONE SHOCK

Group	Type of shock	Time of death after injection	Time of blood samples	Number of animals	Mean clotting times in minutes ± S.D.
1	Controls	—	—	36	3·7 ± 1·5
2	Anaphylactic	<5 minutes	At death	12	3·4 ± 0·8
3	Peptone	<5 minutes	At death	12	3·2 ± 0·9
4	Anaphylactic	Protected by antihistaminic	15 minutes after injection	12	4·0 ± 1·8
5	Peptone	Protected by antihistaminic	15 minutes after injection	12	3·6 ± 1·0
6	Peptone	10 to 30 minutes	At death	12	3·8 ± 0·4

As previously noted, most animals die from anaphylactic shock within 5 minutes of the injection. There was, therefore, a distinct possibility that even if heparin had been liberated during the initial stages of the shock it would not have been able to exert its influence on coagulability

before the animal died. Groups 2 and 3 in Table III are animals in which this state of affairs obtained. It is evident that the clotting times in both groups do not differ from the control values derived from the normal animals of Group 1.

To overcome the difficulty just mentioned, groups of animals were protected from the fatal effects of the shock by an antihistaminic, and the clotting times determined 15 minutes after administration of the antigen or peptone. Columns 4 and 5 of Table III give the results in these animals. Once more the clotting times do not differ from the control values. Similar results are obtained, in the absence of an antihistaminic, in those animals whose death from peptone shock is delayed, and occurs 10 to 30 minutes after the shocking dose, and in which, therefore, any heparin that might be liberated would certainly have had time to exert an anticoagulant action by the time the blood was withdrawn at death. Here again, however (Group 6, Table III), the clotting times are normal. In guinea-pigs, therefore, it is clear that heparin is not liberated in anaphylactic or peptone shock and, furthermore, that the clotting times in shock are no different from those in normal animals.

Discussion

It is now well established that a common factor, and indeed the salient feature, in anaphylactic and peptone shock in animals is the liberation of histamine. It is clear from previous work that the lowered coagulability of the blood in anaphylactic shock in the dog is due to the liberation of heparin from the liver. It is equally clear from the present work that the blood of shocked rabbits has also a lowered coagulability but that this is not due to heparin. Finally, in guinea-pigs there is no alteration of the blood coagulability in shock. It remains to account for these differences in the blood changes in shock, in face of the common factor of histamine liberation.

In the dog in anaphylactic shock the main site of histamine liberation, as of heparin liberation, is the liver; and this organ is extremely rich in mast cells. Riley and West[14] have recently shown a striking correlation between the mast cell and heparin content of a tissue, and the amount of histamine the tissue contains. Riley[15] had previously shown that when lethal doses of the histamine liberator stilbamidine were administered to rats some of it was temporarily trapped in the mast cells, which then disrupted. From these two sets of observations Riley and West concluded that heparin and histamine coexist in the mast cells. While there is as yet no direct evidence that this is so, their hypothesis accords well with what happens in anaphylactic shock in the dog—especially when one considers the extremely rich distribution of mast cells in the liver of this animal, and the fact that in shock it is from the liver that both the histamine and the heparin are derived.

The absence of heparin liberation in the rabbit is not entirely inconsistent with Riley and West's view. The anaphylactic reaction in this animal is mainly confined to the blood, and the main source of histamine appears to be the platelets[16], which are devoid of heparin. Since tissue

mast cells may accordingly not be involved in the shock reactions, the absence of heparin in these conditions would be explained. This, however, does not account for the lowered blood coagulability which is in fact observed in shocked rabbits, and which must clearly be due to some other factor.

The absence of prolonged clotting times in anaphylactic and peptone shock in the guinea-pig is difficult to explain. There is no doubt that death from shock is caused by liberation of histamine from the lungs, which, in this animal, are comparatively rich sources of it[17]. Histological examination has confirmed the presence of mast cells in the guinea-pig lung. If, therefore, histamine and heparin coexist in these mast cells one would expect their disruption to liberate heparin in an amount sufficient to prolong the clotting time of the blood. It may well be, of course, that mast cells show species differences, and that those of the guinea-pig contain little or no histamine.

Alternatively, the histamine liberated by shock in the guinea-pig may come mainly, or even exclusively, from the reacting structures themselves—the bronchiolar muscle cells—and thus be intrinsic histamine in the sense defined by Dale[18].

There is some evidence that the liberation of only small amounts of histamine within the lungs may cause death in the guinea-pig[19,20,21], and that the source of this histamine may be the mast cell. In that event, if the disruption of only small numbers of mast cells is concerned, the amount of heparin liberated may be insufficient to alter the clotting time of the blood.

Summary

1. While it has been established that anaphylactic shock in the dog causes a lowered coagulability of the blood, and that this is due to the liberation of heparin, what happens to rabbit and guinea-pig blood in similar circumstances is not clear.

2. Experiments are described on anaphylactic shock in the rabbit, and on anaphylactic and peptone shock in the guinea-pig, which seek to clarify this confused situation.

3. The results show (*a*) that though in anaphylactic shock the clotting time of rabbit blood is prolonged, this is not due to the liberation of heparin, and (*b*) that in neither anaphylactic nor in peptone shock is the clotting time of guinea-pig blood prolonged.

4. The results are discussed in relation to the hypothesis that heparin and histamine coexist in mast cells.

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