

THE DIMINISHED COAGULATION OF THE BLOOD IN ANAPHYLACTIC SHOCK IN THE DOG.*†

O. H. PERRY PEPPER AND E. B. KRUMBHAAR.

(From the John Herr Musser Department of Research Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.)

In a recent study carried on in this laboratory an attempt was made to formulate a method of procedure for the investigation of the coagulation of the blood in cases of purpura and allied conditions. Following the publication of that work¹ it occurred to us that it might be of interest to apply the method to a study of the changes in the blood following anaphylactic shock, it being well known that one of the phenomena of anaphylactic shock is the delay or loss of the coagulability of the blood.

This was early (1909) described by Biedl and Kraus,² who studied dog's blood, and by Friedberger,³ who found the same changes in the blood of the guinea-pig. Later studies have fully confirmed these observations and many investigators have advanced explanations. The majority, as for example Arthus,⁴ are impressed by the similarity of the changes to those produced by Witte's peptone. Edmunds,⁵ who has studied the influence of toxic protein fractions, finds that only in their failure to produce delayed clotting of the blood does the effect of these bodies differ from anaphylactic shock. That it is not an agonal condition was shown by Weiss and Tsuru,⁶ who failed to find it in chloroform death. Sirensky⁷ found the calcium and magnesium content of the blood unchanged, but the fibrinogen and fibrin ferment slightly decreased. Achard and Aynaud⁸ describe an associated disappearance of platelets, but Biedl and Kraus report contrary findings.

Blaizot⁹ finds that normal or sensitized rabbits which possess demonstrable fibrin ferment in the blood lose it during anaphylactic shock.

These statements are representative of the views expressed in the literature of this subject and it is therefore evident that the phenomenon is as yet unexplained. Our results do not offer a

* Received for publication October 28, 1913.

† Aided by grant from the Committee on Scientific Investigation of the American Medical Association.

¹ *Arch. Int. Med.*, 1913, 11, p. 395.

² *Wien klin. Wchnschr.*, 1909, 11, p. 363.

³ *Ztschr. f. Immunitätsf.*, I, Orig., 1909-1910, 8, p. 636.

⁴ *Presse Medicale*, 1909, 17, p. 305.

⁷ *Ibid.*, 1911, 12, p. 328.

⁵ *Ztschr. f. Immunitätsf.*, I, Orig., 1913, 17, p. 105.

⁸ *Compt. rend. Soc. de biol.*, 1909, 67, p. 83.

⁶ *Ztschr. f. Immunitätsf.*, 1910, 5, p. 516.

⁹ *Ibid.*, 1911, 71, p. 425.

complete explanation but as they are most suggestive, and throw light on one or two phases of the problem, they are here presented. Our work has shown (1) that the ability of the non-coagulating, post-anaphylactic, oxalated plasma to coagulate can be regularly restored by the addition of small amounts of thromboplastin. The coagulation time of the plasma treated in this way is, however, usually long. (2) Furthermore, we have found that the blood of such dogs as do not develop shock, upon administration of the intoxicating dose, presents certain peculiarities of coagulation.

Our technic was as follows: Dogs were given a sensitizing injection of 5 c.c. of normal horse serum and were then kept under constant conditions for at least 3 weeks. At the end of from 3 to 6 weeks a sufficient amount of blood for study was withdrawn under ether anesthesia from the femoral artery. This was received directly from the cannula into 1 per cent sodium oxalate solution in the proportion of one part of blood to ten of solution. The intoxicating dose of horse serum (5 c.c.) was then injected intravenously and the occurrence of shock determined by observing the fall of blood pressure as recorded by means of a mercury manometer and the usual kymograph. Within 5 min. after the injection, shock being fully developed, blood was again collected in the oxalate solution and the animal killed by chloroform. The specimens thus obtained, one before and the other after shock, were examined as follows: (1) the coagulation time upon the addition of CaCl_2 (2 per cent), (2) the influence of the addition of thromboplastin and of fibrinogen was observed, and (3) the content of fibrinogen was measured volumetrically. The coagulation time of the whole blood by means of the Dorrance coagulometer was also determined. The exact technic of these procedures is given in our previous paper.¹

The results of these studies are given in Table 1 (p. 478).

These figures refer to 7 dogs which showed marked anaphylactic shock. Of 5 other dogs used, 2 gave slight signs of shock, as evidenced by a slight transitory fall in blood pressure; and 3 failed to show any signs of shock.

Of the 7 dogs with marked anaphylactic shock all showed by the Dorrance coagulometer either a delay in, or absence of, coagulability of the blood. The study of the oxalated plasma showed that the addition of calcium and of calcium and fibrinogen was of little benefit in restoring its coagulability. The addition of calcium and thromboplastin solution, however, constantly produced a more rapid coagulation than any other method employed. From this we may assume that the failure of the blood of anaphylactic shock

¹ *Op. cit.*

TABLE I.

DATE 1913	Dog No.	BEFORE INTOXICATING DOSE										AFTER INTOXICATING DOSE																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
		Coagulation Time (in Minutes)										BLOOD TAKEN AFTER INJECTION	Coagulation Time (in Minutes)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
		Blood	+Calcium				+Ca +Tp.				Oxalated Plasma				Blood	+Calcium				+Ca + Tp.				+Ca + Fibrinogen																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
			Begin		Com- plete		Begin		Com- plete		Begin		Com- plete			Begin		Com- plete																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
			2 1/2	4	3 1/2	1	2 1/2	3	1 1/2	4 1/2	8		4	2 1/2		4	3 1/2	1 1/2	2 1/2	3	1 1/2	4 1/2	8		4																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															
April 7	23	4	2 1/2	3 1/2	7	11	6 1/2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	

to clot is due either to an abnormally small amount of thromboplastin or to an excess of antithrombin. In the former case, according to Howell's theory, the added thromboplastin would have compensated for the existing deficiency; in the latter it would have been sufficient to neutralize the excessive antithrombin and thus allow coagulation. It is obvious, of course, that the addition of calcium and thromboplastin solution did not reduce the coagulation time of the oxalated plasma to that which existed before the administration of the intoxicating dose. This was not to be expected, however, as similar results have been obtained by others investigating the subject of blood coagulation.¹

As to the second phase of our study, it was found that the blood of the dogs that did not develop shock after the intoxicating dose differed from normal blood in certain important features, coagulability, however, as shown by the oxalated plasma tests, remaining normal. Following exactly the same technic, the oxalated blood was put

¹ Howell, *Am. Jour. Physiol.*, 1911, 29, p. 187; 1912, 31, p. 1; Bayne-Jones, *ibid.*, 1912, 30, p. 1.

into two similar tubes and centrifuged. On removal, one tube presented the usual appearance of a red blood cell sediment with clear, supernatant plasma; in the other, sharply demarcated from the semifluid sediment of red cells, was a solid, cloudy white jelly clot. In a second dog that failed to develop anaphylactic shock, a similar clot was formed in the oxalated plasma of both tubes, indicating a constant association of the two phenomena, which held true also in the third and fourth experiments of this type. In one experiment, in which slight shock was shown by a slight fall in blood pressure, the 1:10 oxalate blood partly clotted. Various control experiments failed to reveal an error in technic capable of explaining this unusual occurrence. It was found, however, that in proportion of 1 part of oxalate solution to 5 parts of blood, coagulation never occurred; whereas in proportion of 1:20 even the normal plasma at times coagulated, tho it never coagulated at 1:10. We therefore felt that if, as is generally believed, oxalate solutions prevent coagulation of the blood by binding the necessary calcium, the failure of certain dogs to develop anaphylactic shock might be shown by these observations to be due to the presence in their blood of an unusual amount of calcium. This would be in accord with reported experiments¹ which seem to indicate that the administration of calcium may prevent anaphylactic shock.

In order to determine this point, quantitative estimations were made of the calcium in blood presenting this peculiar phase of coagulation. As controls, a normal unsensitized animal was studied and several sensitized animals both before and after characteristic shock. The method used was essentially that described by McCrudden,² modified in unessential details for adjustment to the small quantity of calcium present in the necessarily small samples of blood available. The results showed no change from the normal, either in the blood of an animal with typical shock or in that of an animal in which the same intoxicating dose had caused no shock and whose blood clotted in the presence of the usual amount of potassium oxalate. In short, no evidence was found to

¹ Kastle, Healy, and Buckner, *Jour. Infect. Dis.*, 1913, 12, p. 127.

² *Jour. Biol. Chem.*, 1911-1912, 10, p. 187.

substantiate the hypothesis that there is present in the blood of such animals an excess of calcium.

At the time no other hypothesis could be formulated to explain this phenomenon, but recently MacRae and Schnack¹ have published some observations which may have a distinct bearing upon our results. Briefly, they have found that the addition of calcium-free solutions of a thromboplastic substance, as kephalin, may cause clotting of oxalated peptone plasma, if there is not an excess of oxalate present. The presence of such a thromboplastic substance in the sera which we studied may be a possible explanation of our observation that the plasma of sensitized dogs without anaphylactic shock clotted in 1-10 oxalate solution, but was prevented from clotting by a concentration of 1-5. If this be true it would be of interest to determine whether there is any relationship between the excess of some such thromboplastic substance, as kephalin, and the absence of anaphylactic shock.

SUMMARY.

1. In a series of 12 dogs, 7 developed distinct shock, 2 a slight temporary fall in blood pressure, and 3 showed no evidence of the anaphylactic reaction.

2. Of the 7 dogs developing marked anaphylactic shock, all showed a delay in, or absence of, coagulation of the blood.

3. In all of these the addition of calcium and thromboplastin solution to the oxalated plasma restored its coagulability much more efficiently than calcium alone, or calcium plus fibrinogen solution.

4. No noteworthy change was observed in the fibrinogen content of the blood in this group.

5. Such results point to a decrease of thromboplastin or an excess of antithrombin as the important feature responsible for the loss of power of coagulation in anaphylactic shock.

6. The 5 dogs with slight or no shock showed no changes in the coagulability of the blood, except that in 4 the freshly prepared oxalated plasma (1:10) clotted spontaneously. In no case was

clotting observed in richer proportions of oxalate (1:5). The supposition that this coagulation in the presence of oxalate might be due to an excess of calcium in the blood was shown by the result of quantitative calcium determination to be untenable. The only plausible explanation is that suggested by the work of MacRae and Schnack, that is, that some thromboplastic substance, as kephalin, may be present and in the absence of an excess of oxalate is responsible for the clotting of the plasma.