XLIX. THE ANTICOAGULANT ACTION OF ANTITHROMBIN.

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THE presence of a substance in blood, which inhibited coagulation, was noted by early workers on blood coagulation. Schmidt [1892] stated that this substance was present in all cells. To it the term anti-fibrin-ferment was applied by J. Mellanby [1908] and antithrombin by Howell [1910]: the former, who found that the anticoagulant action was abolished by heating to 70°, observed that complete disappearance of thrombin could not be effected by the addition of an excess of antiferment and therefore concluded that the union of antiferment with ferment (thrombin) followed the law of mass action. According to Howell [1912] antithrombin enters into combination with prothrombin and also with thrombokinase, but does not combine with thrombin [cf. Bordet, 1920].

The anticoagulant action of blood upon a mixture of fibrinogen (plasma) and thrombin is observable in the presence of sodium citrate. All the experiments about to be described were made with citrated solutions. Under these conditions of experiment thrombokinase does not convert prothrombin into thrombin: the possibility of the coagulation time being affected by any concomitant interaction of thrombokinase and prothrombin, occurring in the plasma employed, is thus avoided. The action of the anticoagulant must, therefore, be exerted either upon fibrinogen or upon thrombin and is presumably similar to the effect of simple dilution of one or other of the solutions of these two substances employed.

The action of serum in producing delay of coagulation in presence of sodium citrate, is illustrated by the following two experiments (quantities of liquid being given in cc.)¹:

Table I.

Exp.	Plasma	Serum	0.85 % NaCl	Thrombin	Onset of coagulation
1	0.08	—	0.014	0.026	2.5 minutes
2	0.08	0.014	—	0.026	4.5 „

It will be observed that the coagulation time is nearly doubled by the addition of a relatively small amount of serum.

A similar result is obtained if plasma is employed instead of serum.

¹ As source of thrombin the venom of *Echis carinatus* [cf. Barratt, 1913] was used. I am indebted to Sir Charles Martin, F.R.S., Director of the Lister Institute, for kindly providing me with a supply of this venom.

The anticoagulant was, however, found to be present in plasma in amount too small for experimental work: in particular the effect upon fibril formation, which it was desired to observe, could not be ascertained, the changes noted being slight and indefinite.

An attempt was therefore made to determine if the anticoagulant substance could be extracted from plasma. Ultimately it was found that the complete disappearance of this substance, which occurs on heating plasma or serum to 70°, does not take place in presence of a large excess of distilled water. Making use of this circumstance some measure of success was obtained by the following method of extraction which was employed for Exps. 3 to 6, given below.

5 cc. of human plasma were added to 45 cc. of distilled water and the diluted plasma so obtained was poured into 450 cc. of distilled water at 100°. After being kept at about 90° for 5 minutes, the mixture was cooled to 70°, at which temperature it was allowed to evaporate. In the course of 2½ hours its volume was reduced to 20 cc. This was put into a parchment thimble and allowed to dialyse into a litre of distilled water for 48 hours. The liquid contained in the parchment thimble was then evaporated to dryness. The residue was extracted with distilled water, in which it in part slowly dissolved, the insoluble portion being separated by centrifugalisation and the supernatant liquid again evaporated to dryness. The residue thus obtained was then extracted with distilled water, more insoluble material appearing. The process of extraction was repeated several times until at length no further insoluble precipitate was obtainable. In this way a small amount of soluble residue forming a brownish hyaline scale was obtained. For the separation of insoluble protein removal of salts by dialysis was found to be essential.

A portion of this soluble extract, dissolved in 0.85 % NaCl solution, was mixed with citrated plasma. To the mixture thrombin was added (Exp. 3), a control experiment, in which 0.85 % NaCl solution free from extract was used, being also made (Exp. 4). These experiments were repeated with a slightly larger amount of extract (Exps. 5 and 6). The figures in brackets in Table II represent the amount of plasma corresponding to the extract employed.

It will be seen that in each case the addition of extract caused delay in the time of appearance of fibrils.

Table II.

Exp.	Plasma cc.	Extract cc.	0.85 % NaCl cc.	Thrombin cc.	Time of appearance of fibrils mins.
3	0.05	—	0.06	0.01	20
4	0.05	0.06 (0.25)	—	0.01	56
5	0.05	—	0.06	0.01	21
6	0.05	0.06 (0.4)	—	0.01	120

In order to determine whether this delay is due to an action exerted upon fibrinogen or upon thrombin, the effect upon the fibrils of the coagulum was

noted and compared with that occurring (1) when the plasma employed is diluted, and (2) when the thrombin added is similarly diluted: in the former case, in which the concentration of fibrinogen is diminished, the fibrils become short and fine, scanty and barely visible; in the latter, in which the concentration of thrombin is lowered, they are also reduced in number, but exhibit increase in length and thickness [Barratt, 1921].

In Table III the condition of the fibrils in Exps. 3 to 6 is given.

Table III.

Exp.	Approximate number of fibrils in field	Length	Thickness
3	3000	15 μ to 40 μ	0.1 μ to 0.2 μ
4	1000	20 μ to 50 μ	0.1 μ to 0.2 μ
5	4000	30 μ to 60 μ	0.1 μ to 0.2 μ
6	1200	75 μ to 170 μ	0.2 μ to 0.4 μ

It will be seen that a decrease in number of fibrils occurs when extract is added, accompanied by an increase in length and thickness. The anticoagulant substance thus causes diminution in the amount of free thrombin present and is, therefore, an antithrombin in the sense of causing such decrease.

It may be observed that the only means at present available of deciding whether the action of antithrombin upon a mixture of fibrinogen and thrombin in presence of sodium citrate is exerted upon fibrinogen or upon thrombin appears to be by noting the accompanying change of type of fibril formation. In this way an unequivocal indication of the mode of action of the anticoagulant is obtained.

SUMMARY.

(1) Experiments were made to determine the effect of antithrombin, obtained from human blood-plasma after removal of precipitable proteins, upon the formation of fibrils in the coagulation of fibrinogen.

(2) It was found that the anticoagulant action of antithrombin upon a mixture of fibrinogen (citrated plasma) and thrombin *in vitro* is exerted upon thrombin, which becomes diminished in amount: no evidence of any action upon fibrinogen was observed.

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