XXXII. THE ANTICOAGULANT ACTION OF PEPTONE *IN VITRO*.

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THE effect of commercial peptone in causing delay of coagulation of blood *in vitro* has been investigated by Schmidt-Mulheim [1880], Afanassiew [1884], Pollitzer [1886], Halliburton [1888], Camis and Gley [1896], Arthus [1920], and Pickering and Hewitt [1922]. The opinion has been widely held that peptone has no specific action upon shed blood and can produce delay of coagulation or incoagulability of blood only *in vivo*, but the last two observers showed that peptone acts directly upon the constituents of the blood, without, however, determining to which of the constituents this action is directed.

The problem becomes relatively simple if the coagulation of fibrinogen by thrombin is studied in the presence of sodium citrate: under these conditions of experiment it may be assumed that the action of peptone is directed either to fibrinogen or to thrombin.

This action is found to be rapid compared with the process of coagulation. No indication is afforded by comparing the effect of mixing peptone for some time with thrombin before adding fibrinogen, or with fibrinogen before adding thrombin: the time of appearance of coagulation is found to be the same in both cases.

In the course of a search for possible methods of investigation it appeared likely that an indication of the nature of the anticoagulant action of peptone would be revealed by comparison with the effect of dilution of fibrinogen in presence of varying concentrations of thrombin, this being exhibited by curves (Fig. 1), each of which represents the coagulation periods of a series of dilutions of fibrinogen (citrated human plasma) to which a fixed amount of thrombin¹ has been added. Although this expectation was not fulfilled, as will be seen later, it may, nevertheless, be of interest to describe the line of enquiry followed since it reveals the limitations of a method which seemed at first likely to be fruitful of result.

The experiments made are recorded in Table I. They were carried out at room temperature (16° to 18°). In each experiment five parts of plasma (undiluted or diluted as indicated in column 2) were mixed with one part of

¹ As source of thrombin the venom of *Echis carinatus* [cp. 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.

citrated 0.85 % sodium chloride solution containing thrombin or a mixture of thrombin and peptone (columns 3 and 4).

Table I.

Experiment	Concen- tration of plasma employed %	Amount of thrombin (g.) in 1 cc.	Amount of peptone (g.) in 1 cc.	Onset of coagulation min.	Fibrils visible min.
A. 1.	100	167×10^{-6}	—	0.8	1
2.	30	"	—	1.2	none
3.	10	"	—	remained liquid	"
4.	5	"	—	"	"
5.	2.5	"	—	"	"
B. 6.	100	10.2×10^{-6}	—	2.7	3.2
7.	80	"	—	3.7	5.5
8.	60	"	—	5.5	7
9.	40	"	—	6	none
10.	30	"	—	9	"
11.	20	"	—	9	"
12.	10	"	—	9	"
13.	5	"	—	remained liquid	"
14.	2.5	"	—	"	"
C. 15.	90	1.50×10^{-6}	—	13	13
16.	80	"	—	18	18
17.	70	"	—	18	18
18.	50	"	—	25	25
19.	20	"	—	48	51
D. 20.	100	0.635×10^{-6}	—	19	19
21.	30	"	—	120	300
E. 22.	100	10.2×10^{-6}	0.02	22	34
23.	80	"	0.02	52	60
24.	60	"	0.02	55	90
25.	40	"	0.02	140	300
26.	30	"	0.02	remained liquid	none

Fig. 1. The ordinates represent percentage of citrated plasma employed; the abscissae periods of coagulation in minutes. The thin portions of the curves correspond to "setting," the thick portions to the appearance of fibrin fibrils. In the two curves to the left (A, B) it will be observed that "setting" is still recognisable when fibrils have ceased to be visible.

The first twenty-one experiments, arranged in four series, afford data for the four curves shown in Fig. 1. For the first two series, Exps. 1-5 and 6-14, a relatively high concentration of thrombin was employed. In these series coagulation (indicated by fine lines on the figures) ceased to be recognisable when the dilution of plasma (Fig. 1) used was below 30 % and 10 % respectively: before this occurred the appearance of fibrin fibrils (indicated by

thickened lines), which were observed to be numerous, short and fine, had already ceased. In the third series, Exps. 15-19, a curve (C) of increased obliquity is obtained. The last two experiments, 20-21, lie on the curve (D) approaching still more nearly to the horizontal shown on the extreme right of Fig. 1: here, relatively little thrombin was added and the fibrils formed were larger, thicker and less numerous than in the preceding series, "setting" being feeble.

To the above twenty-one experiments giving four normal dilution curves a further series, Exps. 22-26, was now added. In these the conditions of experiment were similar (Fig. 2) to those obtaining in Exps. 6-14, except that 2 % of peptone was present: coagulation was retarded in each of the first four experiments and was absent in the last.

Fig. 2. Ordinates and abscissae as in Fig. 1.

If the action of peptone is upon fibrinogen and is comparable in its effect to dilution of fibrinogen solution (plasma) the coagulation periods in Exps. 22-26 would lie upon the corresponding curve (B) in Fig. 1, it being assumed that this curve could be produced so as to pass to the right close to the axis of abscissae. But actual experiment shows that in dilution experiments the curve cannot be produced so far to the right, both coagulation and the formation of visible fibrin fibrils ceasing while it is still near the axis of ordinates, as exhibited in Fig. 1. This difference would appear to indicate that the action of peptone is not directed to fibrinogen.

If, on the other hand, the action of peptone is exerted upon thrombin causing diminution of the amount of free thrombin present, then the coagulation periods could be plotted against the corresponding dilutions of fibrinogen as in Fig. 1. When this is done the curve shown in Fig. 2 is obtained, which assumes fairly closely the type exhibited in Fig. 1, lying a little to the right of the curve (D) in this figure. The close correspondence of the two curves suggests that the action of peptone is directed to thrombin.

On the basis of these results the conclusion appeared to be indicated that peptone had no effect upon fibrinogen, but entered into some kind of reaction with thrombin. Later investigations, however, necessitated a reconsideration

of this interpretation. In particular it became evident that the tacit assumption made above that the effect of peptone, if directed to fibrinogen and thereby causing a diminution in the amount of fibrinogen present, would be in all respects, that is to say quantitatively as well as qualitatively, identical with that of dilution of fibrinogen, is not necessarily valid when fibrinogen is regarded as a colloid. If the disappearance of fibrinogen could be effected without dilution longer coagulation periods might conceivably be obtained than is possible in dilution experiments.

In this dilemma attention was turned to another means of testing whether the action of an anticoagulant is exerted upon fibrinogen or thrombin, namely by determining the effect on fibril formation. Several attempts had already been made unsuccessfully to ascertain the nature of the action of peptone in this way. Additional experiments, now undertaken, were attended with success and enabled the conditions to be defined under which the action of peptone on fibril formation could be exhibited.

Before proceeding further the factors which normally determine the characters of fibril formation when coagulation occurs must be considered. When thrombin is added to fibrinogen (plasma) in diminishing amounts the fibrils appearing become reduced in number, but increased in length and thickness: on the other hand, when the amount of fibrinogen is diminished (by dilution), the quantity of thrombin added being unaltered, the fibrils formed do not increase in length and thickness, but may be observed to become definitely shorter and finer, this being attended, as in the former case, by a reduction in number and final disappearance [Barratt, 1921]. Both types of change are exhibited in Exps. 1-21: the former, for example, is seen when Exp. 6, in which the fibrils formed were numerous, 5μ to 15μ long and 0.2μ to 0.3μ thick, is compared with Exp. 20, in which the fibrils were less numerous, 40μ to 100μ long and 0.3μ to 0.5μ thick; the latter is observed in each of the four series.

When peptone was employed in Exps. 22-26 the effect upon the characters of the fibrils appearing was equivocal: some increase in length and thickness was observed in most experiments, but the degree of increase was less than that noted in corresponding experiments with nearly the same coagulation period in the normal series 1-21 and was not sufficiently marked to be completely outside the variations normally met with. It was in consequence of the failure to obtain a decisive indication of the influence of peptone upon fibril formation that extended investigation with a series of dilutions of fibrinogen of the type exhibited by Figs. 1 and 2 above was entered upon.

The principal cause of the difficulty experienced in determining the effect of peptone upon fibril formation was found to be the feeble character of the anticoagulant action of the samples employed. If a sample possessing a sufficiently powerful anticoagulant action can be obtained, the effect of peptone on fibril formation is readily shown. The conditions of experiment require to be so arranged that, on the one hand, (1) coagulation occurs very rapidly,

i.e. within one minute or less, in the control experiments in which no peptone is added; while, on the other hand, (2) the delay of coagulation caused by peptone is extremely marked, extending to one or several hours without, however, causing complete disappearance of fibrils. Dilution of plasma should be, as far as possible, avoided.

The effect of peptone upon fibril formation, when these conditions are fulfilled, is shown in Table II, Exps. 27–28: the quantities (present in 1 cc.) of plasma, thrombin and peptone employed are given in columns 2, 3 and 4 respectively. In this table it will be seen that a coagulation period of 50 sec., when thrombin acted upon fibrinogen in the absence of peptone (Exp. 27), was changed by the addition of 4.5 % of peptone into one of 60 min. (Exp. 28). In the former experiment the fibrils seen were very numerous, short and fine: in the latter the length of the fibrils was not markedly changed, but their number was diminished and they were barely visible. With further addition of peptone the fibrils became amicroscopic and when the amount was increased to 7 % “setting” was no longer observed.

Table II.

Experiment	Plasma (cc. per cc.)	Thrombin (g. per cc.)	Peptone (g. per cc.)	Onset of coagulation	Fibrils		
					Number	Length	Thickness
27.	0.80	10.2×10^{-6}	—	50 sec.	very numerous	3μ to 15μ	0.1μ to 0.2μ
28.	0.80	„	0.045	60 min.	moderately numerous, barely visible	4μ to 7μ	0.1μ
29.	0.80	0.037×10^{-6}	—	77 min.	scanty	50μ to 200μ	0.5μ to 0.9μ

The effect of addition of peptone, exhibited in Exps. 27–28, is thus identical, so far as concerns the appearance of fibrils, with that observed when fibrinogen (citrated human plasma) is diluted with citrated 0.85 % sodium chloride solution, as in Exps. 6–14, Table I: in the latter series the fibrils, though diminishing in number and becoming increasingly difficult to observe, remained throughout of nearly the same length, namely 5μ to 15μ . These results indicate unequivocally that peptone acts upon fibrinogen, which, in consequence, becomes diminished in amount just as occurs upon dilution.

For the sake of comparison with the effect of diminution of the amount of thrombin added, Exp. 29 is included in Table II: here the period of coagulation approximates to that of Exp. 28, but the fibrils, much reduced in number, are very markedly increased in length and thickness.

Table II serves to illustrate the action of two classes of anticoagulants, delay of coagulation being attended in the one by increased length and thickness of the fibrils, in the other by an extreme degree of fineness of these structures, which become barely visible, and are usually reduced in length also: in both a marked diminution in number is observed when a relatively considerable

amount of the anticoagulant has been added. Of these two classes the first is exemplified by hirudin, the second, to which peptone belongs, by sodium hydroxide [Barratt, 1921, 1926]: the former acts upon thrombin, the latter on fibrinogen.

Reference may now be made again to the circumstance that when peptone is added to plasma, in presence of a relatively large amount of thrombin, much greater delay of coagulation can be produced than is the case when plasma is simply diluted. The same is observed when sodium hydroxide is used in place of peptone: in this respect the two anticoagulants are in complete agreement. A further point of similarity is in the formation of afibrillary clot: this is readily obtainable with sodium hydroxide [cp. Howell, 1916], less so with peptone, the use of which renders some degree of dilution unavoidable.

SUMMARY.

Experiments made to determine the effect of peptone upon fibril formation in citrated fibrinogen solution (blood-plasma) *in vitro* show that peptone (1) acts upon fibrinogen, which becomes in consequence diminished in amount, and (2) is without action upon thrombin.

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