LVIII. STUDIES OF THE COAGULATION OF THE BLOOD.

PART II. THROMBIN AND ANTITHROMBINS.

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(Received June 26th, 1922.)

IN a recent paper in this *Journal* the present writers questioned the current view that antithrombin is secreted by the liver. It was suggested that the action of that organ in promoting the fluidity of the blood after the injection of "peptone" depends on variation of carbon dioxide content of the blood [Pickering and Hewitt, 1921]. Experiments were devised to test this suggestion with the somewhat unexpected result that in pithed cats respiring air, typical inhibition of the coagulation of the blood followed the rapid injection of "peptone" into animals with the liver out of the circulation. Further, the anticoagulant action of "peptone" was annulled by partial asphyxia and was restored by administration of air. It was found also, when suitable precautions were taken to preserve the surface conditions of the blood, that the addition of "peptone" to blood *in vitro* inhibited clotting when the concentration of "peptone" was no greater than was required to produce a like effect *in vivo*.

Immunity to the anticoagulant action of "peptone" was also found to follow the slow injection of that substance into cats with the liver out of circulation [Pickering and Hewitt, 1922].

Having thus demonstrated that the typical action of "peptone" on the blood of the cat can be obtained without invoking the aid of the liver, it became of interest to examine the action of thrombin on the circulating blood of animals under similar conditions, and also to re-examine the evidence for the extraction of antithrombin from the liver, blood and other tissues. Observations on these latter points are presented in this communication.

THE INTRAVASCULAR INJECTION OF THROMBIN INTO ANIMALS DEPRIVED OF HEPATIC CIRCULATION.

The thrombin was dissolved in mammalian Ringer's solution and was extremely active as a coagulant *in vitro*; thus the addition of 0.25 cc. of a solution, prepared by dissolving 0.0048 g. of solid thrombin in 100 cc. Ringer,

to 0.75 cc. of cat's blood caused coagulation to commence¹ in 45'' on glass, completion¹ of this process being one minute later. On paraffined surfaces these figures were 45'' and 2' 30'' respectively.

Experiment 1. Black cat, 2060 g. Pithed. Artificial respiration was maintained for 20' before the aorta and inferior vena cava were ligatured. The following table shows the coagulation times of blood shed on to glass before and after the injection of thrombin into the circulation.

Table I.

Time	Notes	Commencement of coagulation	Completion of coagulation
0' 0''	Intact animal	7' 40''	12' 0''
1' 0''	Animal pithed, vessels ligatured	—	—
36' 30''	—	7' 50''	11' 48''
36' 45''	7.5 cc. of 0.0048 % thrombin in Ringer injected	—	—
39' 0''	—	1' 0''	7' 10''
44' 0''	—	1' 10''	7' 50''
71' 0''	—	2' 50''	9' 30''
86' 0''	—	1' 45''	8' 50''

Notes: (1) Between 44' and 86' several observations were made. The coagulation times given at 71' show the greatest variations.

(2) *Post-mortem* examination revealed no intravascular clots.

(3) Other experiments of the same nature yielded similar results.

Experiment 2. In this observation 0.01 g. of thrombin was injected into a 2½ kilo. cat under precisely similar conditions to those of Exp. 1. Similar results were obtained. The subsequent injection of 7.5 cc. of 10 % calcium chloride in distilled water caused no material alteration in the coagulation times of shed blood. A further injection of 5 cc. however killed the animal. *Post-mortem* examination did not show any clots in those portions of the vascular system where blood had been circulating.

These experiments show: (1) That relatively large doses of thrombin can be injected into cats respiring air and with the liver out of circulation without causing intravascular coagulation. (2) That subsequent injection of a lethal amount of calcium chloride in distilled water fails to induce thrombosis. (3) That blood shed after the injection of thrombin into animals deprived of hepatic activity coagulates much more rapidly than the normal blood of the same animal under otherwise similar conditions.

It may be remarked that the present authors have found, in the course of a large number of observations on pithed cats, that if the air supply of the animals is regulated and kept constant, then constant times of commencement and of completion of clotting of shed blood can be maintained for considerable periods.

The great rapidity of clotting of shed blood after the injection of thrombin may in part be explained by the fact that in the experiments recorded above no excretion of injected thrombin by the kidneys was possible; thus, directly the blood was shed, the surface conditions of the plasma were altered and the thrombin was able to exert its coagulant action.

¹ "Commencement" of clotting indicates the first visible departure from normal fluidity: "completion" that coagulation had advanced so far that the vessel could be inverted without spilling.

The somewhat delayed coagulation reported by Davis [1912] following the injection of thrombin into intact animals under ether anaesthesia and preceding the typical hypercoagulability has not been observed in this series of experiments. Unfortunately Davis records only the times of completion of clotting of shed blood after injection of thrombin and does not give the coagulation times before, recording instead the "bleeding times," estimated by the rate of decrease of haemorrhage from a small skin wound, such as in the lobe of the ear. Davis employed the method of Duke [1910]. It should be noted that Weil [1920] has shown that prolonged bleeding time may coexist with normal coagulation time. Moreover Mendenham [1915] found that coagulation is hastened by ether anaesthesia. Unless precautions were taken to maintain a constant depth of anaesthesia and a constant carbon dioxide and oxygen content in the blood slight variations of coagulability, like those observed by Davis, might well occur. These would be masked later by the coagulant action of the thrombin.

THE BEARING OF THE FOREGOING EXPERIMENTS ON THE
"THROMBIN THEORIES" OF COAGULATION.

The experiments reported in this paper are directly opposed to a commonly accepted view, advocated by Howell [1918], that the injection of thrombin stimulates the liver to secrete an excess of antithrombin, which latter neutralises the injected thrombin and so maintains the fluidity of the blood. They are also dissonant with the suggestions of Howell [1912] "that prothrombin or thrombin itself constitutes a hormone which excites the secretion of antithrombin," and are concordant with the fact that the typical action of "peptone" on blood can be obtained in cats deprived of hepatic activity. Support is thus given to the conclusion [Pickering and Hewitt, 1922] that the retarded coagulability of shed blood after the rapid injection of "peptone" into cats is due neither to the secretion of antithrombin nor of excess of alkali by the liver. This latter view, advocated originally by Dastre and Flouresco [1897], and later by Mellanby [1909] is also dissonant with the recent work of Gratia [1921] who, using Marriott's colorimetric method [Marriott, 1916], was unable to find any evidence of the slightest difference in the alkalinity of normal and "peptone" plasmas.

In the hypotheses of Morawitz [1904, 1] and of Fuld and Spiro [1904], the formation of thrombin from thrombogen (prothrombin) is said to be the prelude of the actual coagulation of the blood. Thrombin, it is postulated, acts directly on the fibrinogen of the plasma and is the immediate cause of clotting.

So long as it was reasonable to assume that the liver secreted sufficient antithrombin to neutralise the effect of massive doses of thrombin these theories remained tenable. As large amounts of thrombin can be injected without thrombosis into the circulation when such hepatic secretion is impossible, the view that thrombin acts directly on the fibrinogen of *unaltered*

plasmas must be discarded and the views of Morawitz and of Fuld and Spiro become untenable. The theory of Howell [1912] explains the fluidity of normal blood by the presence of antithrombin which prevents the calcium of the blood from activating prothrombin to thrombin. The coagulation of shed blood is attributed to the neutralisation of antithrombin by thromboplastin, derived from platelets and tissue cells. Thrombin, so soon as formed, is again regarded as the immediate cause of clotting, a conclusion which is opposed to the evidence brought forward in this communication.

The most recent development of the thrombin theories, that propounded by Bordet [1920], accepts the belief in the existence of antithrombin and other anticoagulants in normal blood, but dissents from the view of Howell as to the rôle played by cytozyme (thromboplastin) in coagulation, citing the work of Gratia [1920] on the neutralisation of hirudin by cytozyme in support of his contention. There is also another important difference between the views of Howell and of Bordet. The former author accords primacy to antithrombin in the maintenance of fluidity, the latter, together with his co-workers [Bordet and Gengou, 1903; Bordet and Delange, 1913], assigns equal importance to surface conditions. Bordet maintains that contact of the blood with foreign bodies causes the interaction of lipoidal cytozyme (thromboplastin), formed largely from platelets but also from leucocytes and other sources, with serozyme, a product of the plasma formed immediately prior to coagulation. Serozyme and cytozyme are then said to combine, in the presence of calcium, to yield thrombin which in turn acts on fibrinogen to give clots of fibrin. Thus apart from the preliminary processes involving the production of thrombin, the first act of coagulation is believed to be the action of thrombin on fibrinogen. The evidence in this paper shows that if the physical conditions of the blood are preserved by remaining in the living vessels, then thrombin, in amounts sufficient to cause coagulation *in vitro*, does not cause thrombosis *in vivo*. It thus appears that although Bordet is correct in assigning importance to the surface conditions of the blood in the maintenance of fluidity, it is not to the disintegration of formed elements yielding thrombin, under the influence of physical change, that the *inception* of coagulation is due, but rather to physical change destroying the stability of the clotting complexes of the plasma. It is only after such change that thrombin can act as a coagulant. In this view, thrombin would be regarded as an accelerator of coagulation rather than as an initiator of that process. The work of Vines [1921] indicates that the complex associated with the inauguration of clotting exhibits distinctive differences from the prothrombin of Howell.

This view of the action of thrombin is supported by the observation of the present writers [Pickering and Hewitt, 1921] that in blood surrounded by oil, coagulation commences as a reversible gel. Further the work of Howell [1916] indicates that the change from a gel to a typical clot is a physical process due to ageing and condensation.

The addition of thrombin to blood in the gel stage immediately transforms the gel into typical fibrin, the speed of coagulation being identical with the speed of clotting when thrombin, in the same concentration, is added to blood shed on to clean glass by means of a paraffined cannula. This latter observation is concordant with the fact discovered by Gratia [1918], that the coagulation of blood induced by thrombin proceeds at the same rate on paraffined as on glass surfaces.

Bürker [1904] found platelets to remain intact in blood on a paraffined surface, yet under these conditions the first stage in coagulation—the formation of a reversible gel—sets in. This falls into line with the observations of Achard and Aynaud [1908] that if coagulation takes place in a moist vaselined chamber at 16–18°, then the platelets remain independent of the fibrin filaments. These authors maintain that physical changes of platelets are the result, rather than the cause of coagulation. Morawitz [1904, 2] found that thrombokinase (cytozyme) could be obtained from platelets. Cramer and Pringle [1913] showed that thrombokinase is not present in oxalate plasma and concluded that recalcification “induces coagulation primarily by causing the breaking up of platelets and only secondarily by influencing the chemical reaction which takes place in the presence of the substance (thrombokinase) liberated by the platelets,” also the “filtered oxalate plasma... is readily coagulated by both filtered (through a Berkefeld filter) and unfiltered platelet extract in the presence of soluble calcium salts.” On the other hand Gratia [1918] concluded that contact with foreign substances capable of being wetted by blood, does not act solely on platelets and other formed elements but also on the colloids of the plasma. The same writer also states that calcium chloride, if dissolved in distilled water, disintegrates platelets, liberating, presumably, cytozyme (thromboplastin or thrombokinase). Attention is drawn to experiment 2 of this paper in which massive doses of calcium chloride in distilled water were injected into the circulation of an animal deprived of hepatic activity and subsequent to a massive dose of thrombin. In this experiment, if Gratia is correct, platelets were disintegrated; it appears therefore that thrombin plus cytozyme from platelets is unable to cause clotting when the surface conditions of the blood are maintained by contact with the living walls of the blood vessels. It also indicates that platelets in concentrations existing in circulating blood, play only a secondary part in provoking coagulation.

It has been shown that the rapid addition of a tissue extract (a source of cytozyme) to bird's blood *in vitro* has a coagulant action, while the slow addition of similar amounts has an anticoagulant action [Pickering and Hewitt, 1921]. Demonstration has also been given that the intravascular injection of either calcium chloride [Löwit, 1892] or of inactive nucleoprotein [Halliburton and Brodie, 1894], each of which causes leucolysis and therefore liberates cytozyme into the circulation, is not followed by thrombosis.

These facts indicate that the relatively slow liberation of cytozyme by the

disintegration of formed elements in the blood is insufficient to induce thrombosis.

Bloch [1920] suggested that in circulating blood the calcium ions exist in an "inactive and latent state" and that on issuing from the vessels, contact with the *air*, dust and so-called thromboplastic material may transform "inactive" calcium to an active electrolytic precipitant of the colloids of the plasma. The work of Chio [1917] has indicated that the tension of carbon dioxide in the blood may affect the equilibrium of the calcium salts which in turn regulates the physical state of the blood, while Vines [1921] has shown that a change from combined calcium to ionised calcium takes place during clotting.

In Cramer and Pringle's experiments [1913] blood was shed through the air directly into oxalate¹. If Bloch's view is correct change towards clotting had already taken place. Caution should, it is thought, be exercised in deducing any theory of coagulation from the behaviour of blood after exposure to the air, oxalation and replacement of ionised calcium.

In the experiments of Cramer and Pringle referred to, platelets, leucocytes and some erythrocytes were removed from oxalate plasma, and from these was prepared a "platelet extract" whose coagulant action was tested *in vitro*.

The work of Spring [1900], Höber and Gordon [1904], Paine [1911] and Galecki [1912] shows that the speed of addition of a coagulant electrolyte is an important factor in coagulation of inorganic sols, while the present writers have demonstrated that similar conditions determine the coagulation of bird's blood by tissue extract *in vitro* [Pickering and Hewitt, 1921]. If experiments are to show that lysis of platelets is the initial factor in *normal* coagulation, it would be necessary to prove: (a) that the "platelet extract," or the aggregates of platelets, was free from products extracted from leucocytes and erythrocytes; (b) that the addition of the "platelet extract" was made at the same rate as the liberation of the coagulant material occurring normally in shed blood; (c) that the concentration of either "platelet extract" or of aggregates of platelets added, was not greater than the concentration of extract resulting from the disintegration of platelets in normal shed blood; (d) that the lysis of platelets added to oxalate plasma in the presence of ionised calcium took place at the same rate as the disintegration of platelets in normal shed blood.

¹ In a private communication to one of the present authors (J. A. H.) Dr Cramer expressed the opinion that our remarks on p. 719 of Vol. xv of this *Journal* regarding the technique of Cramer and Pringle were liable to misinterpretation; he suggested that we might refer to it in our next communication on the subject in question. We stated "*that blood was not withdrawn through a paraffined or similarly treated cannula.*" The method actually was [Cramer and Pringle, 1913] "*to cut the artery so that the blood flowed directly into the receiving vessel.*" It will be seen that the statement of fact is strictly correct.

THE SIGNIFICANCE OF THE METHODS OF PREPARING ANTITHROMBINS
AND ALLIED SUBSTANCES.

Doyon and his colleagues Morel, Policard, Dubrulle and Sarvonat [1910-1919] describe the following ways of preparing antithrombin. The liver of the dog is frozen and thawed three times over a period of 48 hours; during the intervals between freezing and thawing it is exposed to the air at room temperature. The liver is then perfused with 0.9 % sodium chloride and dilute alkali carbonate. The perfusate has no anticoagulant action until heated to 100° when it yields antithrombin. Alternative methods involve such processes as autoclaving liver at 120° or exposing minced liver to chloroform vapour or allowing intestine to undergo autolysis.

By the use of one or other of these methods Doyon has prepared antithrombins from the majority of the organs of the body, from the muscles of the cray-fish and from macerated earthworms. Recently however Doyon [1921, 1, 2] finds that antithrombin can only be obtained from the liver of graminivorous birds by freezing and thawing, a process which involves breaking up the colloidal complexes of the cells. In this connection it is noteworthy that Howell ascribes the fluidity of shed bird's blood on glass to excess of antithrombin secreted by the liver. Doyon also found that even freezing and thawing, in the case of the rabbit's liver, failed to produce an anticoagulant; yet Davis [1912] has shown that thrombin can be injected into the circulation of the rabbit, in large doses, without causing thrombosis.

The method adopted by Howell [1914] to demonstrate the presence of antithrombin in the blood was by heating oxalate plasma or peptone plasma to 60°. The filtrate delays the action of thrombin on fibrinogen and is consequently said to contain antithrombin. This antithrombin, Howell states, is destroyed by heating at 80°-85°. Collingwood and Macmahon [1914] found that serum, particularly when kept for two days, exhibits an inhibitory influence on thrombin; this antithrombin is destroyed by heating to 60 or 65°. The retarded coagulability of "peptone" plasma is commonly ascribed [Howell, 1918] to excess of antithrombin secreted by the liver. This antithrombin is completely destroyed only at 100° [Nolf, 1908].

Even allowing a wide margin for differences in stability due to the presence of various protective colloids in the respective solutions and also to individual experimental error, it is difficult to believe that liver antithrombin stable in solution at 100°, or even after autoclaving at 120°, is the same substance as the antithrombin prepared from plasma which is destroyed at 80-85°, or that which appears in disintegrating serum and is destroyed at 60°, or the anticoagulant of "peptone" plasma which is active up to 100°. Yet if the current view is accepted that antithrombin obtained from the liver is actually secreted into the circulation, these anomalies must be ignored.

The most recent method for the preparation of antithrombin is the extraction of rapidly dried and hashed lungs by benzene; the extract is dissolved in

0.9 % sodium chloride and purified by precipitation at its iso-electric point. The anticoagulant substance produced is stated by Mills, Raap and Jackson [1921] to be antithrombin.

The earlier methods of manufacturing antithrombin demand drastic disintegration of the colloidal complexes from which the anticoagulant is prepared. The term "manufacturing" is used as such methods cannot imply unaltered extraction. Moreover, Mills, whose technique is less drastic, admits his method involves removal of a phospholipin from a protein-phospholipin complex, so that again there is only a product of cellular break-down. Likewise in serum, in self-digested viscera and in the exudation of decaying liver exposed to chloroform, the anticoagulant may well be considered as a product of autolysis.

When more physiological methods were employed negative results were obtained. Menten [1920] found that perfusion of the liver with normal saline failed to yield any anticoagulant substance. Minot [1916] applied Doyon's chloroform method to solutions of Howell's antithrombin (prepared from oxalate plasma by heating to 60°), and also to plasma and serum both unheated and heated to 60°. In each case no anticoagulant was found. Loeb [1904] was unable to obtain any antithrombin-like material by extraction of linings of blood vessels, a result which throws doubt on the view, recently revived, that antithrombin is secreted by the endothelium of the vascular wall. Rettger [1909] found no evidence of the production of antithrombin after the serial injection of minimal doses of thrombin into the dog and into the rabbit.

Attempts to cause the liver to secrete antithrombin by means of bile, bile salts, secretin and by electrical stimuli gave negative results [Denny and Minot, 1915]. It is noteworthy that Bulger [1918] found an increased coagulation time in cats, rabbits and guinea-pigs suffering from anaphylactic shock, associated with a decrease of antithrombin estimated by Howell's method. Thus the altered coagulability of anaphylactic shock, which closely resembles that of "peptone" shock, also affords no evidence of the secretion of antithrombin.

In short, no direct evidence has been obtained of the existence of antithrombin in living animals.

Two other organic anticoagulants merit notice. Schikele [1912] stated that the extract obtained from the uterus by pressure retarded coagulation. Bell [1914] repeated these experiments but failed to find any anticoagulant. Howell and Holt [1918] extracted dried powdered liver with ether. The solution thus obtained was precipitated by acetone, redissolved in ether and reprecipitated by absolute alcohol at 50°. This last process was repeated from 12 to 20 times and yielded a substance (or mixture) termed by them *heparin*, which is a powerful anticoagulant *in vivo* and *in vitro*; it differs from antithrombin in that it does not neutralise the action of thrombin. Here again the product obtained appears to be the result of more or less drastic

disintegration of cells. Howell and Holt admit that there is no direct evidence of the existence of heparin in the blood. Nevertheless they believe it is an important factor in the maintenance of the fluidity of the blood, and suggest that it inhibits clotting by preventing the activation of prothrombin to thrombin and also by activating pro-antithrombin with the production of antithrombin.

THE PREPARATION OF ANTITHROMBIN FROM VEGETABLE CELLS.

In view of the evidence indicating that antithrombins are artificial products formed by the dissolution of complex substances of animal origin, it became of interest to enquire if the employment of Doyon's technique would produce an anticoagulant from vegetable sources.

The raw materials selected were baker's yeast and a very pure crystalline specimen of edestin. It may at once be stated that from both these vegetable products active anticoagulants were prepared.

In each case the materials were suspended in water and heated to 120° under pressure for 4 hours on three separate occasions; when not being heated they were freely exposed to the air. The product was centrifuged and to the clear supernatant liquid sodium carbonate was added until the solution was neutral to litmus. In the case of the yeast the neutralised solution was dried at room temperature at low pressure and was dissolved in water when required.

The action of the products obtained from yeast will be evident from the following experiment.

Blood was shed from a pithed cat respiring air and with the liver out of circulation, through a paraffined cannula into similarly treated vessels. Thrombin was dissolved in 0.9 % sodium chloride. The yeast extract was dissolved in distilled water. 5 cc. of blood were employed in each experiment.

	Blood on paraffined surface	Blood plus 2 cc. distil- led water	Blood plus 0.75 cc. 0.5 % thrombin	Blood plus 2 cc. yeast extract	Blood plus 2 cc. yeast extract plus 0.5 cc. thrombin	Blood plus 2 cc. yeast extract plus 0.75 cc. thrombin
Commence- ment of clotting	26' 10"	4' 45"	0' 45"	{ Unclotted 2 hours later }	24' 10"	18' 40"
Completion of clotting	29' 50"	8' 45"	2' 45"	{ Small clots next morning }	43' 36"	41' 10"

Note: Similar results were obtained when the yeast extract was dissolved in 0.9 % sodium chloride.

In other experiments of this nature in which non-paraffined vessels have been used, clotting has been observed to occur in fractions. In this, the first clot formed contracted rapidly, while a portion of the fluid remained uncoagulated. Later a second small clot appeared and contracted normally and so on. In clotting induced however by addition of thrombin the clot invariably assumed the typical gel form and later contracted in the normal manner.

Similar results were obtained with rat's blood and with human blood. In the latter case the shed blood had been in contact with cut tissues. With

dog's blood shed through a paraffined cannula into glass vessels, from animals in which the liver was not acting, the anticoagulant action, though evident, was not so pronounced. Employing the same materials as in the experiment just given, delays of 6 and 10½ minutes were obtained in the times of commencement and completion of coagulation.

The action of the yeast extract on the coagulation of recalcified oxalate plasma was investigated.

Fresh oxalate plasma was centrifuged for 30' at 4000 r.p.m. On recalcification with calcium chloride the times of clotting were: commencement 8', completion 9' 15".

To the same volume of plasma was added 0.192 g. of yeast extract; after solution and recalcification the times were 28' and 31' respectively.

Employing the usual animal technique previously described, 0.1 g. of the desiccated material, dissolved in 0.9 % sodium chloride, was injected into the heart. Blood withdrawn 10 minutes after injection yielded a minute clot in 8 minutes, but the great bulk of the blood was fluid after 22 hours. After 26 hours however large gelatinous clots were present. The normal coagulation times of the blood of the animal in question were 8' 15" and 9' 50" for commencement and completion. Other experiments gave similar results.

The crude material employed in the above experiments was extracted with boiling ether and the ether removed at room temperature. The resultant product was also found to possess well marked anticoagulant properties when tested on recalcified oxalate plasma and on unsalted blood; it did not however neutralise the coagulant action of thrombin added to shed blood. It thus resembles in its action the substance extracted by Howell and Holt from the liver and termed heparin.

Treatment of edestin as mentioned above gave a product which behaved like that obtained from yeast except that the coagulant action of added thrombin was not neutralised.

The preceding experiments show that extract of yeast exhibits the typical characteristics of the antithrombin prepared by Doyon. It not merely acts as an anticoagulant *in vivo* and *in vitro* but antagonises the action of thrombin; this and the preparation of a material resembling in its action heparin, when considered in relation to the methods of obtaining antithrombins from the colloids of cells and of serum, indicate that these substances are not secreted by organisms possessing blood streams but are *post-mortem* products of the break-down of colloidal complexes.

Doyon [1921, 1, 2] has recently shown that the nucleic acids prepared by Neumann's method from thymus and from ganglia are anticoagulants *in vivo* and *in vitro*; he assigns the former action to the secretion of a nucleo-protein by the liver. Experiments to be reported in a subsequent paper have demonstrated that in an animal deprived of hepatic activity the anticoagulant action is equally well marked.

SUMMARY.

1. The massive intravascular injection of thrombin into cats deprived of hepatic activity does not produce thrombosis but accelerates the coagulation of shed blood. A phase of retarded coagulability of shed blood preceding hypercoagulability was not observed when the above conditions were adhered to.

2. The view that the continuance of the fluidity of circulating blood after the injection of thrombin is due to the secretion by the liver of an excess of antithrombin is dissonant with the results of the foregoing experiments.

3. In the theories of Morawitz, of Fuld and Spiro and of Bordet, apart from the preliminary processes involving the formation of thrombin, the first act of coagulation is held to be the action of thrombin on fibrinogen. The evidence in this paper indicates that the inception of coagulation is due to physical change destroying the stability of the colloidal complexes of the plasma. Thrombin appears to be an accelerator of clotting rather than an initiator of that process.

4. The addition of thrombin to blood in the state of a reversible gel causes immediate coagulation.

5. Accepting the statement of Gratia that platelets are destroyed by calcium chloride, it is shown that detritus of platelets, in concentrations not greater than can be formed by lysis in circulating blood, plays only a secondary part in provoking coagulation.

6. Attention is drawn to certain inherent difficulties in interpreting experimental results obtained by the use of "platelet extracts."

7. An analysis of the methods of obtaining antithrombins from liver, sundry tissues and from serum, indicates that the anticoagulant substances so formed are *post-mortem* products.

8. The employment of Doyon's technique for preparing antithrombin from liver, yielded from yeast an extract which exhibited the properties of antithrombin. Further extraction with ether yielded a material similar in its action to heparin. The hydrolysis of edestin also gave an anticoagulant.

9. It is concluded that antithrombins are not phylogenetic products of the animal kingdom arising late in evolution as a protection against thrombosis, but are products resulting from the hydrolysis of protein.

The authors would express their thanks to Prof. M. Doyon and to Dr P. A. Levene for placing at their disposal preparations of nucleic acids, and to Prof. B. J. Collingwood for an exceedingly active sample of thrombin.

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