CIV. THE INCEPTION OF BLOOD CLOTTING.

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I. Introduction.

Two explanations of the inception of the spontaneous clotting of pure blood are current. One group of investigators suggests that the initial change is the breakage of blood corpuscles and therefore attributes the inauguration of coagulation to a process akin to cytolysis. Of these inquirers, Morawitz [1904] regards the disintegration of both leucocytes and blood platelets as primary occurrences, but Cramer and Pringle [1913] limit the initial change to the lysis of platelets, whilst Fuchs [1930] maintains that, although the products liberated from leucocytes may contribute to the coagulation of slowly clotting blood, the dissolution of platelets is the primary occurrence when coagulation is rapid. The second group of investigators believes that the inception of blood clotting is primarily a humoral reaction in which changes in the blood-plasma precede the breakage of corpuscles and the release of participants in coagulation from these bodies [Bordet, 1920; Pickering and Hewitt, 1921; Mason, 1921; Pickering and de Souza, 1923; Mills and Mathews, 1924; Mills, 1928; Pickering, 1928]. A plasmatic reaction in the inauguration of blood clotting is also implied by those physiologists who accept the teaching of Wooldridge [1893] that blood platelets are artefacts, but the latter conclusion is rejected by almost all those haematologists who have made a special study of the blood platelets [*vide* Roskam, 1927; Cramer and Bannerman, 1929 and the recent review of this subject by Mackay, 1931]. Fundamentally different explanations are also offered for the maintenance of the fluidity of the blood after the introduction into the blood-stream of certain bodies which induce clotting in shed blood. One group of investigators postulates that fluidity is maintained *in vivo* by the hepatic secretion of an excess of an anticoagulant [Morawitz, 1904; Mellanby, 1909; Collingwood and MacMahon, 1912-13; Howell, 1925, 1928; Fuchs, 1930]. But several other workers either wholly or partly reject this suggestion and suggest that the essential difference in the condition of circulating and shed blood is the alteration or disintegration of plasma complexes in the latter fluid [Bordet, 1920; Pickering and Hewitt, 1921; Mason, 1921, 1924; Pickering and de Souza, 1923; Pickering, 1925, 1928; Mills, 1928].

The production of intravascular clotting by the intravenous injection of extracts of various tissues is well known but its relationship with natural thrombosis is still obscure.

A controversy has existed for more than half a century on the mode of action of the participants in blood clotting commonly known as thrombokinese and thrombin in this country. These bodies are described as enzymes by one group of inquirers [Morawitz, 1904; Mellanby, 1909; Waldschmidt-Leitz *et al.*, 1929], but several other investigators claim that they react quantitatively in the coagulation of the blood [Rettger, 1909; Bordet, 1920; Howell, 1925, 1928; Mills and Mathews, 1924; Mills, 1928].

A large proportion of the experiments upon which these divergent conclusions are based were conducted with either blood or blood-plasma to which anticoagulants had been added, and an adequate comparison of the results obtained *in vitro* with those obtainable *in vivo* is often lacking. It seemed advisable to re-investigate some of the principal phenomena of blood clotting during the maintenance of the fluidity of the blood in paraffined vessels, without the addition of an anticoagulant, and to compare the results so obtained with parallel reactions *in vivo*.

II. General technique.

Cats, anaesthetised with chloralose, were used. Artificial respiration was only employed in the experiments on intracardiac blood after the isolation of the heart from the circulation by ligatures. Substances introduced into the blood-stream were injected into the jugular vein. A hypodermic syringe was used to inject material into the isolated heart. Shed blood was obtained from the carotid or femoral arteries. A freshly prepared paraffined cannula and a fresh incision into the artery were used for each bleeding. The animals were kept on the operating table for an hour before blood was shed to avoid the fluctuations in the speed of blood clotting which commonly occur shortly after the administration of an anaesthetic. Only relatively small quantities of blood were used in the experiments on shed blood to avoid the hypercoagulability of the blood produced by haemorrhage. It was found that amounts up to 15 cc. of blood could be withdrawn from an animal in good condition without an appreciable variation in the speed of blood clotting. Every experiment on changes induced in the coagulability of blood was accompanied by a control experiment. Except where otherwise stated, all experiments on shed blood were conducted in paraffined vessels.

The blood-serum employed was prepared from blood shed through a paraffined cannula by centrifuging broken-up blood clots directly the coagulation was complete. It was immediately used. The fresh tissue juices were prepared by crushing and grinding the tissues with sand in a mortar, after washing the tissue in normal saline and removal of connective tissue, and the juices were obtained by centrifuging. They were immediately used. The animals' own tissues were employed.

By the expression "commencement of clotting" is implied the first visible departure from fluidity other than the formation of isolated filaments of minute dimensions on the surface of the blood exposed to the air, by "completion," that coagulation had advanced so far that the vessel could be inverted without spilling. The speeds of clotting are given in minutes and seconds. Each experiment is representative of a number yielding concordant results.

III. *The coagulation of blood-plasma after the removal of leucocytes.*

The experiments illustrated in Tables I and II show that the removal of the leucocytes from the blood-plasma in an intact condition does not retard the coagulation of that fluid. It thus appears that the disintegration of leucocytes is not an essential factor in the coagulation of the blood.

The leucocytes were removed from the plasma by centrifuging the blood in paraffined vessels under liquid paraffin at the temperature of the laboratory. Histological examination did not reveal any damage to the leucocytes and plasma was selected in which an abundance of platelets remained intact.

Table I. *Illustrating the speeds of the coagulation of blood-plasma on glass before and after the removal of the leucocytes.*

Temperature 18°.				
No. of exp.	...	1	2	3
		Whole blood	Blood-plasma containing leucocytes and platelets	Blood-plasma containing platelets but no leucocytes
Commencement of clotting		6' 0"	6' 10"	6' 0"
Completion of clotting		6' 40"	6' 35"	6' 30"
No. of exp.	...	4	5	6
Commencement of clotting		4' 0"	3' 55"	3' 50"
Completion of clotting		4' 20"	4' 10"	4' 8"

The rapidly clotting blood was obtained from an animal after haemorrhage. 0.5 cc. of blood or plasma was used in each experiment.

Table II. *Illustrating the speeds of the coagulation of blood-plasma in a paraffined vessel before and after the removal of leucocytes.*

Temperature 18°.				
No. of exp.	...	7	8	9
		Whole blood	Blood-plasma containing leucocytes and platelets	Blood-plasma containing platelets but no leucocytes
Commencement of clotting		56' 0"	57' 10"	53' 30"
Completion of clotting		62' 10"	61' 20"	60' 50"

0.5 cc. of blood or plasma was used in each experiment. A repetition of the experiment with a hypercoagulable blood obtained after haemorrhage gave similar results.

IV. *The rôle of blood platelets in the inception of clotting.*

The next group of experiments (Nos. 10-19) shows that the complete disintegration of blood platelets neither produces intravascular clotting nor hastens the coagulation of normal blood which has been shed through a paraffined cannula into paraffined vessels. They also illustrate that the lysis of platelets accelerates the clotting of blood which has been disturbed either by contact with glass (which is wetted with blood) or by the presence of tissue débris. It is concluded that the breakdown of platelets is not the primary occurrence in the clotting of pure blood; but that the products liberated by the disintegration of platelets rapidly induce coagulation in blood-plasma which has been disturbed either by contact with a surface which it wets or by the action of tissue débris.

Exp. 10. 5 cc. of a 10 % solution of saponin in normal saline were injected into the jugular vein of an anaesthetised cat weighing $3\frac{1}{2}$ kg. Blood shed from the carotid artery 10 mins. later showed complete lysis of platelets, indications of damage to some of the leucocytes and partial haemolysis of the erythrocytes. *Post mortem* examination revealed absence of intravascular clotting. The times of coagulation at 18° of blood shed on glass before the injection of saponin were: commencement of clotting 5' 30", completion of clotting 6' 10"; after the administration of saponin: commencement of clotting 4' 20", completion of clotting 5' 10".

Exp. 11. 0.5 cc. of distilled water was injected into each of the ventricles of the heart of a cat after the isolation of the heart from the general circulation by ligatures. Histological examination of blood aspirated from the ventricles revealed almost complete lysis of platelets. There was no intracardiac clotting.

In each of the experiments summarised in Tables III, IV and V, blood from the carotid artery was shed into two paraffined vessels. The blood in one vessel was mixed with sufficient saponin to disintegrate all the platelets.

Table III. *Illustrating the speeds of clotting in paraffined vessels at 19° of arterial blood and of arterial blood in which the platelets had been disintegrated by saponin (0.04 cc. of a 10 % solution of saponin to 0.5 cc. of blood was used).*

No. of exp.	...	12	13	14	15
		A small drop of pure blood	0.5 cc. of pure blood	A small drop of blood + saponin	0.5 cc. of blood + saponin
Commencement of clotting		57' 0"	64' 0"	57' 30"	64' 10"
Completion of clotting		60' 0"	66' 0"	60' 30"	68' 0"

There was no indication of hypercoagulability of this blood since the clotting times on glass at 39° were: commencement of clotting 3' 30", completion of clotting 4' 0". The same concentration of saponin was used in the experiments noted in Tables IV and V.

Table IV. *Illustrating the speeds of clotting in glass vessels at 19° of arterial blood and of arterial blood in which the platelets had been disintegrated by saponin before the blood had been placed on glass.*

No. of exp. ...	16	17	18	19
	0.5 cc. of pure blood	0.5 cc. of blood + saponin	2.5 cc. of pure blood	2.5 cc. of pure blood + saponin
Commencement of clotting	6' 15"	4' 0"	6' 40"	4' 0"
Completion of clotting	9' 5"	4' 15"	7' 25"	7' 10"

Table V. *Illustrating the speeds of clotting in paraffined vessels at the temperature of the laboratory (19°–20°) of extremely hypercoagulable blood, unmixed with saponin and after admixture with saponin.*

(The blood had been rendered hypercoagulable by contact with tissue débris.)

No. of exp. ...	20	21	22
	The blood in a glass vessel	The blood in a paraffined vessel	The blood + saponin in a paraffined vessel
Commencement of clotting	Not determined	16' 25"	4' 30"
Completion of clotting	0' 3"	18' 25"	10' 30"

V. *The difference in the capacity of fresh blood-serum to clot shed and unshed blood.*

Exps. Nos. 23 to 26 illustrate that fresh blood-serum promptly clots both pure blood and pure blood-plasma which have been shed into paraffined vessels. Those numbered 27 and 28 show that all the blood in the ventricles of the heart of male cats and of non-pregnant female cats, after isolation of the heart from the circulation by ligatures, remains completely fluid *in vivo* after the injection of fresh serum, although the presence of active thrombin can be demonstrated by the capacity of the intracardiac blood to clot oxalated plasma. A fundamental difference is thus apparent in the resistance of unshed and shed blood to coagulation by the thrombin of serum which cannot be explained as due to the neutralisation of thrombin *in vivo* by an anticoagulant but is consistent with the conclusions of Pickering [1925, 1928] and of Mills [1928] that dissociation of plasma-colloids is a pre-requisite for the coagulation of the blood by thrombin. Exp. No. 29 shows, however, that if the heart is

Table VI. *Illustrating the rapid clotting by fresh serum of pure blood and of pure blood-plasma shed into paraffined vessels.*

Temperature 19°.				
No. of exp. ...	23	24	25	26
	5 cc. of blood + 1 % of serum	15 cc. of blood + 0.3 % of serum	2 cc. of plasma + 0.1 % of serum	10 cc. of plasma + 0.2 % of serum
Commencement of clotting	—	0' 8"	2' 15"	3' 0"
Completion of clotting	2' 30"	8' 30"	2' 50"	3' 7"

killed by the action of the serum, the serum introduced into the heart may or may not be inactivated.

Exp. 27. The heart of a non-pregnant female cat was isolated from the general circulation by ligatures. 1 cc. of fresh serum was injected into each ventricle. Twelve minutes later a few drops of blood were withdrawn from each ventricle and added to oxalated plasma. The blood from each ventricle rapidly clotted the oxalated plasma, coagulation commencing in less than 2' 30". Fifteen minutes later the animal was killed and *post mortem* examination showed the absence of clots from the ventricles.

Exp. 28. The experiment described above was repeated with a male cat and gave similar results.

Exp. 29. In another male cat 1 cc. of fresh serum was injected into each ventricle of the isolated heart. The heart stopped beating. Blood withdrawn from the right ventricle did not clot oxalated plasma but that withdrawn from the left ventricle produced a filamentous clot in oxalated plasma. There was slight intracardiac clotting in the right ventricle but the blood in the left ventricle was completely fluid.

A marked difference in the resistance of intracardiac blood to coagulation *in vivo* is apparent when fresh blood-serum is injected into the isolated hearts of pregnant cats, even when relatively small amounts of serum are used. Massive clotting may be produced in one ventricle whilst the blood remains fluid in the other ventricle. This may be partly due to changes in the equilibrium of the plasma-colloids since the content of fibrinogen and of lipoids as well as the suspension stability of the blood is altered in pregnancy, and one of us (J. W. P.) has frequently observed hypercoagulability of the blood of women in an advanced stage of pregnancy.

Exp. 30. Blood was obtained from the carotid artery of a cat in an advanced stage of pregnancy. Clotting times on glass at 19° were: commencement of coagulation 2' 15", completion of coagulation 6' 40". Hypercoagulability was thus manifested by the rapidity of the inception of clotting. The heart was isolated from the general circulation and 0.5 cc. of fresh serum was rapidly injected into each ventricle. Eight minutes later blood was withdrawn from each ventricle. The blood from the left ventricle completely clotted oxalated plasma in 6' 30", that from the right ventricle in 8' 30". *Post mortem* examination showed a massive clot and some fluid blood in the right ventricle and no clots in the left ventricle. The fluid blood from both ventricles clotted when shed.

VI. *The behaviour of fresh tissue juices in relation to extravascular and intravascular clotting.*

The experiments numbered 31–36 show that freshly prepared tissue juices obtained by crushing the lining of the aorta, testes, muscles or kidneys, rapidly induce coagulation in pure blood shed into paraffined vessels. Exps. Nos. 37–40 illustrate that relatively large amounts of the fresh juices of testes, muscles or kidney may be injected into either the general circulation or into the heart isolated from the circulation of non-pregnant cats without producing intravascular clotting. Exp. No. 41 demonstrates that the fresh juice of the kidney may induce localised intravascular coagulation in the pregnant cat. A fundamental difference is thus apparent in the resistance of unshed and shed blood to coagulation by tissue juices, which cannot be ascribed to neutralisation of

the coagulant material by an anticoagulant either secreted by the liver or liberated from the cells of the intima of the aorta, since these influences are excluded in Exp. No. 38, and Exp. No. 31 shows that the fresh juices of the aortic lining hasten the coagulation of the blood. It is suggested that the absence of coagulation *in vivo* from the non-pregnant animal is due to the specific resistance of the plasma complexes to dissociation by disturbing material and that this resistance is lowered in pregnant animals (*cf.* Exp. No. 30).

Table VII. *Illustrating the rapid coagulation of pure blood shed into paraffined vessels after the addition of fresh tissue juices.*

Temperature 19°.				
No. of exp.	...	31	32	33
		5 cc. of blood of non-pregnant female + 0.05 cc. of juice from aortic intima	2.5 cc. of blood from a male + 0.05 cc. of testis juice	5 cc. of blood from a male + 0.05 cc. of testis juice
Commencement of clotting		4' 0"	—	1' 50"
Completion of clotting		5' 15"	0' 5"	2' 40"
No. of exp.	...	34	35	36
		2.5 cc. of blood of non-pregnant female + 0.05 cc. of muscle juice	2.5 cc. of blood of non-pregnant female + 0.05 cc. of kidney juice	5 cc. of blood of pregnant female + 0.1 cc. of kidney juice
Commencement of clotting		2' 30"	2' 40"	—
Completion of clotting		5' 45"	3' 0"	1' 10"

These observations are consistent with those of Morse [1919], who obtained material which hastened blood clotting from scrapings of the aorta, and with those of Mason [1924] who found that fresh extracts of the aortic intima as well as those of brain, kidney, lung, liver, testes and spleen accelerated the coagulation of oxalated plasma after "recalcification." They are also consistent with the finding of Pickering and Hemingway [1926] that extracts obtained from the tissues of the brain and lungs accelerate the clotting of blood shed into paraffined vessels.

Table VIII. *Illustrating the results obtained by the rapid intravascular injection of the fresh juices of testis, muscle and kidney.*

(10 cc. of tissue juice were injected into the jugular vein in each experiment except in No. 38, in which 0.5 cc. of testis juice was injected into each ventricle of the heart after isolating the heart from the general circulation by ligatures.)

Exp. 37	Exp. 38	Exp. 39	Exp. 40	Exp. 41
♂ (4½ kg.) testis juice injected. No intravascular clotting	♂ Testis juice injected. No clotting in the ventricles of the heart	♀ (2½ kg., non-pregnant). Muscle juice injected. No intravascular clotting	♀ (4 kg., non-pregnant). Kidney juice injected. No intravascular clotting	♀ (4 kg., in advanced stage of pregnancy). Kidney juice injected. Massive clots in portal vein

The normal blood volume of the cat has not yet been accurately estimated but may be assumed to amount approximately to 50 cc. of blood per kg. of body weight. A comparison of Tables VII and VIII shows that concentration of tissue juices employed *in vivo* were much greater than those that sufficed for the rapid and complete coagulation of blood shed into paraffined vessels. Larger doses could not be employed *in vivo* as respiration failed and the heart ceased beating. It may be added that an increase of the amounts of tissue juice added to shed blood, beyond those noted in Table VII, accelerates extravascular clotting on a paraffined surface.

There was no indication of a negative phase of coagulation in any experiments either *in vivo* or on shed blood. In Exp. No. 41 the blood remained fluid in all the larger vessels except in the portal vein, and was also fluid in the right ventricle of the heart. The left ventricle, the auricles and aorta were empty. Other pregnant animals showed intravascular clotting limited to the veins in the abdomen or to the left ventricle of the heart and like results were obtained with muscle juice.

A sharp contrast is apparent between the maintenance of the fluidity of the blood in non-pregnant cats after the rapid intravascular injection of massive amounts of the fresh juices of kidney and testes and the extensive intravascular clotting which follows the intravascular injection of extracts of these organs, which have been prepared by the methods of Wooldridge [1886, 1888, 1893] or of Halliburton [1891]. In the former technique strong acidification is employed, in the latter strong solutions of common salt are used. Both these procedures may involve either the dissociation of the colloidal complexes of tissue material or the denaturation of proteins, and in this connection the suggestion of Mason [1924] should be noted that the coagulants of tissue are normally united with protective colloid which is dissociated from the coagulant fraction during blood clotting. The repetition of precipitation and re-solution used in the "purification" of the extracts might remove a protective colloid, and so enhance the activity of the disturbing material.

VII. *The mode of action of thrombokinase and of thrombin in blood clotting.*

More than two hundred experiments were performed in an attempt to obtain sufficient information to decide whether the material liberated by the disintegration of blood platelets or from crushed tissues, known as thrombokinase in this country, and the participant in blood clotting called thrombin (the thrombase of Anwyl-Davies and Mellanby [1923]) react as enzymes or quantitatively in blood clotting. A conclusive answer to these questions was not found, but some light is thrown on them. There was no indication that the material released by the complete lysis of all the platelets in the blood hastens blood coagulation either *in vivo* or in blood shed into paraffined vessels (*vide* Exps. 10-15). It thus appears that the débris of blood platelets, when in a fresh condition, does not behave as an enzyme in blood which has not been disturbed by contact with a surface that it wets, and this conclusion is consistent with the observations of Fonio [1913] that massive doses of disintegrated blood platelets can be injected intravenously without producing intravascular clotting and with the medicinal use of this procedure without the occurrence of thrombosis.

It has already been noted, in Table IV, that blood in which the platelets have been disintegrated before shedding on glass clots more rapidly on glass than normal blood. But the decrease in the time required for the inception of clotting (2 mins.) is not greater than may be attributed to the almost instantaneous nature of the breakdown of the platelets. Our observations thus appear to be consistent with the conclusions of Bordet and Delange [1913] and of Bordet [1919, 1920] that quantitative reactions occur in the clotting of blood by the action of the *débris* of blood platelets.

The most cogent evidence that thrombokinase is an enzyme is provided by the observations of Mellanby [1917] on the coagulation of blood *in vitro* after the addition of testis extract; but no evidence is offered of identity in the behaviour of this material with that of the *débris* of blood platelets. It appears that conclusions based on these observations are valid only in the problem of the coagulation of shed blood after admixture with tissue juices and the experiments already illustrated in this paper suggest that a much wider range of experimentation is necessary before deciding whether the inception of intravascular and extravascular clotting can be ascribed to the same cause.

There was no indication in any of our experiments that fresh tissue juices inaugurate intravascular clotting by enzyme action. Much larger amounts of the fresh juices of testis, kidney and muscle than those required to produce rapid and complete clotting in shed blood were introduced into the blood-stream or into intracardiac blood without producing clotting in non-pregnant cats and the limitation of intravascular clotting to the blood of the abdominal veins or of the cardiac ventricles of the pregnant animals suggests local variations in the condition of the blood, rather than the occurrence of enzyme action (examples of the experiments are given in Table VIII).

The very rapid coagulation of blood shed into paraffined vessels after the addition of very small amounts of fresh tissue juices, illustrated in Table VII, suggests, however, catalytic action and these observations are consistent with the conclusions of Mellanby in so far as they apply to the inception of coagulation in shed blood by the action of tissue juices. But these experiments do not discredit the alternative explanation offered by Mills [1928] that enzyme action is simulated in blood clotting by a progressive release of the participants in clotting by the coagulation of the fibrinogen of the fibrinogen-prothrombin complex of the plasma.

Many attempts were made to confirm the statement of Rettger [1909] that quantitative reactions occur in the coagulation of the blood by the addition of fresh serum, but without any success. The experiments illustrated in Table VI show that very rapid and complete clotting follows the addition of minute amounts of fresh serum to blood shed into paraffined vessels. In only one experiment was there any indication which suggested a quantitative reaction.

Exp. 42. A medium sized blood clot was dropped into 2.5 cc. of blood shed into a paraffined vessel. Two minutes later the clot had doubled in size but the rest of the blood remained

completely fluid for nearly an hour. Coagulation commenced on the surface of the blood exposed to the air in 54 mins. and was complete in 65 mins. The control blood commenced to clot in 55 mins. and its clotting was complete in 65 mins.

It might be assumed that the thrombin of the serum in the blood clot was used chemically in the production of the increase in size of the clot but it appears equally probable that the thrombin was inactivated by adsorption on the surface of the clot. A study of the experiments of Rettger suggests that weak sera were used in his experiments and it appears probable that the explanation just offered accounts for the results he obtained. A large number of attempts were also made to obtain evidence of the alleged quantitative reaction in the coagulation of oxalated and citrated plasma by fresh serum. Reactions which simulated combination of the serum and the plasma were obtained but it was also apparent that fresh serum is inactivated by exposure, for 2-5 minutes, to 0.1 % potassium oxalate and a like reaction occurs after exposure to citrate. Inactivation of the serum thus accounts for the incompleteness of the coagulation when small amounts of serum are used. The experiments we have performed are thus consistent with the conclusion that fresh serum reacts as a catalyst in the clotting of shed blood, but they do not discredit the alternative explanation offered by Mills and mentioned above.

VIII. *The significance of the experiments in the problem of the maintenance of the fluidity of the blood.*

The experiments already described show that much larger amounts of fresh serum than are required to produce extravascular blood clotting can be injected into the hearts of non-pregnant cats, after the isolation of the heart from the general circulation, without producing intracardiac clotting (*vide* Exps. Nos. 27, 28), and a like resistance *in vivo* is displayed to the inception of intracardiac clotting by the action of fresh tissue juices (Exp. 38). The isolation of the heart from the general circulation precludes the inactivation of either the thrombin of the serum or of the thrombokinase of the tissue juices by the hepatic secretion of an excess of anticoagulant, and the confirmation of the conclusions of Morse [1919] and of Mason [1924] that the lining of the aorta contains a very active coagulant discredits the suggestion of Popielski [1913] that the fluidity of circulating blood after the intravascular injection of a coagulant is maintained by the release of an anticoagulant from the vascular endothelium. Moreover, the demonstration of the presence of active thrombin in the blood aspirated from the heart after the intracardiac injection of fresh serum (*vide* Exp. 27) indicates that the fluidity of the blood can be completely maintained *in vivo* without the inactivation of thrombin.

A specific resistance of plasma-colloids to dissociation and coagulation *in vivo* is indicated rather than the maintenance of the fluidity of the blood by the ingress of anticoagulants into the blood-stream. It has been pointed out by Pickering [1928] that blood-plasma possesses only a limited capacity of resisting disturbance and he suggests that changes in the surface condition

of shed blood lead to the dissociation of plasma-colloids whereby material in equilibrium is replaced by bodies which are susceptible to rapid change. On this assumption, the lack of resistance of shed blood to coagulation by fresh serum, by the material liberated by the lysis of platelets and by that present in fresh tissue juices becomes intelligible.

IX. *The significance of the experiments in the problem of natural thrombosis.*

Thrombosis has been defined as intravascular clotting arising during life-time. This definition is, however, inadequate, since the most simple forms of thrombi do not contain blood clots. But both red and mottled thrombi are largely composed of clotted blood, and red thrombi display a similar histological structure to that of the intravascular clots produced by the intravenous injection of tissue extracts [Mason, 1924]. An intimate relationship between natural thrombosis and experimental intravascular clotting thus seems apparent. The great resistance displayed by both male and non-pregnant female cats to intravascular clotting by fresh tissue juices suggests that ingress of such bodies into the blood-stream after trauma does not explain post-operative thrombosis, whilst the production of intravascular clotting in pregnant cats, limited to veins in the abdomen or to the left ventricle of the heart, by the intravenous injection of fresh tissue juices, suggests that localised variations in the condition of the venous blood occur during pregnancy, which conduce to thrombosis.

SUMMARY.

1. The disintegration of leucocytes is not an essential occurrence in the inception of blood clotting.

2. The complete disintegration of blood platelets neither produces intravascular clotting nor hastens the coagulation of normal blood which has been shed into paraffined vessels.

3. The lysis of blood platelets hastens the clotting of blood shed on to glass and also hastens the coagulation of blood shed into paraffined vessels when the blood is contaminated with tissue juices.

4. Fresh blood-serum promptly clots both pure blood and pure blood-plasma which have been shed into paraffined vessels, but the rapid intravascular injection of amounts of fresh sera sufficient to produce extra-vascular clotting does not produce intravascular coagulation in non-pregnant cats, if the amount administered is not lethal.

5. The rapid injection of fresh blood-serum into the ventricles of the hearts of pregnant cats, after the isolation of the heart from the general circulation, may or may not produce intracardiac coagulation of the blood.

6. The addition of very small amounts of the fresh juices of testis, muscle and kidney to blood shed into paraffined vessels rapidly produces complete coagulation, but the rapid intravascular or the rapid intracardiac injection (after the isolation of the heart from the general circulation) of these juices does not produce clotting *in vivo* in non-pregnant cats.

7. The production of intravascular clotting by the intravenous injection of the fresh juices of kidney and muscle into pregnant cats is described.

8. The significance of the data recorded in the explanations of the inception of the coagulation of the blood, of the maintenance of the fluidity of the blood *in vivo* and of the genesis of natural thrombosis are discussed.

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