XCI. THE FLUIDITY AND COAGULATION OF THE BLOOD.

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ONE of the landmarks in the history of the coagulation of the blood is the observation of Fredericq [1878] that heat coagulation occurs when the blood in the excised jugular of a horse is heated to 55–57°, after the subsidence of the corpuscles. As the fibrinogens of the laboratory prepared by the method of Hammarsten [1879] and of Schmidt [1892] coagulate at 56°, it is commonly believed that they are identical with the precursor of fibrin in the blood. This was not however accepted as conclusive by two prominent physiologists, Wooldridge [1885, 1893] and Michael Foster [1888], each pointing out that fibrin ferment (thrombin) could be injected into the circulation in massive amounts without causing thrombosis, while similar quantities added to solutions of the fibrinogens of the laboratory provoke clotting. An attempted explanation of this anomaly, in the terms of Howell's [1911] views on coagulation, was advanced by Gasser [1917], who suggested that thrombin, when intravenously injected, combines with antithrombin to form an inactive substance termed metathrombin, it being assumed, without any direct evidence, that the introduction of thrombin into the circulation causes the liver to secrete an excess of antithrombin. But Pickering and Hewitt [1922, 2] have shown that massive doses of thrombin can be injected into the circulation of animals deprived of hepatic activity without causing intravascular clotting and these authors have deduced evidence that antithrombins are *post-mortem* products. Thus the lack of coagulant action of massive doses of thrombin in circulating blood is at least suggestive of some essential difference in the condition or composition of the fibrinogens of the laboratory and the precursor of fibrin in the living blood.

The salts employed in the preparation of fibrinogen by Hammarsten's method cannot be regarded as without influence on the structure or condition of the latter material, as Howell [1914, 1] has found it is not possible by this method to obtain solutions of fibrinogen of uniform properties, part of the

¹ The theory of the fluidity and coagulation of the blood enunciated in this paper owes its origin and elaboration solely to J.W.P. but I am in entire agreement with it. The experimental work has been carried out in collaboration, the operative part by D. H. de S., the observations on clotting by both. D. H. de S.

protein being denaturated by repeated precipitation. The method of Schmidt [1892], which involves dilution with excess of distilled water, is not well calculated to preserve the surface conditions of colloidal complexes, and Mellanby [1909] has found that the fibrinogen prepared by this process is always contaminated with prothrombin. Only after prolonged adsorption by barium sulphate were Dale and Walpole [1916] able to disassociate prothrombin from fibrinogen. Accepting these data, we shall write of a fibrinogen-prothrombin complex in blood, rather than of two disunited substances fibrinogen and prothrombin.

Nolf [1921] has shown that fibrinogen dissolved in faintly alkaline isotonic solution and fibrinogen dissolved in the protein residues of the plasma behave differently with regard to precipitation by chloroform. This, he explains, as due to protection afforded by the stable colloids in the protein residue, which restore the condition of the prepared fibrinogen to that of the fibrinogen of circulating blood.

In view of the obvious differences of circulating and shed mammalian plasma, it appeared desirable to enquire into the condition of fibrinogen prior to bleeding, rather than subsequent to that act. To this end experiments were devised: (1) to study the effect of heating the blood in the heart to 56–63°, without exposure to the air; (2) to observe the results of heating intraventricular blood to 56–63°, after disturbance of its surface conditions; (3) to examine the coagulability of the fluid blood remaining in the heart, after the formation of mechanically produced intravascular clots and (4) to enquire into the effects on the coagulability of blood produced by heating it to temperatures less than the coagulation temperature of fibrinogen.

GENERAL TECHNIQUE.

Cats and rabbits were used for the experiments, the former were anaesthetised with A.C.E., the latter with ether. In the case of the cats the animals were pithed under the anaesthetic and artificial respiration was maintained. The great vessels round the heart were then tied by a mass ligature, the heart removed and immediately placed either in isotonic saline or in neutral olive oil. Under these conditions the hearts were heated without admission of air into their interiors for the periods and to the temperatures shown in the protocols.

Control experiments were made with a thermometer inserted, through the aorta, into the left ventricle and ligatured in position, so as to prevent the entrance of fluid from the bath into the cavities of the heart. These showed that four minutes sufficed for the intraventricular temperature to exceed 56°, when the heart was placed in a bath at 60° and that after seven minutes the temperature of the ventricular cavity was that of the bath.

The mechanically produced thrombi were obtained by the introduction of cotton threads into the cavities of the heart *in situ*. The contraction of the living heart muscle prevented haemorrhage from the punctures. By the term

"commencement" of coagulation is implied the first visible departure from fluidity, other than the formation of isolated filaments of minute diameter, by "completion" that coagulation has advanced so far that the vessel could be inverted without spilling.

Each experiment described is representative of a number yielding concordant results.

THE HEATING OF BLOOD IN EXCISED HEARTS.

Exp. 1. The heart of a cat was excised and placed immediately in an oil bath at 60°. The temperature of the bath fell to 56° and remained at that temperature for five minutes. The bath was raised to 60° and maintained at that temperature for twelve minutes. When removed from the bath, the heart showed a small clot in the right ventricle, with a considerable amount of fluid blood. This clotted on glass and some of it which remained in contact with the surface of the ventricle developed a large clot. The blood from the right ventricle also gave a precipitate when one-fifth saturated with ammonium sulphate and also when half saturated with sodium chloride. The small amount of blood in the left ventricle was completely fluid and subsequently clotted on glass.

Exp. 2. Cat. The clotting times of blood from carotid before excision of the heart were commencement 5' 30" and completion 6' 20". The heart was excised and placed in a normal saline bath at 62°. For the next eleven minutes the temperature of the bath varied between 60° and 63°, it was then kept at 63° for five minutes. All the blood in the right ventricle was fluid on shedding but subsequently developed small clots in contact with glass. These subsequently increased in size. The time of commencement of clotting was 4' 30", of completion of that process 7' 0", the times being in each case taken from the moment of shedding. The left ventricle was empty.

Exp. 3. Rabbit died under ether. Heart immediately excised and placed in oil at 65°. During the next seventeen minutes the temperature of the oil varied from 60–67°. It was then kept at 66° for six minutes. Examination of the right ventricle showed clots on the chordae tendinae and a considerable amount of fluid blood in which four small clots formed after it was shed on to glass. The residual fluid was not precipitated by either one-fifth saturation with ammonium sulphate or by half saturation with NaCl. The blood from the left ventricle behaved in a similar manner.

Exp. 4. Cat. The conditions were the same as in *Exp. 2*, except that the heart had stopped beating for eleven minutes before excision. After removal from the bath all blood was found to be fluid, except for some small clots in the left auricle. The fluid blood failed to clot.

Exp. 5. Cat. Heart excised 19 minutes after death from A.C.E. No heat applied. All blood fluid but subsequently completely clotted on glass. The coagulation times of blood from the carotid before death were: commence-

ment 7' 15" and completion 7' 55". Blood from the excised heart showed commencement of clotting 5' 10" and completion 8' 0".

A comparison of Exps. 4 and 5 shows that blood shed from a dead heart at room temperature clots but that the fluid taken from the cavities of a dead heart which has been heated from 56–63° behaves like blood which has been heated to a like degree either in a test tube or in a vein exposed to the air, *i.e.* the fibrinogen is destroyed. Blood taken from the cavities of a moribund heart (as in Exp. 3) which has been heated above the coagulation temperature of fibrinogen has the bulk of its fibrinogen destroyed. When however the freshly excised and still beating heart is immersed in a hot bath and is heated for 15–20 minutes to 56–60° fibrinogen remains in the blood contained in the ventricles. Such blood clots spontaneously when shed on to glass and is precipitated by one-fifth saturation with ammonium sulphate and by half saturation with sodium chloride (Exps. 1 and 2). Evidence is thus forthcoming that fibrinogen exists in blood, when unexposed to air or foreign surfaces, after heating above the coagulation temperature of the fibrinogens of the laboratory.

The next experiment illustrates the effect of sodium citrate on intracardiac blood heated to 56.5°.

Exp. 6. Cat. 3 kilo. A.C.E. pithed. Artificial respiration. 10 cc. of a 20 % solution of sodium citrate was injected into the jugular. The animal exhibited pronounced opisthotonus and its heart stopped. Blood withdrawn by aspiration from the right ventricle failed to clot, but clotted on re-calcification. The heart was excised and heated in a normal saline bath for 8½ minutes to 56–56.5°. On opening the ventricles the blood was found to be fluid. It did not clot on recalcification.

This experiment indicates that the intravascular injection of sodium citrate alters the condition of the fibrinogen moiety of the fibrinogen-prothrombin complex of the blood or of a protective colloid associated with fibrinogen in circulating blood.

A THEORY OF THE FLUIDITY AND CLOTTING OF THE BLOOD.

In introducing this theory a preliminary paragraph is perhaps not inopportune. Any hypothesis is merely a conceptual device for the correlation of experience and can lay no claim to finality. It is in this sense that the theory to follow is suggested. Hypotheses exhibit signs of senile decay when, to maintain them, it is necessary to postulate, as knowledge progresses, an increasing number of substances not actually observed or isolated. Such indications are evident in the current thrombin theories of coagulation.

The essential characteristics of the fibrinogen of the laboratory are:

- (1) It coagulates by heating to 56–57°.
- (2) It is clotted by moderate amounts of thrombin.
- (3) It is precipitated by one-fifth saturation with ammonium sulphate [McLean, 1920] and by half saturation with sodium chloride.

None of these features is exhibited by intravascular blood, provided its surface conditions have not been disturbed.

The disparity of this conclusion with the classic experiment of Fredericq on heating the "living test tube" to 56° becomes intelligible on consulting Fredericq's original memoir; for he found, almost invariably, a clot adjacent to the junction of the plasma and settled out corpuscles. Fibrin ferment (thrombin) was also present, although absent from the circulating blood of the horse. The colloids of this plasma had therefore progressed towards clotting.

The differences of the fibrinogen of the laboratory from that of undisturbed intravascular blood may be explicable in one of two ways:

(a) That fibrinogen does not exist in circulating blood, but that a precursor of it is present:

(b) That the fibrinogen in circulating blood is associated with a protective colloid, which renders it resistant to changes by the agencies named.

The following facts indicate that the latter interpretation is the more probable.

1. Fibrinogen is closely associated with prothrombin. On heating it can be resolved into two proteins exhibiting different coagulation temperatures [Huiskamp 1905; Iscovesco, 1906].

2. The protection against precipitation by chloroform resulting from dissolving laboratory fibrinogen in the protein residues of plasma [Nolf, 1921].

3. Both fibrinogen and prothrombin can be obtained from plasma after dilution with excess of distilled water, yet whole blood, even after contact with cut tissue, when so diluted and evaporated down to its original volume does not spontaneously clot [Pickering and Hewitt, 1921]. Lack of activation of prothrombin due to absence of "thrombokinase" cannot be pleaded as an explanation of these results, because the blood employed had been in contact with cut tissues, exhibited complete haemolysis following the dilution with distilled water and coagulated normally on glass when undiluted. The inhibition of clotting is however intelligible on the view that colloidal material is associated with the fibrinogen-prothrombin complex in circulating blood and the action of excess of distilled water may be regarded as stabilising such material.

It is suggested that the essential factors in the maintenance of the fluidity of circulating blood, in the clotting of shed blood and in the formation of intravascular clots are (1) a fibrinogen-prothrombin complex, (2) a protective colloid united to the fibrinogen-prothrombin complex in circulating blood and (3) salts of calcium.

No series of antibodies is postulated, as in the thrombin theories, but when the surface conditions of the blood are disturbed, either by shedding or by the introduction of disturbants *in vivo*, disunion of the protective colloid and the fibrinogen-prothrombin complex occurs. The inception of these changes is marked when blood slowly clots in oil by the appearance of a reversible gel [Pickering and Hewitt, 1921] and similar reversible material was found by

Pickering [1923] in the filaments formed immediately after bleeding in films of human blood exposed to moist air. From a comparison of the times of appearance of the reversible gels with the pre-clot changes in shed blood observed by Vines [1921, 1] and by Novy and De Kruif [1917], it would appear that the disunion of the protective colloid and the fibrinogen-prothrombin complex corresponds in time with the changes in the content of ionised calcium in shed blood, observed by the former worker, and with the development of toxicity recorded by the latter.

The next stage in the coagulation of the blood is the activation of prothrombin, yielding a *post-mortem* product thrombin. This may be effected by calcium ions [Howell, 1914, 1], or in blood in contact with damaged tissues by calcium ions and the hydrophile material called thrombokinase. On this view, both thrombin and thrombokinase would be regarded as accelerators of coagulation, rather than as initiators of that process. The prime factor in fluidity and clotting would thus be the surface conditions of the protective colloid, as when these remain undisturbed or if they are artificially stabilised then fluidity is preserved.

Working with fibrinogen purified by adsorption by barium sulphate and with prothrombin prepared by a modification of Mellanby's method, Dale and Walpole [1916] found that thrombokinase was necessary for the clotting of fibrinogen. Gratia [1918] found that calcium chloride, dissolved in distilled water, causes the lysis of platelets and it is generally recognised that disintegrated platelets yield thrombokinase. It is also generally accepted that prothrombin exists in circulating blood. Löwit [1892] found that the massive intravascular injection of calcium chloride fails to induce thrombosis and Pickering and Hewitt [1922, 2] obtained the same result with lethal intravenous injections of large amounts of calcium chloride, dissolved in distilled water, even after the intravascular injection of massive doses of thrombin into cats, deprived of hepatic activity. It would thus appear, accepting Dale and Walpole's results, that thrombokinase activates the prothrombin of the laboratory, but may not always invoke clotting under the conditions of circulating blood. This is intelligible as an example of protection *in vivo*, against the disturbant properties of the thrombokinase.

In the development of coagulation prothrombin is converted into thrombin and the union of fibrinogen and prothrombin is broken up. In this sense, the process of clotting is the lysis of a colloidal complex. Evidence is not wanting as to the similarity of protection against the precipitation or the coagulation of blood and protection against haemolysis. Numerous observers have shown that serum protects against haemolysis by several agencies, and Nolf [1921] has shown that the proteins of serum protect fibrinogen against precipitation by chloroform. The addition of egg-albumin to shed blood has long been known to delay clotting, and egg-albumin protects against haemolysis by eosin in the presence of sunlight [Schmidt and Norman, 1920], and also against haemolysis by saponin and by a heterologous serum [Pickering and Taylor, 1923].

The addition of an equal volume of 0.5 % solution of sucrose to blood inhibits coagulation. Eisler [1909] found that sucrose protects against specific haemolysis and observed that the addition of moderate amounts of electrolytes restores haemolytic action. Isotonic sucrose also inhibits the haemolytic action of saponin and sapotoxin [Furuhata, 1918]. "Peptone" inhibits the clotting of blood, and Schmidt and Norman [1920] have shown that "peptone" protects against haemolysis by eosin, in the presence of sunlight. Coagulability is restored to "peptonised" blood by the addition of moderate amounts of sodium chloride, by neutralisation with a weak acid and by the passage of carbon dioxide.

We have seen that the addition of moderate amounts of electrolytes restores haemolysis after protection by sucrose, and Pickering and Taylor [1923] have found that either acid or neutral egg-albumin affords less protection against haemolysis by saponin than does alkaline egg-albumin.

If the view is accepted that the problems of the fluidity and clotting of blood are comparable to those of the protection against, and the inception of, haemolysis, then they may ultimately be resolved into questions of the interfacial permeabilities of the fibrinogen-prothrombin complex of the plasma.

There remains for consideration a number of generally recognised phenomena in the fluidity and coagulation of blood, some of which have been neglected by the supporters of the thrombin theories, but all of which can be interpreted on the hypothesis just enunciated.

(1) *The fluidity of circulating blood.* It may be assumed that the surface conditions of the vascular wall are such as not to disturb those of the protective colloid of the plasma.

(2) *The condition of the plasma in the excised jugular of the horse.* Here partial clotting occurs with the formation of thrombin [Fredericq, 1878], but the bulk of the plasma remains fluid. This the thrombin fails to clot, in like manner to the failure of thrombin to clot circulating blood. This may be attributed to absence of disturbance of the protective colloid in those portions of the plasma in contact with the undamaged vascular wall.

(3) *The slowing of clotting on oiled and paraffined surfaces.* This is intelligible on the view that oil or paraffin only slowly causes surface change in the protective colloid of the plasma.

(4) *The fluidity of bird's blood in vitro.* This can be explained by regarding the protective colloid in bird's blood as more stable than that in mammalian blood. The observation of Rettger [1909] that bird's blood *in vitro* is clotted by a feather is explicable by disturbance of surface conditions by that agency. Coagulation by the rapid addition of tissue extract (thrombokinase) may be regarded as acting in a like manner.

(5) *The effect of re-precipitating and washing fibrinogens.* Howell [1916] found that the fibrinogens prepared from the oxalated plasmas of the bird and of the terrapin, by a single precipitation with sodium chloride, frequently

failed to clot on the addition of thrombin; but that the fibrinogens prepared by two or more precipitations were clotted by thrombin, provided the precipitates were sufficiently washed. This is intelligible on the view that the repetition of precipitation and washing disassociates the fibrinogen from the protective colloid.

(6) *The coagulant action of a thread.* The following type of experiment was devised to elucidate this.

Exp. 7. Cat. A.C.E. pithed. Artificial respiration for 20' before operating on the heart. Three cotton threads were introduced into the right and left ventricles, by passing threaded needles through the heart. The coagulation times of carotid blood, shed immediately before the introduction of the threads, were commencement of clotting 5' 50" and completion of that process 7' 0". A mass ligature was placed round the heart, which was excised while beating. Examination showed large clots surrounding the threads and some fluid blood. The latter on shedding on glass commenced to clot in 1' 0" and was completely clotted in 1' 20".

The preceding experiment indicates a sharp distinction between the residual fluid blood after the formation of mechanically produced thrombi and the blood remaining fluid after the formation of thrombi by the slow intravascular injection of tissue extract. In the former case the residual blood is hypercoagulable, in the latter it exhibits inhibition of clotting, the negative phase of Wooldridge. The hypercoagulable blood when shed closely resembles, in the speed of its clotting, blood shed after the intravascular injection of thrombin, while blood in the negative phase behaves like blood to which an anticoagulant has been added.

The production of a thrombus by a thread is intelligible as an example of the processes whereby a thread hastens the coagulation of shed blood. The electropositive ions in the fluid adjacent to the thread (the latter when wetted being electronegative) may well be regarded as disturbants in the first instance of the protective colloid and later of the fibrinogen-prothrombin complex. The fact that a considerable amount of coagulable blood was found associated with the thrombus indicates that any thrombin formed had no coagulant action on that portion of the blood whose surface conditions had not been disturbed. This may be attributed to the presence of undisturbed protective colloid, preventing the action of thrombin on the fibrinogen of the blood. In like manner may be explained the fact that the localised clot formed when an artery is ligatured does not lead to general thrombosis.

(7) *The action of organic intravascular coagulants.* Tissue extracts, some snake venoms and certain synthesised substances [Pickering, 1896], when rapidly injected into the circulation, produce intravascular clotting. This is intelligible on the view that the hydrophile qualities of these substances alter the surface conditions of the colloidal complexes of the plasma. Change in the distribution of electrolytes may also play a part.

(8) *The addition of a small quantity of water.* This hastens coagulation

in vitro and well may be a disturbant of any protective colloid present. It is noteworthy that Howell [1912] found that the addition of a small quantity of water to the shed blood of the terrapin induced clotting, but that addition of isotonic saline had no such effect.

(9) *The action of neutral salts on shed blood.* Relatively stable unions of these salts with either the protective colloid or the fibrinogen-prothrombin complex or both would account for the inhibition of clotting obtained. This view is concordant with the observation of Wooldridge [1893] that thrombin is absent from salted plasmas.

(10) *The action of oxalates and citrates*¹. This is probably dual in nature, a union of the salts with the protective colloid and/or with the fibrinogen-prothrombin complex, and also the removal of calcium, or, in the case of citrates, change in the ionisation of the calcium present. The former may stabilise the protective colloid, the latter may modify the subsequent activation of prothrombin. In the case of citrated blood or plasma changes in dispersion may also play a part.

Exp. 6 shows that the intravascular administration of sodium citrate disturbs the colloidal complexes of the plasma, for the fibrinogen of intraventricular citrated blood coagulates at the same temperature as does the fibrinogen of the laboratory.

The action of citrates *in vitro* is progressive. Freshly citrated blood is coagulated by addition of a non-retracted clot and by tissue extracts, but blood 18 to 23 hours after citration is not clotted by these agencies [Folley, 1918]. These facts are intelligible as due to the gradual adsorption of citrate by the protective colloid of the plasma, yielding, after prolonged action, a more stable protective complex than is at first formed. The fact that blood citrated for 18 to 23 hours can be clotted by the addition of small amounts of sodium chloride (Folley) is significant in connection with the clotting of "peptone" plasma by sodium chloride and in relation to the antagonistic action of certain concentrations of electrolytes to protection against haemolysis.

(11) *The inhibition of clotting by "peptone," nucleic acids and hirudin.* The anticoagulant action of "peptone" and of nucleic acids has been explained as due to excessive secretion, by the liver, of antithrombin or of antithrombogenic [Nolf, 1910]. But Pickering and Hewitt [1922, 1] have shown that inhibition of clotting occurs after the rapid intravascular injection of "peptone" into cats deprived of hepatic activity, and unpublished work by the same authors indicates that nucleic acids (freed from proteoses) exhibit a like effect under like conditions. It has also been shown that the addition of "peptone" to blood or plasma *in vitro* inhibits clotting, provided adequate precautions have been taken to preserve the surface conditions of the latter fluids [Pickering and Hewitt, 1922, 1].

On the view of coagulation enunciated, the anticoagulant action of "peptone" and of nucleic acids may be ascribed to the union of these sub-

¹ Work is in progress in this laboratory on citrates and fluorides.

stances with the protective colloid of the plasma, yielding a more stable protective complex than that existing in circulating blood. Several of the generally recognised reactions of peptonised blood point to this conclusion. It is clotted by dilution with water, by the passage of carbon dioxide and by filtration through a clay cell. Each of these agencies, as Wooldridge [1893] pointed out, tends to dissolution. The disruption of a relatively stable union of a protective colloid and "peptone," under the conditions of shed blood, may be expected to cause clotting. "Peptonised" blood ultimately clots, when exposed to the air in glass vessels. This is intelligible as the gradual resolution, under the stress of disturbed surface conditions, of the union of the "peptone" with the protective colloid of the plasma. The addition of tissue extract rapidly coagulates "peptonised" blood. This may be attributed to lysis by the hydrophile material of the tissue detritus. On the other hand, thrombin fails to clot "peptonised" blood [Wooldridge, 1893]. On the view advocated, the difference between "peptonised" and normal shed blood is that in the former the protective colloid is stabilised by union with "peptone," in the latter it has undergone surface change. Thrombin only acts as a coagulant after surface change has disunited the union of protective colloid and the fibrinogen-prothrombin complex of the plasma. In "peptonised" blood this union is preserved and thrombin has consequently no action.

Hirudin exhibits its anticoagulant action after injection *in vivo* and admixture *in vitro* and its union with the protective colloid of the plasma is more stable and less toxic than are the unions of plasma with "peptone" or nucleic acids. Its action and its neutralisation by tissue extracts are explicable on the views stated. At a later stage in coagulation, hirudin also apparently combines with thrombin [Vines, 1921, 2]. The fact that "peptone" has no anticoagulant action on blood shed in the absence of precautions to preserve its surface conditions points to the union of the "peptone" with the unchanged protective colloid of the plasma. Hirudin, however, may unite with the colloids of the plasma, after disturbance of their surface conditions. Nucleic acids occupy an intermediate position, for they exhibit anticoagulant action after intravascular injection, after addition to blood shed on paraffined surfaces and also when blood is shed on glass. The degree of anticoagulant action is greatest when nucleic acids are injected *in vivo*, least when added to blood shed on glass, the action on blood shed on paraffined surfaces occupying an intermediate position.

(12) *The negative phase of coagulation.* No satisfactory explanation is afforded by the thrombin theories. A negative phase in the coagulation of bird's blood *in vitro* has been produced by Pickering and Hewitt [1921] by the slow addition of tissue extract, drop by drop, to that fluid. In the paper recording this fact attention was directed to the negative phase or tolerance exhibited in the precipitation of colloids by the slow addition of electrolytes, in precipitation of gelatin by the slow addition of alcohol, in the fractional neutralisation of certain toxins by antibodies (known as the Dansyz [1902]

reaction), and in the fractional neutralisation of the toxic qualities of arsenious acid by ferric hydroxide. It was submitted that these phenomena are of one class and are explicable in the same manner as physico-chemical processes.

On the view that a protective colloid is associated with the fibrinogen-prothrombin complex of the plasma, the *rapid* addition of tissue extract *in vivo* or *in vitro*, possibly by creating inequality of electric charge, tends to dissolution of the protective colloid and to coagulation; the *slow* addition of the same substance tends to uniformity of charge and to the formation of unions with the protective colloid, which unions (of protective colloid and tissue extract) exhibit physical resistance to further disturbance by the tissue extract.

In this connection, the reversed or negative action of "peptone" on the coagulability of blood demands notice. "Peptone" when introduced into the circulation slowly, drop by drop, not only does not delay clotting but produces partial or complete immunity against the anticoagulant action of subsequent injections of "peptone." This occurs both in intact animals and in those deprived of hepatic activity. This immunity reaction is intelligible on the view that the slow introduction of the "peptone" promotes unions with protective colloid of the plasma, which unions exhibit physical resistance against further unions with "peptone."

(13) *The action of silica sols.* These afford a marked contrast to the action of tissue extracts. Gye and Purdy [1922] found that lethal intravenous injections produced extensive thrombosis, that fractional injections induced hypercoagulability and that their addition to shed blood did not alter the speed of clotting. If we accept the conclusion of these observers that the lethal injections damaged the vascular wall, then the thrombosis is explicable. For damage to the vascular wall alters those surface conditions, which are essential to the preservation of the stability of the protective colloid of the plasma. The absence of a negative phase and the lack of coagulant action *in vitro* indicate that sols of silica neither unite with nor directly disintegrate either the protective colloid or the fibrinogen-prothrombin complex.

(14) *The influence of temperature.* That cooling retards clotting and heating hastens that process are generally accepted, but no unanimity exists among observers as to the variations in the speed of spontaneous clotting arising from increasing temperatures. This may be accounted for by the wide divergences in the methods adopted to determine coagulation time, and by the varying degrees of contact of the blood with damaged tissues. In the experiments to be described attempts were made to eliminate or minimise this.

Exp. 8. Blood was shed from the carotid of a pithed cat through an evenly paraffined cannula into wide mouthed glass vessels each of the same calibre. The vessels were submerged above the level of the blood in them in water-baths, raised to the desired temperatures. Evaporation was minimised by covering the vessels with moist filter papers. As it is known that hypercoagulability may result from successive haemorrhages, observations at any one temperature were repeated at different stages of the bleeding.

The graph (Fig. 1) shows the speeds of clotting corresponding to temperatures ranging from 33–57°. The points on the upper line of the graph represent the times of completion of coagulation, those on the lower line show the times of commencement of that process.

Fig. 1.

Exp. 9. Human blood was obtained by bleeding from serial punctures in the fingers, after cleaning with chloroform and ether and coating the skin with paraffin wax. The paraffined surface was cleansed after each bleeding. The points in the graph (Fig. 2) have the same significance as in Fig. 1. In this experiment contact with damaged tissue is not eliminated, but contact with clotted blood and foreign material is minimised.

Fig. 2.

Irregular results were obtained in other experiments in which the same puncture was repeatedly used, when bleeding was not on paraffined surfaces and when pressure was exerted to squeeze out the blood.

The following is indicated by Fig. 1: (a) In the absence of contact with cut tissues and at temperatures ranging from 33 to 42°, the speed of spontaneous coagulation of the blood of the cat shed on glass increases regularly

with rises of temperature. (b) From 42 to 45° increase of temperature increases the speed of clotting, but the acceleration is not so marked as with increases at lower ranges of temperature. (c) At 47° marked slowing occurs in the commencement of coagulation and the end point of clotting is not well marked. The type of clot underwent profound change on heating to 47–48°. At lower and higher temperatures (below 56°) clotting was completed by the formation of a continuous gel, so that the vessel could be inverted without spilling blood. At 47–48° a succession of small clots was formed and the phase of a continuous gel was absent. The small clots contracted and progressive lysis of these clots was observed. It is significant that these changes in coagulability and the appearance of lysis of clots occur at a temperature which corresponds with that at which death and the onset of heat rigor is manifested in mammalian muscle [Brodie and Richardson, 1899]. In this connection, the views of Nolf [1909] on the supposed metabolic importance of the lysis of clots and the assumed secretion of antifibrinolysin by the liver may be recalled.

The second graph (Fig. 2) illustrates the retardation of the speed of clotting at 46° obtained with human blood which had passed through punctured tissue. With this blood the end point of coagulation was marked by the formation of a continuous gel.

These results are concordant with the view that physical or chemical changes or both occur either in the protective colloid or in the fibrinogen-prothrombin complex at the time of the death of the blood. Whether the lysis of the complexes of plasma is due to death by heat future experiment must determine. From a number of preliminary experiments on heating pithed animals to 47–49° it is evident that coagulability is not only modified but that intravascular clots may be produced. It is proposed to return to this subject later.

SUMMARY.

(1) Blood in the freshly excised heart, with its cavities unexposed to air, can be heated for 15 to 20 minutes to 56–60° without the destruction of fibrinogen. Such blood coagulates when shed, is precipitated by one-fifth saturation with ammonium sulphate and by half saturation with sodium chloride.

(2) Blood in a dead heart when heated under like conditions has its fibrinogen destroyed. Blood in a moribund heart has its fibrinogen partially destroyed.

(3) If intraventricular blood is citrated and recalcified *in vivo*, its fibrinogen is completely destroyed on heating, without exposure to the air, to 56–56.5° for 8½ minutes.

(4) A theory of the fluidity and the coagulation of the blood is enunciated. The observations recorded and the generally recognised phenomena of fluidity and clotting are correlated by this hypothesis.

(5) Attention is directed to the similarity of phenomena in protection against the spontaneous coagulation of blood and those of protection against haemolysis.

(6) The fluid blood adjacent to mechanically produced thrombi is hypercoagulable. Attention is directed to the contrast in the condition of this blood with that of the fluid blood associated with the thrombi formed in the negative phase of clotting. Reasons are given why the thrombus produced by ligaturing a living artery does not produce general thrombosis.

(7) Observations are recorded on the influence of heat on the coagulability of the shed blood of the cat, after precautions had been taken to prevent contamination with tissue detritus. The influence of heat on the coagulability of human blood after contact with the surfaces of a puncture, is also described. Both these bloods exhibit marked changes in coagulability on heating to 46–48°, *i.e.* at the temperature of death and the onset of heat rigor in muscle.

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