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Blood Coagulation and Lipid Metabolism

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BLOOD coagulation is the result of a complex series of reactions. Normally, once initiated, the whole process proceeds at an accelerating rate until completed. By the use of appropriate reagents, however, the process can be stopped rather easily at certain definite points, and it is common practice to discuss blood coagulation as though it takes place in several stages.

FIRST STAGE

The formation of thromboplastin can be considered the first stage. While there is no unanimity of opinion regarding the order in which the various plasma factors react to form thromboplastin, there is general agreement as to the importance of these factors. At least three bleeding conditions have been observed in which the common defect is in the formation of plasma thromboplastin. Each appears to be due to the lack of (or inactivation of) specific proteins present only in minute amounts. Since any hypothesis of blood coagulation must take these facts into account, a suggested scheme for the events in the first stage of coagulation is shown in Chart I antihemophilic factor (AHF), plasma thromboplastin component (PTC) and possibly other proteins (Stuart Factor, Hageman Factor, PTA) react with a lipid material from platelets to form thromboplastic activity. The reaction can be initiated by contact with a "wettable surface" or inflamed or injured area. This suggests that lysis of platelets may be the initiating factor. MacFarlane[1] gives evidence which suggests that PTC and AHF join with calcium ions to form an intermediate product which then combines with platelets.

AHF is present in normal fresh plasma but is absent or inactive in normal serum. It is not readily adsorbed on the usual prothrombin-adsorbing reagents. PTC is present in both normal plasma and serum and is readily adsorbed on prothrombin-adsorbing reagents. According to the work of Tocantins and Carroll[2] a thromboplastic inhibitor exists in plasma. They have isolated this material from both normal and hemophilic plasma. It appears to inhibit the development of thromboplastin as well as inactive thromboplastin after the latter has been formed. Thromboplastin can be obtained from tissues but this material appears to need factors from plasma and serum to make it fully active. Heparin inhibits the formation of thromboplastin[3] and the action of thromboplastin itself.

SECOND STAGE

The conversion of prothrombin to thrombin normally depends on the presence of thromboplastin and thus can be considered the second stage. The work of several laboratories has

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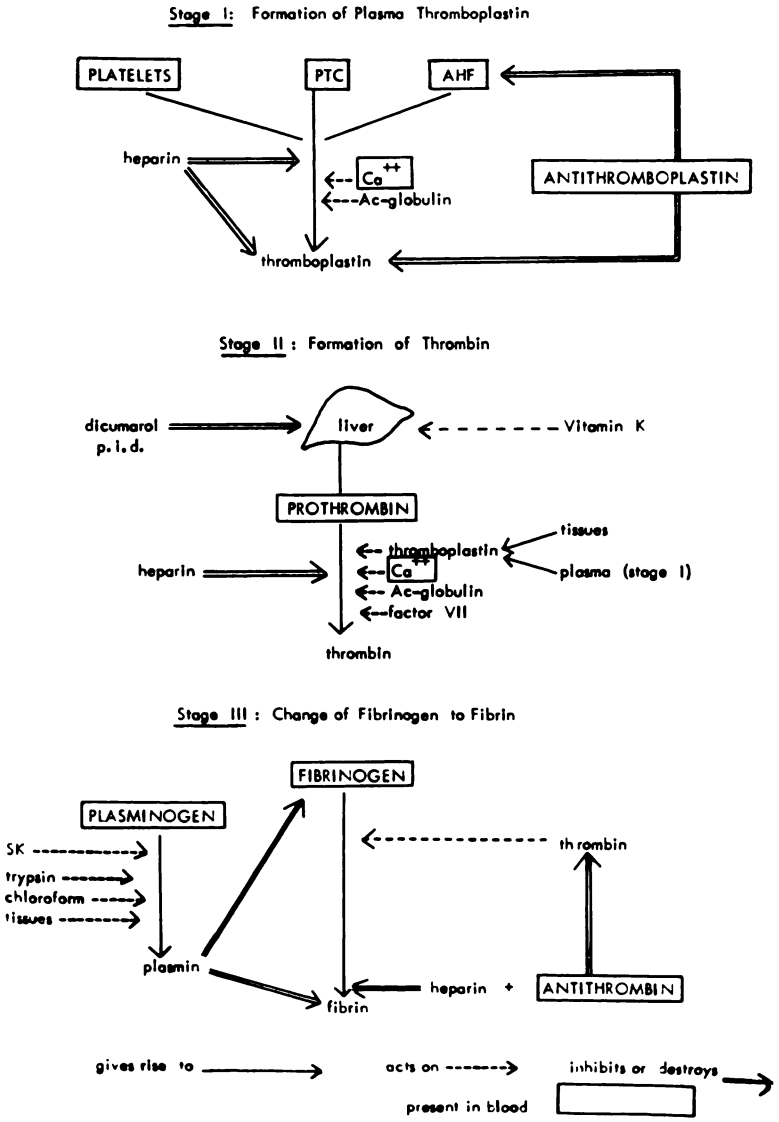

Chart 1. Schematic representation of the various reaction involved in the coagulation of blood. SK = streptokinase.

indicated the existence of certain plasma and serum factors which accelerate the conversion of prothrombin to thrombin. The plasma factor was discovered independently in widely separated laboratories and has been referred to by a variety of names. It appears to be associated with the globulin fraction of plasma and is normally present in only trace amounts.

Ware, Fahey and Seegers[4] found that the activity of this plasma AC-globulin was greatly increased during coagulation and that plasma AC-globulin fractions were made more potent by small amounts of thrombin. It is now generally accepted that serum contains material not normally active in plasma. The active material is readily adsorbed on prothrombin adsorbing reagents and is more stable than the plasma fraction. The term "factor VII" is most commonly used to designate the serum-active factor (SPCA, proconvertin, autoprothrombin are other names for this factor).

Alkjaersig, Johnson and Seegers[5] have shown that a derivative of purified prothrombin acts as an accelerator and have suggested that the

serum factor is derived from prothrombin during the coagulation process. In support of this hypothesis, it may be pointed out that during the administration of Dicumarol or Tromexan both prothrombin and factor VII levels are decreased, with factor VII often being the first to show change.

THIRD STAGE

Once thrombin is formed it acts enzymatically to convert soluble fibrinogen to insoluble fibrin. This reaction can be considered the third stage. Associated with events in the third stage of coagulation is the activation of plasminogen to plasmin and the subsequent lysis of fibrinogen and fibrin. This area has become so specialized and complex that it is often considered as a separate field, and will not be discussed in detail at the present time. One point, however, is relevant to the present discussion. It has been postulated that plasmin may function normally to prevent excess deposition of fibrin on the endothelium of the vessels. Greig and Runde[6] reported that plasmin is inhibited in the presence of fat and suggest that this inhibition might be a contributing factor to the development of atherosclerosis.

Plasma and serum contain a substance known as antithrombin, the physiological importance of which has not yet been determined. Attempts to correlate decrease in antithrombin with tendency toward thrombosis have been unsuccessful. Antithrombin was originally believed to be an albumin but recent work indicates that it is associated with the alpha globulins[7] Fig. 1.

The development of a synthetic substrate for thrombin[8] P-toluenesulfonyl-arginine-methyl ester (TAMe) has made it easier to distinguish between inhibition of thrombin and destruction of thrombin. Heparin-cofactor and antithrombin activities appear to be properties either of the same substance or of two closely related substances. It has so far been impossible to separate the two activities. Whenever one activity is concentrated the other is concentrated; destruction of one is associated with destruction of the other. As shown in Figure 2 heparin added to blood

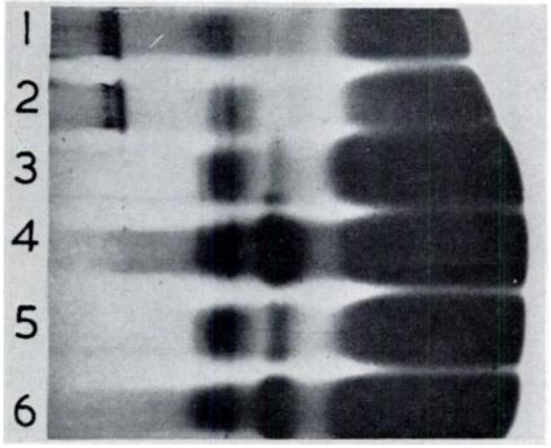

FIG. 1. Electrophoretic patterns on starch; 1, defibrinated plasma; 2, after adsorption with aluminum hydroxide; 3 and 5, fractions from citrate eluates; 4 and 6, fractions from phosphate eluates. Sample 2 had no antithrombin potency. Samples 4 and 6 had double the activity of samples 3 and 5.

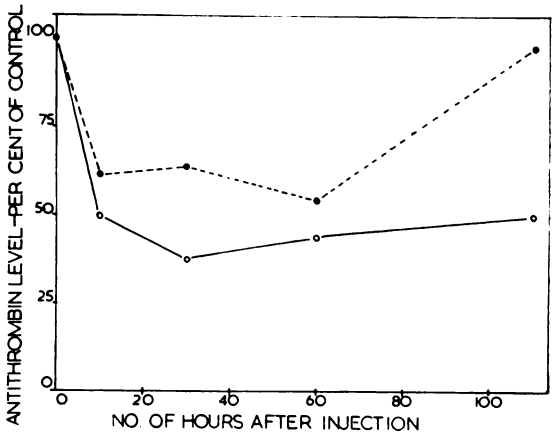

FIG. 2. The decrease in antithrombin level of plasma following an intravenous injection of heparin. ○—○ 500 units per kilo; ●—● 200 units per kilo.

in vitro, or injected intravenously, decreases the antithrombin titer. This may seem paradoxical. However, while the capacity of heparinized blood to destroy thrombin is reduced from normal the total thrombin *inhibitory* effect is greatly increased. Heparin achieves its inhibitory action by interfering with the thrombin-fibrinogen reaction, not by destroying thrombin.

ROLE OF FATS

There is accumulating evidence that the consumption of excessive amounts of fat or

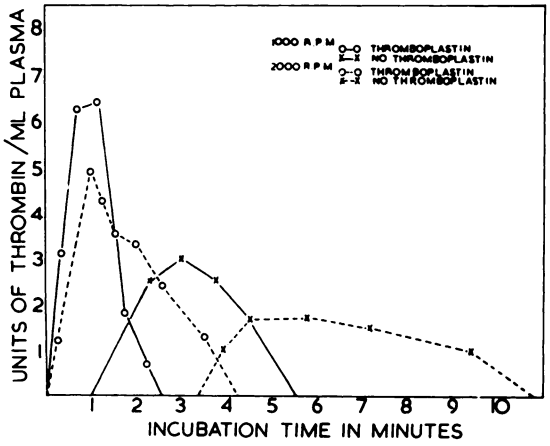

FIG. 3. The effect of rate of centrifugation and addition of thromboplastin on the thrombin generation of recalcified citrated dog plasma.

impairment in fat metabolism are factors in the development of atherosclerosis and thrombosis. The assumption, valid or not, that there is a relationship between blood clotting and thrombosis has led to an increasing number of studies on the role of fat in blood coagulation.

The results of tests carried out on whole blood have been controversial. Waldren, Beidelman and Duncan[9] found a shortening of the clotting time in siliconed tubes following a fat meal. Manning and Walford,[10] in similar experiments, failed to find any significant change. Merskey and Nossel[11] found that their results varied depending on the type of meal ingested.

More uniform results have been obtained when the tests have been carried out on plasma. Robinson *et al.*[12] found there was an increase in the concentration of free fatty acids in plasma following a fat meal. This increase is within the range where Poole[13] and Pilkington[14] demonstrated an *in vitro* effect with a variety of added fatty acids. Thus, some of the increase in coagulability may be the result of an increase in fatty acid concentration and the variability in results may be a consequence of the type of fatty acid adsorbed.

More emphasis however has been placed on the role of phospholipids in blood coagulation. O'Brien[15] observed that when the platelets are able to act there is no detectable difference in the clotting time of lipemic blood after a meal of fat compared with that from the same patient fasted. When platelets are removed or unable to act, lipemic blood clots more rapidly than the equivalent fasting sample. Thus an extremely important point to consider is the method of preparing the plasma. Pitney and Dacie[16] showed that the amount of thrombin generated when plasma is recalcified is influenced by the degree of centrifugation. This has been confirmed in our laboratory. Figure 3 illustrates the effect of variation in rate of centrifugation on the thrombin generation of recalcified citrated plasma.

O'Brien[15] introduced what is known as the "Stypven®" time. He showed that the addition of Russell viper venom to plasma caused a greater decrease in the clotting time of citrated plasma four hours after a fat meal than after fasting. This has been confirmed by others.[17] As early as 1941 Macfarlane, Treven and Atwood[18] showed that crude lecithin, chylomicra or milk-fat augmented the thromboplastic activity of Russell viper venom. Merskey and Nossel[11] showed that the decrease in "Stypven" time was independent of the type of fat ingested. Thus the "Stypven" time may merely reflect the degree of lipemia and be unrelated to any real change in the coagulability of blood.

Poole[19] found that when plasma was freed from chylomicra by high speed centrifugation its clotting time was increased. The clotting time of such plasma could be returned to normal by the addition of a washed suspension of chylomicra. With Robinson[20] Poole showed that the active fraction of chylomicra was phosphatidyl ethanolamine, and later[21] that the clotting time of high-spun plasma could be greatly decreased by adding phosphatidyl ethanolamine to it. Since the lack of platelets is the most noticeable characteristic of high-spun plasma it was logical to suppose that the thromboplastic activity of platelets might be due to their phosphatidyl ethanolamine content. Bell and Alton[22] used a chloroform extract of acetone dried brain and O'Brien[23] a phosphatide fraction from eggs as substitute for platelets in the thromboplastin generation tests. Thus it has been fairly generally assumed that the activity

of platelets is due to their content of phosphatidyl ethanolamine. Recently however Spaet and Marcus[24] and Troup and Reed[25] have fractionated lipid extracts of platelets and red cells and claim that phosphatidyl serine is much more active in the formation of thromboplastin than phosphatidyl ethanolamine. Thus, there is still some question regarding the exact identity of the active platelet lipid. The answer awaits the development of more accurate methods of identification.

ANTICOAGULATION

While we are concerned with the coagulant properties of lipids we should not completely ignore their anticoagulant properties. During the past ten years Tocantins and his colleagues have carried out an extensive study on a lipid anticoagulant fraction from brain. They have succeeded in obtaining a highly purified substance which, on the basis of chemical tests, has been identified as phosphatidyl serine.[26] The extract is active both *in vitro* and *in vivo*. The anticoagulant activity of their phosphatidyl serine from brain appears to be in direct contrast to the coagulant activity of the phosphatidyl serine fraction prepared from platelets by others.[24] Recently however Barkhan and associates[27] demonstrated that phosphatidyl serine from brain can act *in vitro* either as an accelerator or as an inhibitor of thromboplastin formation depending on experimental conditions. Thromboplastin formation was inhibited when the phosphatidyl serine in aqueous solution was placed in the reaction mixture. When the phosphatidyl serine was first suspended in plasma which had been decalcified by ethylene diamine tetraacetic acid (EDTA) and adsorbed by aluminum hydroxide, and then added to the reaction mixture thromboplastic formation was enhanced. This potentiating effect was not found when citrated or oxalated plasma was used. Thus the same substance may exhibit either coagulant or anticoagulant characteristics *in vitro*, depending on the concentration of the substance and the type of test used. Further work may reveal that the clotting activities of the various phosphatides depend upon the type, and degree of saturation of the fatty acids incorporated in the molecule.

CONCLUSIONS

It is reasonable to conclude that lipids, especially the phosphatides, play an important part in the reactions which result in the formation of a blood clot. Not all lipids are coagulants. Changes in the level of fatty acids and phosphatides result in changes in a number of *in vitro* coagulation tests. However, variations in the same coagulation test can be demonstrated simply by varying the rate of centrifugation, the type of decalcifying agents, or even the order in which the reagents are added. Thus at present we have no clear understanding how changes in static blood are related to the development of an intravascular clot. It is unlikely that any changes in the coagulability of blood brought about by variations in the lipid level are important in the presence of a healthy endothelium or a normal blood flow rate. Such changes, however, may be significant when superimposed on degenerative changes in the vessel wall or when circulation is impaired. The clinical importance of lipids on blood clotting can only be ascertained when there is a better knowledge of the relationship between blood clotting and vascular changes.

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