The Activation of the Contact Phase of Coagulation by Physiologic Surfaces in Plasma: The Effect of Large Negatively Charged Liposomal Vesicles

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The endogenous, negatively charged surface that induces activation of the contact coagulation factors was investigated in plasmas taken from women in late pregnancy and control subjects of child-bearing age. The plasmas from the two groups of subjects were incubated at 4°C for 24 hours either in plastic or in glass tubes and the factor VII coagulant activity (VIIc) was assayed in the treated plasmas. The activation of factor VII under these conditions involves the generation of enzymes derived from factor XII (XIIa). The contact surface is rate-limiting for the activation of factor VII in the plasmas in both groups of subjects and can be supplemented by large multilamellar liposomal vesicles carrying the appropriate density of negative charge. The size of these vesicles is within the range of sizes of the large lipoprotein particles (chylomicrons, very low and intermediate-density lipoproteins). The relationship between the density of negative charge on the liposomal vesicles and VIIc was similar in the late pregnancy and the control plasmas incubated in plastic tubes. At a saturating density of negative charge the observed relative VIIc was similar in both sets of plasmas. The incubation of late pregnancy or control plasma in plastic tubes in the presence of sodium stearate caused VIIc to increase with increasing concentration of the added fatty acid. These results suggest that large lipoprotein particles carrying the appropriate free fatty acid at a sufficient density of negative charge could provide the contact surface that induces the generation of factor XIIa and the subsequent activation of factor VII. Moreover, plasmas from women in late pregnancy have a higher concentration of potential surface and a higher density of negative charge than the plasmas from nonpregnant women.

The Northwick Park Heart Study,1,2 has demonstrated a strong association between factor VII coagulant activity (VIIc), measured at entrance to the study, and the incidence of ischemic heart disease within the subsequent 5 years. The same study has also shown a positive correlation between VIIc and both the triglyceride and the cholesterol concentrations in plasma. Further epidemiologic observations also suggest that high VIIc may be of causal significance in ischemic heart disease. For instance, increasing age,3 obesity,4 diabetes,5 and the use of oral contraceptives6 are all associated with an increased risk of coronary artery disease as well as with high levels of VIIc. It is also clear that all of these conditions are associated with increased plasma concentrations of cholesterol and triglycerides. To understand the relation between hyperlipidemia and factor VIIc we have previously7 induced hypercholesterolemia in the rabbit and examined its relation to the flux within the coagulation pathway. In rabbits fed a 1% cholesterol-supplemented diet, the increase in plasma cholesterol concentration was associated with an increase in VIIc7 and an increase in the rate of thrombin generation in vivo.8 The major part of the excess cholesterol in the hypercholesterolemic rabbit is associated with the large lipoprotein particles such as chylomicrons, very low-density lipoprotein (VLDL) and the intermediate-density lipoprotein (IDL) fractions. The increase in VIIc in the hypercholesterolemic rabbit can be attributed to the increased reactivity of factor VII arising from an increase in the ratio of the two-chain, more reactive, form of VII (αVIIa) to the single-chain form.9

Late pregnancy is associated with increased plasma concentration of triglycerides and of cholesterol, caused by an increase in chylomicrons and the VLDL and IDL fractions.10 Late pregnancy is also associated with increased VIIc11 that has been attributed to an increased reactivity of factor VII.12

The plasma proteins factor XII (Hageman factor [HF]), factor XI, prekallikrein, and high molecular weight kininogen constitute the contact activation system of blood coagulation.13 The contact system can be initiated in vitro when citrated plasma is incubated in plastic tubes in the presence of certain negatively charged surfaces such as kaolin or glass,14 dextran sulfate,15 ellagic acid-metal complex,16 or sulfatide vesicles.17 HFα (the two-chain form that is enzymatically active) appears to play the primary activating role through the limited proteolysis of prekallikrein18,19 and the reciprocal activation of factor XII by kallikrein. HFα can activate factor XI20 and this (XIa) activates factor IX, thus initiating the intrinsic coagulation pathway.21 High molecular weight kininogen acts as cofactor in all these reactions.22,23

In plasma, the activation of factor XII by kallikrein24 results in the formation of an 80-Kd enzyme, HFα25 which consists of a 52-Kd heavy chain and a 28-Kd light chain held together by a disulfide bond. The heavy chain contains the surface binding domain,26 and the light chain contains the catalytic domain.27 The heavy chain can be further split to a 40-Kd fragment and finally yields fragments of either 3,000 or 800-d, each of which remains disulfide linked to the 28-Kd light chain of HFα.28 These last fragments (HFα or βXIIa) have only residual coagulant activity, since they have lost the contact surface binding capacity.29 However, HFα can convert factor VII29,30 to a VIIa and this can be seen in vitro as an increased VIIc following the prolonged incubation of plasma at low temperature.31

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Although contact activation is a prerequisite for the in vitro intrinsic blood coagulation, the significance of contact activation during coagulation in vivo has yet to be established, since persons deficient in factor XII, prekallikrein, or high molecular weight kininogen do not commonly exhibit bleeding disorders. Most of the surfaces known to be deficient in factor XII, prekallikrein, and high molecular weight kininogen do not commonly exhibit bleeding disorders. Assuming that there is a physiologic contact surface, its identity and the mechanism of contact activation is unclear. Some studies have suggested that the zymogens factor XII and prekallikrein express a weak proteolytic activity in the presence of a suitable surface. The reaction rates are greatly enhanced by the reciprocal proteolytic activation of factor XII and prekallikrein. In contrast, other studies have suggested that in the presence of a negative charged surface, factor XII undergoes autoactivation and that in the presence of high molecular weight kininogen acts upon its substrates, factor XI and prekallikrein.

In the present paper we attempt to identify the nature of the physiologic surface that activates the contact coagulation factors. For this, we have compared the reactivity of factor VII in plasmas from women in late pregnancy, following the prolonged incubation in the cold either in glass or plastic tubes, and compared these with the reactivity of factor VII in plasmas from nonpregnant women of child-bearing age after identical incubations. Moreover, incubation mixtures, in which the negatively charged surface is rate-limiting for the activation of factor VII, are supplemented with liposomal vesicles of the appropriate size and negative charge. Also, the effect of stearate in supplementing the negative charge in these incubation mixtures has been investigated.

MATERIALS AND METHODS

Phosphatidylcholine (PC) from frozen egg yolk (type XIII-E), phosphatidylserine (PS) from bovine brain, cholesterol (CH), and sodium stearate were from Sigma Chemical Co (Poole, Dorset, UK). No impurities could be detected in these lipids by thin layer chromatography using standard solvent systems. Dihexadecylphosphate (DHP) was from Aldrich (Gillingham, Dorset, UK). Rabbit brain thromboplastin was obtained from Diagen (Thame, Oxon, UK).

Blood samples. A 10-mL sample of blood was taken between 10:00 and 11:00 AM from pregnant volunteers attending Northwick Park Hospital antenatal clinic. These women were in the last 6 weeks of normal gestation. The same volume of blood was taken at the same time of day from control subjects who were healthy volunteers of child-bearing age not on oral contraceptives. The blood was taken by a vacutainer system into 10% (vol/vol) of 3.8% trisodium citrate. The citrated blood was centrifuged at 3,000 g for 15 minutes at room temperature. The plasma was removed with a plastic Pasteur pipette and used without delay.

Preparation of liposomes. PC:CH, PC:DHP:CH, or PC:PS:CH liposomes were prepared either by using the procedure described by Batzi and Korsm for preparation of single-bilayer vesicles of relatively narrow range of small size, or by the procedure of Bangham et al for preparation of multilamellar vesicles of relatively wide range of large size. For the single-bilayer small vesicles, the dilute suspension in 0.15 mol/L NaCl was concentrated and washed free of ethanol by ultrafiltration on an Amicon (Woking, Surrey, UK) ultrafiltration device using an XM-100 A membrane with rapid stirring under N2 pressure (101 KPa). The average diameter of vesicles prepared by this technique is 25 nm. For the preparation of large size multilamellar liposomes, the lipid mixture, dissolved in chloroform, was deposited on the sides of a glass conical tube by evaporating the solvent under a stream of N2 at 37°C. The dried lipid was then hydrated with 50 mmol/L-imidazole/HCl containing 100 mmol/L NaCl buffer, pH 7.4 (glyoxaline buffer) and the mixture was dispersed by vigorous shaking with a vortex mixer for 20 seconds and allowed to stand for 30 seconds. This process was repeated ten times. The preparation was then placed on a reciprocal shaker (two cycles/second, 5 cm amplitude), for 30 minutes at 37°C. The lipid concentration was 60 µmol/mL of buffer and this was diluted appropriately on addition to plasma. The large multilamellar vesicles had an average diameter of 1,320 nm as determined by negative stain and 1,610 nm as determined by freeze-fracture techniques. All lipid ratios or percentages in the liposomes are expressed on a molar basis. The sodium stearate (1 mmol/L) was suspended with albumin (60 µmol/L) in distilled H2O and the suspension was added to the plasma in the final concentrations indicated.

Isolation of various plasma lipoproteins. Late pregnancy plasma was used to isolate the various lipoprotein fractions. Chylomicrons were collected from the surface layer after centrifugation at 22,000 g for 30 minutes. This fraction was centrifuged at 205,000 g for 24 hours to obtain the chylomicon fraction in the floating layer free of the contaminating lipoproteins (d < 1.006). VLDL (d < 1.006), IDL (1.006 < d < 1.019), LDL (1.019 < d < 1.063) and HDL (1.063 < d < 1.21) fractions were isolated from the plasma by sequential preparative ultracentrifugation in a Beckman L8-70M ultracentrifuge using a T770.1 rotor (Beckman Instruments, High Wycombe, Bucks, UK). Portions of the various lipoprotein fractions and the various lipoposomal vesicles were assayed for total cholesterol by an enzymic method with reagents and standards from Sigma and Boehringer (Mannheim, FRG), respectively.

Preparation of proteins. Sweet corn inhibitor was prepared from freshly picked sweet corn cobs by the method of Mahoney et al. The purified inhibitor was a single-chain protein with a molecular weight (mol wt) of 12,000 on sodium dodecyl sulfate (SDS) gel electrophoresis. Human factor XII was isolated from fresh frozen human plasma. The single-chain factor XII was converted to HF, by digestion at 37°C with trypsin for 15 minutes at an enzyme-substrate ratio of 1:100 (mol/mol), in 50 mmol/L Tris/HCl buffer, pH 8.0, containing 75 mmol/L NaCl. The reaction was terminated by the addition of a twofold excess of soybean trypsin inhibitor and the HF was isolated by chromatography on DEAE-Sepharose. The purified protein moved as a single band on SDS gel electrophoresis (mol wt, 30,000). Human C1 inhibitor (C1INH) was prepared as previously described except that DEAE-Sepharose was used instead of DEAE-cellulose. The purified protein moved as a major band on SDS gel electrophoresis (mol wt, 104,000) with two minor contaminants (mol wt, 70,000 and 30,000) that were the cleaved inhibitor.

Assay of VIIc. Bovine plasma depleted of the vitamin K-dependent proteins was prepared by two successive additions of barium sulfate powder (50 mg/mL) to fresh oxalated plasma. Factor VII-deficient plasma was prepared by the addition of human factor II, IX, and X to the barium sulfate-adsorbed plasma. Standard plasma was a pool of normal citrated plasma diluted to a factor VII potency identical to that of standard plasma from Immuno A.G. (Vienna). Duplicate coagulation assays were performed in an H. Amelung KC10 Coagulometer (American Hospital Supply, Didcot, Oxon, UK) at 37°C. To 0.1 mL of a dilution of the plasma sample, 0.1 mL of factor VII-deficient plasma and 0.1 mL of a 1:32 dilution of rabbit brain thromboplastin were added and the reaction was then
started by the addition of 0.1 mL of 25 mmol/L CaCl₂. The mean clotting time for the duplicate assays of each dilution were obtained and activity in the test plasma as a percent of that in standard plasma was calculated by means of a microcomputer program. The addition to plasma of either large multilamellar or the small unilamellar liposomes or of the stearate suspension, at the concentrations used in the cold activation mixtures, did not influence the measurement of factor VII activity.

Other assays. C1INH antigen was determined by electroimmunoassay using rabbit anti-human C1INH (Dako Ltd, High Wycombe, Bucks). Factor XII coagulant activity was measured as described previously.

RESULTS

Table 1 shows VIIc in 50 plasma samples from women in late pregnancy and in 18 plasma samples from nonpregnant women of child-bearing age. VIIc is significantly higher in the fresh plasmas from late pregnancy as compared with those values in the plasmas from the control subjects.

VIIc is increased further in late pregnancy plasmas or in the plasmas from nonpregnant control subjects by incubation in glass tubes, at 4°C, for 24 hours (Table 1). The extent of cold activation of late pregnancy plasmas is considerably higher than that of plasmas from nonpregnant control subjects. Incubations of the plasmas from the two groups, in plastic tubes at 4°C for 24 hours, did not increase VIIc in the control plasmas, but in the plasma taken from the pregnant women VIIc increased considerably (Table 1).

The possibility that differences in the extent of activation of factor VII between late pregnancy and control plasmas were due to variations in the concentration of either C1INH or factor XII was considered. In late pregnancy plasmas C1INH was 77% (P < 0.001) and the factor XII coagulant activity was 172% (P < 0.001) of the corresponding values in plasmas from control subjects. However, in experiments involving plasmas from subjects in late pregnancy there was no difference in the rate of cold activation of factor VII (either in glass or plastic) when the C1INH varied (by the addition of purified C1INH) between 77% to 160% of that in plasmas from control subjects. The same plasmas were diluted 1:1 with either buffer or factor XII-deficient plasma and were incubated in glass. The VIIc was similar in both incubation mixtures.

The sequence of biochemical steps involved in the increase of VIIc during cold activation. Nine late pregnancy plasmas were diluted with an equal volume of glyoxaline buffer and incubated in plastic tubes, at 4°C, for 24 hours in the presence and in the absence of human HF. VIIc was assayed in the treated plasmas and is plotted in Fig 1 against the concentration of HF present in the incubation mixture. The activity increased with the concentration of HF. The difference in VIIc observed in the absence and in the presence of 125 mmol/L of HF was highly significant (P < 0.001), as is the difference (P < 0.001) between the VIIc observed in the presence of 12.5 and 125 mmol/L HF (Fig 1). To confirm that the increase in VIIc arises from the conversion of factor VII to αVIIa catalyzed by the generation of XIIa (enzymes derived from factor XII), nine late pregnancy plasma samples, diluted as above, were incubated in glass tubes in the absence and in the presence of increasing concentrations of sweet corn inhibitor. The increase in VIIc caused by the cold activation was progressively decreased by increasing the concentration of the inhibitor (Fig 2).

The contact surface in plasmas from late pregnancy. The fact that the late pregnancy plasmas "cold activate" both in plastic and glass tubes (Table 1) suggests that these plasmas contain a negatively charged surface that can activate the contact coagulation factors and generate factor XIIa. To explore this possibility further, various dilutions of the same late pregnancy plasmas were cold activated either in plastic or in glass. The VIIc observed at each dilution was compared with the corresponding value for the nondiluted plasmas cold activated under the same conditions (Fig 3). It can be seen that in the dilutions of plasma cold activated in glass, the VIIc observed corresponded to the dilution of the plasma, whereas in the samples that were cold activated in plastic tubes, the VIIc decreased by a factor that was not commensurate with the dilution. This would suggest that on dilution the contact surface becomes rate-limiting. To test this hypothesis, liposomal vesicles made of negatively

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<th>Table 1. VIIc* in Fresh and in Cold-Activated Plasma from Women in Late Pregnancy or Nonpregnant Control Subjects</th>
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<td>Plasma</td>
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<td>Late pregnancy (N = 50)</td>
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<td>Nonpregnant control subjects (N = 18)</td>
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NOTE. VIIc is expressed as percent of mean activity (SD) in a standard plasma. Since the distribution in some groups was not normal, the range of values is also given.

*The plasmas were incubated for 24 hours at 4°C either in plastic or glass tubes.

Fig 1. Effect of human HF on VIIc in cold activated late pregnancy plasmas. Plasmas from nine women in late pregnancy were diluted 1:1 with buffer and incubated in the absence and in the presence of various concentrations of HF, for 24 hours at 4°C. VIIc was assayed and expressed as percent of the activity in incubations conducted in plastic tubes in the absence of HF. The mean values of the corresponding nine incubations conducted in plastic (O) and that for incubations conducted in glass with no HF (Q) are given and the vertical lines indicate the so.
charged and neutral phospholipids were added to the plasma dilutions to supplement the contact activating surface. When increasing concentrations of small unilamellar (average size, <25 nm) or large multilamellar (average size, >25 nm) prepared from PC/CH (1:0.5) were incubated in plastic tubes with late pregnancy plasma, diluted with equal volume of buffer, at 4°C for 24 hours, no increase in VIIc was seen at any concentration of liposomes. However, when the experiment was repeated with negatively charged vesicles (average size, >25 nm) prepared from PC:DHP:CH (0.85:0.15:0.5) there was an increase in VIIc that depended on the concentration of liposomes (Fig 4). Small unilamellar vesicles prepared also from PC:DHP:CH (0.85:0.15:0.5) failed to increase VIIc above the value observed in the absence of liposomes.

These results suggest that the contact surface present in late pregnancy plasmas is provided by the plasma lipoproteins. However, chylomicrons, VLDL, and IDL isolated from the late pregnancy plasma by floatation techniques failed to produce an increase in VIIc when they were added separately instead of the negatively charged large vesicles.

The effect of negative charge. To investigate the effect of negative charge on the activation of factor VII, the late pregnancy plasma samples were diluted with an equal volume of a vesicle suspension or buffer and incubated in plastic tubes as before. The vesicle suspension contained the same concentration of large vesicles composed of a constant molar ratio of phospholipid to cholesterol (1:0.5) to which increasing amounts of DHP had been added. At a DHP concentration one tenth of the PC, the VIIc after cold activation was lower than that seen in the late pregnancy plasma (diluted 1:1 with buffer) incubated in glass (Fig 5). On increasing the content of DHP in the phospholipid to 20%, the VIIc increased to greater than twice the value seen in the diluted plasma incubated in glass. A further increase in the molar ratio of DHP to PC was accompanied by a smaller increase in VIIc.
ACTIVATION OF THE CONTACT COAGULATION FACTORS

The activation of the contact coagulation factors was studied in plasmas from late pregnancy women and from nonpregnant control subjects. Nine plasmas were incubated in plastic (O) or glass tubes (θ) in the absence or in the presence of a fixed amount (1 mmol/L with respect to cholesterol) of large size multilamellar liposomes. These were made of phosphatidylcholine:cholesterol (1.0:0.5) at constant concentration of cholesterol and with varying molar ratios of phosphatidylcholine to dihexadecylphosphatidylcholine. VIIC is expressed as percent activity in the corresponding incubation conducted in plastic in the absence of liposomal vesicles. Each point is the mean value of nine comparisons with the vertical lines indicating the SD. Figure insert is the same plot except that log VIIC (mean) is plotted. The arrow shows the density of negative charge for half-maximal activation of VII.

When the above experiment was repeated using similarly diluted plasmas from nonpregnant women, a corresponding relationship between negative charge and the extent of factor VII activation was observed (Fig 6). Four mean values are used to determine the regression slope seen in each of the inserts. However, we have also used the values of the individual plasmas and obtained highly significant positive correlations (n = 36, n = 0.950 for the results of the experiment in Fig 5 and n = 44, r = 0.960 for the experiment in Fig 6). From these regressions we calculate densities of negative charge for half maximal activation of 12.4 and 17.5, respectively, for the late pregnancy and the control plasmas. These values are identical to the values determined from the means as shown in Figs 5 and 6. The half-maximal activation of late pregnancy plasmas occurs at a lower density of negative charge than that required to achieve half-maximal activation in the plasmas from control subjects (compare the inserts in Fig 5 and Fig 6).

The incubation in plastic tubes, at 4°C for 24 hours of late pregnancy plasmas, diluted with equal volume of an albumin suspension of stearate (see Methods) results in a concentration-dependent increase in VIIC, which followed saturation kinetics (Fig 7). Plasma from control subjects also showed a stearate concentration-dependent increase in VIIC (Fig 7), but this increase follows a semi-logarithmic relationship like that seen in Figs 5 and 6 for the relation between VIIC and the density of negative charge. In a number of experiments (not shown) in which late pregnancy or control plasmas were incubated in plastic tubes, the VIIC at the maximal concentration of stearate used (0.3 mmol/L) was always lower or
equal to the VIIc in parallel incubations conducted in glass tubes in the absence of the fatty acid. However, in the presence of the optimal concentration of large multilamellar liposomes, with an adequate negative charge density, the VIIc always exceeded the value obtained in similar incubations conducted in glass, in the absence of liposomes.

DISCUSSION

The data presented in this report demonstrate that in plasmas from women in late pregnancy or from control subjects, a negatively charged surface (endogenous plus exogenous) can initiate the contact phase of blood coagulation and generate activated factor XII, which also leads to conversion of single-chain factor VII to the more reactive αVIIa. This is seen as an increase in VIIc after cold activation. Since the within-24-hours time-dependent increase in VIIc in these incubations conducted either in glass or plastic tubes is linear (results not shown), we can assume that the increase in VIIc after cold activation represents the rate of cleavage of factor VII. The likelihood that a form of activated factor XII (HFa or Hf) is the agent responsible for this conversion is supported by the observation that VIIc increases after the addition of HF to the plasma. Conversely, the addition of a specific inhibitor of XIIa derived from sweetcorn abolished the cold activation of factor VII. A similar conclusion has been reached by Ratnoff and Monene.

Plasmas from control subjects incubated in plastic fail to show a significant increase in VIIc whereas plasmas taken from subjects in late pregnancy incubated under the same conditions show considerable increase in VIIc. Moreover, following cold activation in glass tubes, the increase in VIIc is considerably greater in the late pregnancy plasmas than in the plasmas taken from control subjects. This, taken with the finding that the modulation of either factor XII or C1INH had little or no effect on VIIc, suggests that for both kinds of plasma the contact surface (endogenous plus exogenous) is rate-limiting for the activation of factor VII (Figs 3-7). This would appear to be inconsistent with the results of Fig 3 showing that when late pregnancy plasmas are incubated in the presence of a constant exogenous contact surface (incubations in glass), VIIc decreases linearly with the dilution of plasmas. However, since the contribution of the endogenous contact surface diminishes sharply with the dilution of these plasmas (see incubations in plastic), the composite effect of the exogenous plus the endogenous contact surface (incubations in glass) results in rates of activation of factor VII that correspond to the dilution of plasma. In contrast, when various dilutions of plasma from control subjects were incubated in glass, the VIIc observed was higher in all cases \((P > .01)\) than would have been expected from the dilution. When these same plasma samples were incubated in the presence of an additional glass surface (glass beads) the VIIc was significantly raised \((P = .01)\) by the presence of the beads. The results (Figs 5 and 6) showing that at optimal amounts of contact surface, the relative increase in VIIc is similar in both kinds of plasmas are consistent with the idea that the in vitro rate of factor VII activation is limited by the amount of contact surface.

The other principal aim of this report has been to identify the physical nature of the surface in plasma that promotes the activation of factor XII, and is particularly clearly seen in late pregnancy.

Late pregnancy is associated with an increase in the plasma concentration of cholesterol and triglycerides due mainly to an increase in the population of large lipoprotein particles, like chylomicrons, VLDL, and IDL fractions. It is possible that one of these components, or a combination of them, may be responsible for the presence of the raised contact activating surface in late pregnancy. The range of sizes of these lipoprotein particles encompasses the average size of the negatively charged multilamellar vesicles successfully used to supplement the endogenous contact surface. Vesicles of the same composition but of an average size less than that of IDL failed to supplement the endogenous contact surface. Our experiments also show that the vesicles should be negatively charged, since neither the unilamellar nor the multilamellar large vesicles made of neutral phospholipid were capable of supplementing the endogenous contact surface. The finding that the lipoproteins isolated by floatation techniques did not supplement the endogenous contact surface was surprising. However, it is possible that the isolation procedure resulted in the loss of relatively low molecular weight, negatively charged components, a view supported by our finding that stearic acid stimulates cold activation. It is also well recognized that the plasma-free fatty acid levels in late pregnancy are considerably above those in the plasma of nonpregnant women.

It had previously been shown that the activation of isolated factor XII can be achieved in the presence of sulfatide-containing vesicles, which provide the contact activation surface. Liposomal vesicles of a hydrodynamic radius of 35 ± 0.5 nm made of 100% sulfatide or 42% sulfatide-58% phosphatidylethanolamine are capable of promoting activation of factor XII, whereas 100% phosphatidylethanolamine, 100% phosphatidylserine, and 100% phosphatidylcholine vesicles of the same size are not. We have observed in the present system significant activation of VII in the presence of large multilamellar vesicles made of PS:PC:CH (0.15:0.85:0.5) (results not shown). The size of the negatively charged vesicles may be of importance in providing binding sites for all three proteins (factor XII, prekallikrein, and high molecular weight kininogen) for a significant rate of factor XII activation. This requirement may be satisfied by the large lipoprotein particles with the appropriate negative charge provided by free fatty acids that also circulate with the blood. However, in view of our inability to demonstrate directly that large lipoprotein particles are responsible for the factor XII-dependent activation of factor VII, the present experiments with large negatively charged liposomal vesicles or stearate suggest, but do not prove, that the above complex represents the endogenous contact surface.

Stearate added to late pregnancy plasmas or to plasmas from nonpregnant women in incubations conducted in plastic tubes produced an increase in VIIc. However, the stearate-concentration-dependent increase in VIIc followed different kinetics in the plasmas from the two conditions. In the case of plasmas from women in late pregnancy the increase in VIIc
followed saturation kinetics as though the negative charge was rate-limiting. By adding sodium stearate, more particles acquire a negative charge and interact with the contact coagulation factors. In contrast, in the plasmas from the nonpregnant control subjects, the surface appears to be limiting even in the presence of the highest concentration of stearic acid. In this case the relation between VIIc and stearic acid concentration is semilogarithmic. This phenomenon may explain why certain fatty acids added to citrated plasma shorten the clotting time or shorten the thrombus-formation time of blood in an in vitro system.

The above sequence of events may explain the increased VIIc and increased hypercoagulability in the hypercholesteremic rabbit. In this experimental animal model the diet-induced increased concentration of large lipoprotein particles provides the increased concentration of the contact surface, hence raising the flux in the intrinsic pathway of coagulation and thereby causing an elevation in VIIc. This increase in VIIc is due to an increase in the ratio of aVIIa:VIIc. Similarly in humans, conditions that are associated with an increased VIIc are also associated with an increase in the plasma concentration of large lipoprotein particles and free fatty acids. For instance in diabetes, in obesity, in women using oral contraceptives, and in hypertriglyceridemia, where there is an increase in the concentration of the large lipoprotein particles there is also an increase in VIIc. It is also relevant to this argument that aVIIa is only poorly inhibited by antithrombin III, therefore small changes in the flux of the coagulation pathway will be reflected in measurable changes in VIIc. This is consistent with the strong positive association between VIIc and the plasma concentration of triglycerides and of cholesterol shown in epidemiologic studies and could explain the relation between dietary fat intake and VIIc.

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