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Free fatty acids and albumin as mediators of thrombin-stimulated fibrinogen synthesis

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PICKART, LOREN R., AND M. MICHAEL THALER. *Free fatty acids and albumin as mediators of thrombin-stimulated fibrinogen synthesis.* Am. J. Physiol. 230(4): 996–1002. 1976.– Mobilization of FFA in mice, triggered with an injection of thrombin, was followed within 24 h by a 2.5-fold increase in fibrinogen synthesis and a 30% increase in plasma fibrinogen concentration. In mouse liver slices, incubated in plasma, additions of palmitate or stearate in amounts similar to those expected in vivo in FFA mobilization stimulated fibrinogen synthesis 5.6- to 6.1-fold while unsaturated and short-chain FFA were less effective. Palmitate and linoleate also augmented albumin synthesis although not as strongly as fibrinogen synthesis. These observations raised the possibility that the greater effectiveness of saturated FFA in stimulating fibrinogen synthesis may reflect higher FFA/albumin ratios within hepatocytes in the presence of saturated FFA. Injection of exogenous defatted albumin into mice before thrombin injection prevented the FFA-associated rise in fibrinogen synthesis and plasma concentration. In incubated liver slices, defatted albumin abolished the FFA stimulation of fibrinogen synthesis when FFA/albumin ratios were maintained in the physiological range. These studies indicate that the FFA/albumin ratio may play a major role in the replenishment of fibrinogen after periods of rapid defibrinogenation.

fibrinogen synthesis; free fatty acid/albumin ratio

THE BLOOD CLOTTING PROTEIN, fibrinogen, is unique among the major plasma proteins in being consumed in the fulfillment of its biological function. As a consequence, plasma is subject to rapid defibrinogenation during periods of extensive clot formation (25). The resultant deficit in plasma fibrinogen is rapidly restored by a large increase in hepatic fibrinogen production after clotting. Thrombin-induced defibrinogenation is followed by a rapid stimulation of synthesis which leads to a state of secondary hyperfibrinogenemia (25, 30, 37). A similar response has been demonstrated in rabbits in which the percentage of fibrinogen in hepatic export protein increased from 5 to 48% within 6 h after treatment with corticotropin (3). These observations indicate that the synthesis of fibrinogen is rapidly responsive to changes in the metabolic environment.

The homeostatic mechanisms regulating fibrinogen synthesis are poorly understood. Hypofibrinogenemia, per se, does not stimulate fibrinogen synthesis (6, 20, 37). Thrombin enhances fibrinogen synthesis in intact animals, but it has no direct effect on liver tissue in vitro (30, 37). Similarly, both fibrinogen degradation products and serum increase fibrinogen concentrations in vivo (6, 25, 50) without affecting synthesis in vitro (31, 37, 38). These observations suggested that enhancement of fibrinogen synthesis may be factors released during thrombin treatment. We are presenting evidence that free fatty acids (FFA) are mobilized by thrombin and appear to be responsible for the subsequent increase in fibrinogen synthesis. Stimulation of fibrinogen synthesis was effected by FFA in intact mice and in mouse liver slices and could be inhibited by defatted albumin.

MATERIALS AND METHODS

Preparation of human defatted albumin. Human albumin (Cohn fraction V) was obtained from Cutter Laboratories, Berkeley. Only fresh stocks, from 1 day–2 wk old, were utilized for experiments. The albumin was transported on ice, then stored at −20°C until defatted. Lipids were removed by precipitating 1 vol of a 10% solution of albumin in saline with an equal volume of ice-cold 10% trichloroacetic acid (TCA), followed by centrifugation for 10 min at 10,000 *g*. The precipitated protein was dissolved in 3 vol absolute ethanol, recentrifuged for 20 min at 30,000 *g*, and the ethanolic supernatant was stirred into 30 vol of ice-cold diethyl ether. The flocculent precipitate which formed was washed twice with cold diethyl ether, being sedimented after each wash at 3,000 *g* for 10 min. The pellet of delipidated albumin was lyophilized overnight, dissolved in saline, and dialyzed for 4 days at 2°C. The molar ratio of FFA to albumin was reduced from 1.9 in the initial preparations to 0.14 in the final defatted albumin. FFA removed from albumin contained oleate 31.1%, palmitate 25.1%, stearate 16.2%, linoleate 15.4%, palmitoleate 7.1%, myristate 3.6%, and traces of other FFA 1.5%.

Rabbit antihuman fraction V albumin antisera, prepared by standard methods (23), revealed no immunological alterations in the defatted albumin. Double diffusion immunoprecipitation by the Ouchterlony procedure (34) with the antisera in the center well and the undefatted and defatted albumin in adjacent peripheral wells produced a smooth albumin precipitation line with no evidence of a spur at the precipitation junction of the two albumin preparations.

On polyacrylamide gel or paper electrophoresis (47), the defatted albumin moved slightly slower than fraction V, but when premixed with undefatted fraction V gave no new bands. In one set of experiments, fraction V and defatted albumin in isotonic saline (40 mg/ml)

were incubated for 30 min at 37°C with 0.1 μCi [1-^{14}C]-palmitate. The mixture was then subjected to paper electrophoresis for 16 h, and the albumin band was located on the paper by excitation at 340 nm. The band was cut out, along with adjacent areas of the strip, and the fatty acids were extracted by the method of Nestel and Steinberg (32). Aliquots of the extracts were counted for radioactivity in the standard liquid scintillation system. Ninety-four percent of the radioactivity was located with the albumin band; the remainder was uniformly distributed in the trailing zone. Thus, the defatting procedure did not affect the binding of trace amounts of palmitate to albumin.

Preparation of dextran. Dextran, mol wt 75,000 (Sigma Chemical Co., St. Louis) was exhaustively dialyzed against distilled water for 3 days at 3°C, then lyophilized to dryness. For use, an appropriate amount was dissolved in 0.85% NaCl and sterilized by filtration through 0.22-μm Millipore filters (Millipore Corporation, Bedford).

Serum FFA and albumin in thrombin-treated mice. In intact mice (37) and rats (30), 50–5,000 U of thrombin administered intraperitoneally have been shown to stimulate fibrinogen synthesis. The lowest reported effective dose of thrombin, 50 NIH U, was selected as the standard dose in all experiments with intact mice.

Swiss-albino mice, 25 g in weight, were maintained on an ad libitum laboratory chow diet and were housed in a windowless room equipped with lighting which provided 12-h periods of light and darkness. Experiments were performed at the same time of day to avoid changes due to diurnal variation and irregular postprandial periods. The mice were kept in a relatively unexcited state by minimizing handling and manipulation of cages and by shielding from loud noises. Individual animals were injected with 50 U of thrombin in 0.5 ml normal saline or in saline containing 50 mg defatted albumin. Control animals received injections of normal saline only. Eight animals from each group were sacrificed by aspiration of blood from the heart at 0-, 2-, 4-, and 6-h intervals after treatment. Blood from four animals in each group was pooled, allowing for analysis of duplicate samples. The clotted blood was centrifuged briefly, and serum FFA were determined by the method of Dole and Meinertz (17). Serum albumin was measured by paper electrophoresis.

Synthesis of fibrinogen and albumin in mice. Preliminary experiments indicated that enhanced incorporation of labeled precursor into fibrinogen is initially detectable after an interval of 6–8 h following thrombin injection in mice. Miller and John (30) have shown that the plasma fibrinogen level is not augmented in thrombin-treated rats before 16 h, rising rapidly thereafter to a plateau which is maintained for at least 8 h. The experiments detailed below were designed to measure fibrinogen incorporation rates during the period of thrombin-induced hyperfibrinogenemia. This was achieved with injections of radioactive precursors 8 h after thrombin treatment and measurement of plasma fibrinogen concentrations and specific activity 16 h later.

Maintenance and handling of mice were as described. Animals were grouped in threes. Within each group, one control animal received normal saline, 0.5 ml ip, and two were treated with 0.5 ml saline containing 50 U of thrombin. Thirty minutes after initial injection, the control animal and one thrombin-treated animal received another injection of 0.5 ml saline, while the remaining thrombin-treated animal was injected with 0.5 ml saline containing 50 mg defatted human albumin or 50 mg dextran. Defatted albumin was used to avoid the thrombogenic effect of albumin with an FFA content greater than 1.5 mol/mol of albumin (48).

Eight hours after injection, all animals received 1 μCi [U-^{14}C]aspartic acid (New England Nuclear Corp., Boston, sp act 150 mCi/mmol) or [1-^{14}C]glycine (New England Nuclear, sp act 100 mCi/mmol) in 0.5 ml isotonic saline. Experiments were terminated on the following morning (24 h). All animals were injected with 100 U of heparin intraperitoneally 30 min prior to sacrifice, anesthetized with chloroform, and blood was aspirated by cardiac puncture, using plastic disposable syringes. Changes in plasma volume due to albumin or dextran injection were monitored with hematocrits. The blood was mixed with ACD anticoagulant solution in graduated centrifuge tubes kept on ice, centrifuged immediately at 3,000 *g* for 20 min in a refrigerated centrifuge, and the plasma was removed for isolation of fibrinogen and albumin.

For measurements of serum FFA, a parallel series of experiments was performed, except that radioactive precursors were not used, and mice were not heparinized before sacrifice.

Synthesis of fibrinogen and albumin in liver slices. Sodium salts of fatty acids were dissolved in absolute ethanol, 0.1-ml aliquots placed in empty, sterile, wide-bottom flasks, and dried under nitrogen. The plasma used in incubations was freshly prepared from blood obtained after an overnight fast from healthy young human males. The blood was drawn by venipuncture, anticoagulated with heparin, 100 U/ml, and the plasma was separated by centrifugation. Plasma and labeled precursor were then added, and all flasks were preincubated at 37°C in a rotary metabolic shaker at 120 rpm to dissolve the fatty acids. Fatty acid was omitted from control flasks. Albumin or dextran, 28 mg/ml, was introduced into plasma and preincubated in a similar manner. Swiss albino mice, 25 g in weight, were the source of liver used in all incubations. After decapitation, the liver was removed, blotted dry, and sectioned on an automatic tissue sectioner set to produce 1-mm-thick slices. The preincubated human plasma was iced, and 100-mg portions of slices were suspended in 3 ml of plasma containing 3–5 μCi of a labeled amino acid. Each flask was incubated for 2–4 h at 37°C in 95% O_2-5% CO_2 in the rotary shaker. Incubations were terminated by placing flasks in ice water. The tissue was sedimented for 10 min at 2,000 *g* at 3°C, and the clear supernatant which consisted of the incubating plasma was used for isolation of labeled fibrinogen and albumin. The labeled amino acids used in incubations were [1-^{14}C]glycine (New England Nuclear, sp act 100 μCi/

mmol) and [U-^{14}C]aspartic acid (New England Nuclear, sp act 150 μCi/mmol). The initial and final pH of incubating plasma were both 7.4 ± 0.1 U. All incubations were in triplicate; individual experiments were repeated at least 3 times.

Determination of plasma fibrinogen and albumin concentration. The absolute plasma fibrinogen concentration was determined by the Saifer-Newhouse method (43). This photometric micromethod is based on an analysis of factors which influence fibrin yield (pH, clotting time, temperature, thrombin, and buffer concentration) at various plasma concentrations. The colorimetric reaction makes use of a ninhydrin method which is highly sensitive, reacts with the alpha-amino groups of fibrinogen, and employs fibrinogen standards prepared under conditions identical with those used in the analysis of unknown plasma samples. A correction factor for occluded nonfibrinogen proteins permits calculation of "true" fibrinogen plasma concentrations. The values obtained correspond with results of direct gravimetric macroanalysis (±5%). The plasma or serum albumin concentration was measured by paper electrophoresis in a Gelman cell, using a dye elution technique (19).

Isolation of fibrin and albumin for specific activity determination (37). Approximately 85% of the total fibrinogen pool is distributed in the intravascular compartment (1). The concentration of fibrinogen and its specific acitivity in plasma are, therefore, a suitable indicator of total fibrinogen synthesis.

Fibrinogen was isolated as fibrin from the plasma of experimental animals and from plasma used in incubation experiments by methods previously described (37). The final fibrin solution in 0.1 M ammonium hydroxide was dried in scintillation vials and dissolved in hyamine for counting in a toluene-based Liquifluor. Counting was continued to a value which deviated less than 1% from the mean. The protein concentration in the solutions was determined by the ninhydrin method with purified fibrin as a standard (43). Specific activity of the fibrin was expressed as counts per minute per milligram of protein.

Clottable protein as an indicator of fibrinogen specific activity may lead to considerable error, since the clots have been reported to occlude about 22% nonfibrinogen protein (43). The amount of occluded protein was greatly reduced when the fibrinogen/nonfibrinogen protein ratio was increased by fractionation with ammonium sulfate. Tracer amounts of ^{131}I-labeled serum added to plasma before clotting provided a comparison of the specific activity of the plasma and the final clot which showed that occluded protein accounted for less than 0.5% of the total protein in the clot.

Albumin was isolated from the 25% saturated ammonium sulfate supernatant which remained after the removal of fibrinogen. The supernatant was dialyzed exhaustively against 0.85% saline containing 1% of an aqueous solution of 1% sodium fluoride, 1% E-amino caproic acid, and 1% disodium ethylenediaminetetra acetic acid. The albumin was extracted from the dialyzed supernatant by the TCA-ethanol-diethyl ether method used to prepare defatted human albumin, and the specific activity was determined in a manner similar to the fibrin.

RESULTS

Serum FFA and albumin concentration in thrombin-treated mice. Serum FFA were elevated in all animals treated with thrombin or thrombin-albumin combinations (Fig. 1*A*). Six hours after thrombin injection, FFA concentrations reached 2.3 times basal values. In contrast, albumin concentrations remained relatively stable. The average serum albumin concentration in control mice was 0.51 μmol/ml, compared with 0.44 μmol/ml at 2 h, 0.43 μmol at 4 h, and 0.47 μmol/ml at 6 h after thrombin was administered. Albumin injections which were intended to raise the serum albumin concentration by approximately 20% at near equilibrium with the total albumin pool increased the serum albumin concentration to 0.54, 0.58, and 0.62 μmol/ml after 2, 4, and 6 h, respectively. Enlargement of the total albumin available to the animal by nearly 22% in 6 h had no effect on the thrombin-mediated increase in serum FFA (Fig. 1*A*).

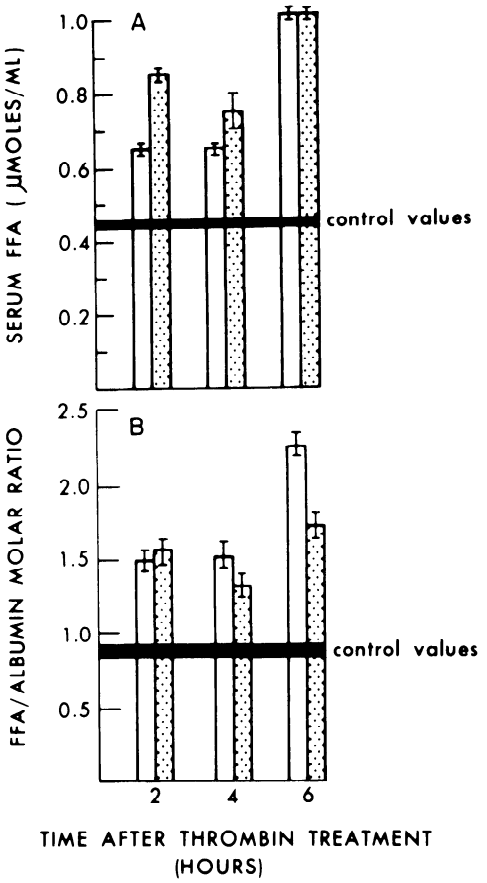

FIG. 1. *A:* effect of thrombin (open columns) and thrombin-albumin (hatched columns) and serum FFA in mice. Thrombin (50 U), or defatted albumin (50 mg) plus thrombin (50 U), was administered intraperitoneally. Control animals (8) were injected with saline. Each column represents mean of 8 animals. Bars indicate SE. *B:* effect of thrombin and albumin-thrombin on serum FFA/albumin molar ratios in mice. Experimental conditions and numbers of animals as in Fig. 1.

TABLE 1. *Effect of defatted human albumin on thrombin-induced fibrinogen and albumin biosynthesis in mice*

	No. of Expt	Fibrinogen Concn, mg/ml	Fibrinogen Sp Act, counts/min per mg	Total Incorporation into Fibrinogen Concn times Sp Act counts/min per ml	Albumin Concn, mg/ml	Albumin Sp Act, counts/min per mg
[^{14}C]glycine						
Control (saline)	7	3.6 ± 0.2	59.3 ± 4.1	214 ± 23	33.2 ± 2.4	9.1 ± 1.3
Thrombin	7	4.7 ± 0.3	107.2 ± 10.1	503 ± 68	31.4 ± 1.7	11.8 ± 1.2
Thrombin and albumin	4	3.3 ± 0.1	67.0 ± 6.3	221 ± 37	39.8 ± 3.2	10.2 ± 0.9
Thrombin and dextran	3	4.8 ± 0.2	112.7 ± 15.8	551 ± 88		
[^{14}C]aspartic acid						
Control (saline)	6	3.5 ± 0.2	24.8 ± 3.8	87 ± 11	33.6 ± 3.0	16.2 ± 2.1
Thrombin	6	4.7 ± 0.3	53.3 ± 7.7	250 ± 36	31.2 ± 2.9	19.8 ± 2.5
Thrombin and albumin	4	3.2 ± 0.1	30.9 ± 4.1	99 ± 21	40.1 ± 2.7	15.4 ± 1.6
Thrombin and dextran	2	4.9 ± 0.1	54.0 ± 3.2	264 ± 29		

Values are means ± SD. Sera were obtained 24 h after thrombin injection. Each experiment represents the pooled plasma from four mice. * Differences in total incorporation into fibrinogen in the control and thrombin-albumin groups were not significant. Differences between both the control or the thrombin-albumin group and the thrombin or thrombin-dextran groups were significant at the 0.01 level.

Serum FFA/albumin molar ratios increased in thrombin-treated mice as a result of the rise in FFA without a corresponding change in serum albumin (Fig. 1*B*). In mice treated with thrombin and defatted albumin, FFA/albumin ratios decreased in proportion to the added albumin at the time of its equilibration with the total albumin pool (6 h).

Fibrinogen and albumin synthesis in thrombin-treated mice. In mice injected with 50 U of thrombin, the plasma fibrinogen concentration increased from 3.6 ± 0.2 to 4.7 ± 0.3 mg/ml, while the rate of synthesis, as reflected by the specific activity of highly purified fibrin from plasma, increased from 59.3 ± 4.1 to 107.2 ± 10.1 counts/min per mg in 24 h (Table 1). Defatted human albumin (50 mg), administered intraperitoneally 30 min after the thrombin, prevented the rise in plasma fibrinogen concentrations and almost completely inhibited thrombin-induced stimulation of fibrinogen synthesis. The average plasma albumin concentration in albumin-treated mice was increased from 33.0 to 39.9 mg/ml, i.e., a 21% rise in 24 h.

The intraperitoneal injection of 50 mg dextran had no effect on incorporation of either amino acid precursor into fibrinogen (Table 1). Plasma volumes were not significantly affected under the experimental conditions: hematocrit values in control, albumin-treated, and dextran-treated animals averaged 45.7 ± 2.0, 46.9 ± 2.2, and 44.7 ± 1.9% of total blood volumes, respectively.

Fibrinogen and albumin synthesis in liver slices. The addition of 0.3 μeq sodium palmitate per milliliter of incubating plasma increased the incorporation of [^{14}C]glycine and [^{14}C]aspartic acid into fibrinogen by 560% above control values (Table 2). Sodium stearate was even more effective in stimulating fibrinogen synthesis. The unsaturated FFA sodium oleate, linoleate and linolenate, and the saturated short-chain fatty acid hexanoate had approximately half the stimulatory activity of saturated long-chain FFA. When the FFA/albumin molar ratio was lowered with defatted albumin, the production of fibrinogen remained at basal rates even in the presence of palmitate. However, when the FFA/albumin ratio was increased to 1.26 with commercial crystalline albumin carrying 1.91 mol FFA per mole albumin, fibrinogen synthesis was increased by 350%.

TABLE 2. *Effect of FFA and albumin on fibrinogen synthesis in liver slices*

Addition	Final FFA/Albumin Molar Ratio	Fibrinogen Synthesis % Increase Over Basal Synthesis
Stearate (18.0)	1.31	610 ± 62
Palmitate (16.0)	1.31	560 ± 67
Oleate (18.1)	1.31	342 ± 51
Linoleate (18.2)	1.31	268 ± 32
Linolenate (18.3)	1.31	260 ± 36
Hexanoate (6.0)	1.31	258 ± 23
Glucose	0.85	32 ± 14
Defatted albumin*	0.58	0 ± 8
Defatted albumin plus palmitate†	0.85	20 ± 11
Crystalline albumin‡	1.26	350 ± 63

* FFA/albumin ratio, 0.14. † Palmitate added to a concentration of 0.3 μeq/ml. ‡ FFA/albumin ratio, 1.91.

Sodium palmitate also enhanced synthesis of albumin in liver slices, although to a much lesser extent than fibrinogen (Fig. 2). Both fibrinogen and albumin syntheses were increased by sodium palmitate in a dose-dependent manner when the FFA was kept in the normal physiological concentration range. However, palmitate was much more effective in stimulating fibrinogen synthesis compared with albumin synthesis. In contrast, sodium linoleate stimulated the synthesis of fibrinogen and albumin approximately equally. Nearly twice as much albumin was synthesized in the presence of linoleate compared with palmitate (Fig. 2). The absolute plasma concentration of fibrinogen and albumin was not detectably altered during the short incubation periods.

Treatment of plasma with glucose, 1 mg/ml, increased both fibrinogen and albumin synthesis by approximately 30%. Fibrinogen and albumin synthesis were not affected, in vitro, by products of the action of plasmin on fibrin, as demonstrated by incubating liver slices in plasma treated with urokinase (10 ploug U/ml), or with a mixture of urokinase and epsilon-amino caprylic acid (10^{-2} M), or with peptides produced from the urokinase activation of plasminogen in plasma after a 3-h incubation at 37°C.

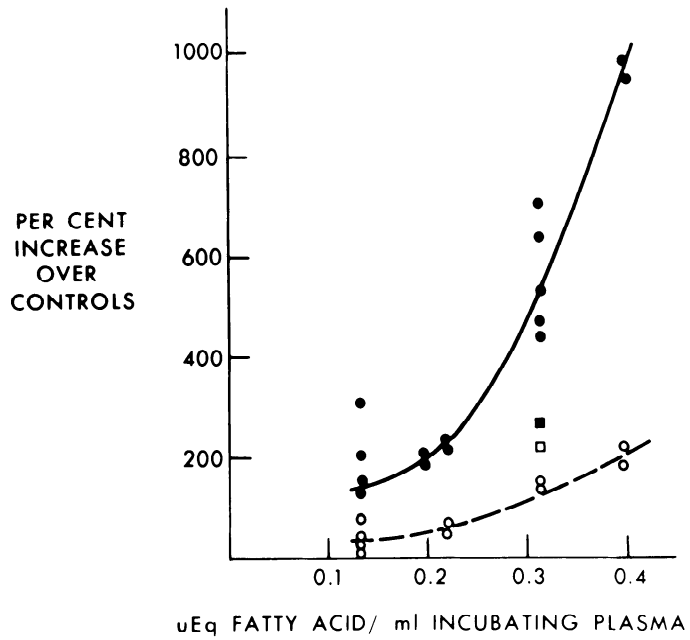

FIG. 2. Effect of added palmitate (circles) and linoleate (squares) on incorporation of [^{14}C]aspartic acid into fibrinogen (solid symbols) and albumin (open symbols) by liver slices incubated in plasma. Palmitate was added over a concentration range, 0.131-0.512 μg/ml. Before addition of exogenous FFA, basal count rate was 15-25 counts/min per mg above background.

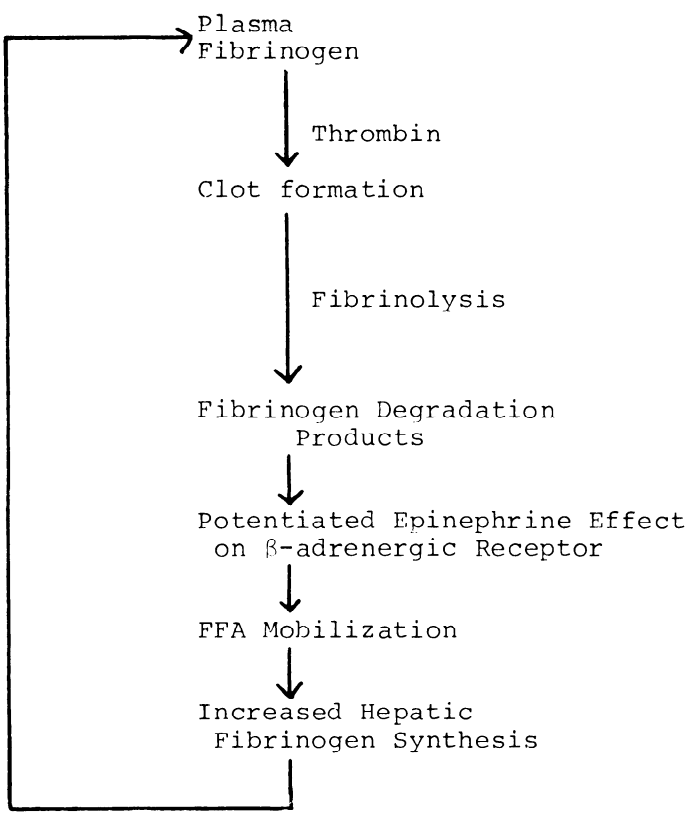

FIG. 3. Proposed in vivo mechanism for replacement of plasma fibrinogen after depletion by clot formation.

Composition of fibrinogen and albumin synthesized in vitro. Fibrinogen and albumin from different species have similar amino acid compositions (2, 8, 35, 36). A differential labeling experiment was devised to determine whether amino acids were incorporated by plasma-incubated liver slices into fibrinogen and albumin in the proportions expected from their composition. The ratio of the weight percentages of aspartic acid in fibrinogen/albumin is about 1.30; the ratio for glycine is about 2.90. Thus, when glycine is the precursor instead of aspartic acid, the ratio of radioactivity (fibrinogen/albumin) should increase by 2.90/1.30 = 2.23 when compared to the aspartic acid incorporation. The average ratio of radioactivity incorporated by the liver slice into fibrinogen and albumin was 1.7 with [^{14}C]glycine and 0.7 with [^{14}C]aspartic acid. Therefore, the proportional incorporation of glycine and aspartic acid into fibrinogen and albumin, respectively, was 1.7/0.7 = 2.43, in close agreement with the predicted ratio of 2.23.

DISCUSSION

There is a close relationship between FFA mobilization and a rise in plasma fibrinogen in a wide variety of experimental and clinical situations. This association suggested the possibility that FFA may be involved in the stimulation of fibrinogen synthesis. Both plasma FFA and fibrinogen are increased during stress which can be psychological (e.g., electroshock (26, 29), overcrowding or anxiety (7, 28), or physical (e.g., toxins, burns, tissue injury (39, 48), and as a result of metabolic changes induced by large doses of certain hormones (epinephrine or corticotropin (39, 48), hydrocortisone (12, 48), growth hormone (10, 48), cigarette smoking (21, 24), ascorbic acid deficiency (5, 44), and nephrosis (39, 46). Conversely, elevated FFA and fibrinogen concentrations can be reduced together by insulin treatment in diabetes (45, 49) and by clofibrate (Atromid-S) treatment of patients with arteriosclerotic heart disease (14). The mobilization of FFA by epinephrine and the trauma-induced hyperfibrinogenemia are both abolished by hypophysectomy or adrenalectomy (13, 22, 48).

In 1967, Pickart and Pilgeram (37) demonstrated that thrombin injections stimulated fibrinogen synthesis in intact mice and suggested than an in vivo mobilization of FFA by thrombin may have been responsible for the stimulatory effect. Results consistent with this conclusion were obtained when human and mouse liver slices were incubated with FFA (38). Subsequently, Cucuianu et al. (15) reported doubled plasma fibrinogen concentrations in palmitate-infused dogs which survived the FFA-induced thrombotic episodes.

The present investigation demonstrates that thrombin causes an increase in circulating FFA (Fig. 1*A*) and confirms the previous observations that FFA stimulate the production of fibrinogen in the mammalian liver. This effect was readily demonstrable 24 h after FFA release was triggered with a single thrombin injection. Measurements of specific activity indicated a 2.5-fold increase in fibrinogen synthesis and a 30% increase in the plasma fibrinogen concentration (Table 1). A similar increase in fibrinogen synthesis was observed in liver slices upon addition of FFA to incubating serum in concentrations which were in the range of increments in serum FFA of intact mice following thrombin treatment (Table 2).

When the effect of saturated and unsaturated FFA was examined in incubated liver slices, linoleate was approximately half as active as palmitate and one-third

as active as stearate in augmenting fibrinogen synthesis (Table 2). These differences in activity may reflect differential affinities of various fatty acids for albumin, resulting in unequal unbound fatty acid concentrations. Thus, while results of in vitro studies must be cautiously interpreted, these observations suggest that the saturated fatty acids, stearate and palmitate, may play a major role in rapid accumulation of fibrinogen in the intact organism. This interpretation is consistent with results of studies in which rats fed a diet rich in saturated fats developed severe hyperfibrinogenemia due to increased fibrinogen synthesis (41), and with clinical investigations where provision of 6 g of linoleate in the daily intake of elderly patients caused a significant reduction in plasma fibrinogen (18).

Injections of defatted albumin inhibited the FFA-stimulated increase in fibrinogen synthesis in vivo (Table 1). Alterations in plasma volume could not be responsible for this action of albumin, since hematocrits were not altered significantly and dextran treatment was ineffective in reducing total fibrinogen synthesis. The decrease in specific activity of highly purified fibrin following albumin injection could not be due to dilution of the precursor pool with amino acids derived from the injected albumin, since the effect was the same whether labeled glycine or aspartic acid was used as precursor. Had precursor dilution occurred, it would be reflected in much lower aspartate incorporation compared with glycine, because albumin contains approximately 4 times as much aspartic acid as glycine (2, 8, 35, 36), whereas tissue concentrations of aspartic acid are much lower than glycine (17).

FFA/albumin ratios in intact animals treated with defatted albumin reflected changes in serum albumin (Fig. 1*B*). Experimentally induced variations in serum albumin and, consequently, in FFA/albumin ratios permissible in vivo were relatively limited, compared with in vitro preparations, where more extensive manipulation of FFA/albumin ratios was possible (Table 2). Fibrinogen production in liver slices was clearly a function of the FFA/albumin ratio in medium (Table 2), indicating that inhibition of fibrinogen synthesis by albumin was due to its ability to block entry of FFA into hepatocytes. Addition of defatted albumin prevented FFA-induced stimulation of fibrinogen synthesis. In contrast, crystalline albumin, which carried nearly 14 times as much FFA as did defatted albumin, enhanced fibrinogen synthesis (Table 2).

We conclude from these results that the relationship between FFA and albumin is important in regulating the stimulation of fibrinogen synthesis produced by thrombin. A similar regulatory role has been postulated for the FFA/albumin ratio in other in vitro systems, e.g., synthesis of the apoprotein moiety of lipoproteins in perfused liver (42), and synthesis of two related dehydrogenases in isolated fat cells (40). The effect of the interaction between albumin and FFA on fibrinogen production may explain the paradoxical observations reported by Baar (4) that hyperfibrinogenemia in patients with thermal injuries could be prevented with infusions of a mixture of plasma and albumin, while infusions of serum were ineffective. Although serum is free of fibrinogen, fibrinogen degradation products in serum can increase fibrinogen concentrations in experimental animals (6, 50).

Within hepatocytes, the FFA/albumin ratio can be expected to reflect both FFA utilization and the prevailing rate of albumin production. The liver oxidizes saturated FFA less efficiently than unsaturated and short-chain FFA (9, 11). Unsaturated FFA stimulated albumin synthesis nearly twice as effectively as did saturated FFA (Fig. 2). These observations may provide an explanation for the greater activity of saturated, compared with unsaturated FFA, in stimulating fibrinogen synthesis. Prior to its export from the liver, newly synthesized albumin may bind intracellular FFA, and interfere with their ability to activate fibrinogen synthesis. Albumin may also serve to deplete the intrahepatic FFA pool, should the protein be exported while bound to FFA. Such a mechanism may be particularly effective in regulating the intrahepatic concentrations of saturated FFA, since recent evidence indicates that cytoplasmic FFA-binding protein in liver possesses high affinity for unsaturated FFA and low affinity for saturated FFA (33). Thus, a specific carrier system may mediate the metabolic activities of unsaturated FFA, leaving saturated FFA to albumin.

On the basis of available information and the additional data described in this report, a scheme can be proposed for in vivo regulation of fibrinogen synthesis after its depletion by clotting mechanisms (Fig. 3). The action of thrombin on the coagulation of fibrinogen and the subsequent action of the fibrinolytic system on the fibrin clot result in the release of small peptides (e.g., fibrin split products (FSP), fibrin degradation products (FDP)), which raise the plasma fibrinogen concentration in the intact animal (6, 25). This effect of FDP must be indirect, since perfusion of isolated liver with FDP is not followed by increased fibrinogen synthesis (31). Malofiejew (27) demonstrated that FDP may influence the receptivity of various tissues (rat uterus, guinea pig intestine, rat heart) to specific biologic or pharmacologic stimuli. FDP was shown to potentiate the action of epinephrine on adrenergic receptors in rat uterus severalfold. An analogous effect of FDP on beta-adrenergic receptors in fat cells would enhance the mobilization of FFA from depot fat by epinephrine. Since hepatic uptake of FFA is a direct function of the FFA/albumin ratio, FDP-facilitated release of FFA would result in increased intrahepatic FFA concentrations, thus shifting hepatic protein synthesis toward fibrinogen. In addition to their stimulatory effect on fibrinogen production, FFA activate Hageman factor, an essential component of the clotting and fibrinolytic systems (48). Thus, the proposed scheme suggests a central role for FFA in the regulation of processes which lead both to depletion and to resynthesis of fibrinogen during clot formation. The mechanisms whereby FFA exert these effects on hepatic protein metabolism may be clarified with in vitro studies using a variety of compounds which share important physicochemical properties with FFA.

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