Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 1530–1534, March 1982
Cell Biology

Regulation of fibrinogen synthesis by plasmin-derived fragments of fibrinogen and fibrin: An indirect feedback pathway

(rat hepatocytes/leukocytes/hepatocyte-stimulating factor)

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Communicated by Harvey A. Itano, October 26, 1981

ABSTRACT **The effect of plasmin-derived fibrinogen fragments on the biosynthesis of fibrinogen was investigated in cultured monolayers of rat hepatocytes. Incubating the cells with several concentrations of either fibrinogen or fibrin fragment D or E had no effect on the synthesis and secretion of fibrinogen by these cells. However, if the fragments were incubated with isolated peripheral blood leukocytes, they caused these cells to secrete a factor that when added to the hepatocytes caused an increase in fibrinogen synthesis 4- to 6-fold over controls. Moreover, the hepatocyte-stimulating factor also affected the production of several other proteins produced by the hepatocyte. These results demonstrate that both fragments D and E can stimulate hepatic fibrinogen synthesis via an indirect leukocyte-mediated pathway.**

When fibrinogen or fibrin is acted upon by plasmin, a series of well-defined proteolytic cleavages occur that ultimately result in the formation of two different molecular species that come from different parts of the molecule. The fragments are designated E, which is derived from the amino-terminal region of the molecule and has a M_r of 40,000, and D, which is derived from the carboxy-terminal region and has a M_r of 80,000 (1, 2). Slightly more than a decade ago, Barnhardt *et al.* (3) infused fibrinolytic fragments into dogs and found a significant increase in fibrinogen synthesis as judged by an increased immunofluorescence of liver slices taken several hours after the infusion. The observation that some of the plasminolytic fragments derived from fibrinogen or fibrin may be involved in the regulation of fibrinogen synthesis was suggestive of a novel regulatory pathway that could be an important part of the inflammatory response.

A number of reports has appeared (4–8) which seem to verify and expand the study of Barnhardt *et al.*—that is, they suggest that either fibrinogen degradation products or fibrin degradation products (FDP or fdp, respectively) play an important role in regulating fibrinogen synthesis. On the other hand, several other studies (9–12) have been unable to demonstrate any effects of FDP on fibrinogen production. Unfortunately there is no consistent explanation for these conflicting observations. Thus, whether FDP play any significant role in regulating fibrinogen is still unresolved.

In this report we have characterized a role that FDP play in regulating fibrinogen synthesis by exposing cultured hepatocytes to purified fragments of fibrinogen and fibrin. We provide evidence that the fragments do not stimulate fibrinogen synthesis by direct interaction with the hepatocyte. However, the fragments do affect fibrinogen synthesis by stimulating leukocytes to produce a potent hepatocyte-stimulating factor (HSF), which then acts on the hepatocytes to increase fibrinogen synthesis. We also demonstrate that both fragments are equally potent in causing the production of HSF. These results show the pathway by which FDP stimulate fibrinogen production.

MATERIALS AND METHODS

Preparation of Rat Hepatocyte Monolayer Cultures. Hepatocytes were prepared from adult (200–300 g) male Sprague–Dawley rats by the collagenase perfusion procedure of Seglen (13) with several minor modifications described by Weigel *et al.* (14). Full details of preparing and maintaining the primary hepatocyte cultures are described elsewhere (15). The hepatocytes were cultured in Williams essential medium (GIBCO) with 0.1 mM ornithine/insulin (0.02 unit/ml)/5% fetal bovine serum/penicillin (100 μg/ml)/streptomycin (100 μg/ml)/50 nM dexamethasone.

Preparation of Human Leukocyte Suspensions. Fresh human peripheral leukocytes were prepared for each experiment. Approximately 200 ml of blood was drawn into heparin-coated plastic syringes. Twenty-five milliliters of freshly drawn blood was placed into a 50-ml sterile centrifuge tube and 2.5 ml of sterile 0.6% dextran (dextran 600 in 0.15 M NaCl) was added. After 45 min at 25°C, the plasma (containing platelets, some erythrocytes, and leukocytes) was carefully removed from the settled erythrocytes and was then centrifuged at 400 × *g* for 5 min. After the supernatant—which had some suspended platelets—was aspirated, the resulting leukocyte pellet was resuspended, and any remaining erythrocytes were lysed by a 30-sec exposure to 0.2% NaCl. After the addition of an equal volume of 1.58% NaCl, the cells were centrifuged and washed two times with sterile phosphate-buffered saline (P_i/NaCl). The final cell pellets were resuspended in sterile P_i/NaCl at 4×10^7 cells per ml. All procedures were carried out under sterile conditions.

Production of HSF. Typically 1 ml of washed leukocytes was incubated with various test substances in a shaking water bath for 3 hr at 37°C. The cells were then pelleted by centrifugation at 9650 × *g* in a Beckman microfuge. To remove any fibrinogen or fragment D or E that was added to the leukocyte suspension, the supernatants were shaken for 20 hr at 4°C with 100–300 μl of goat anti-rat fibrinogen (GARF) coupled to Sepharose 4B (GARF-Sepharose). After the GARF-Sepharose was pelleted (9650 × *g*; 30 sec), the supernatants were checked for the presence of fibrinogen crossreactivity (both fragments D and E crossreact with the monospecific GARF) by direct enzyme-linked immunosorbent assay (ELISA) and then were tested for the presence of HSF as described below.

Hepatocyte Bioassay for HSF Activity. We have previously described a bioassay for HSF that uses cultured rat hepatocytes (15). The assay consists of determining the amount of fibrinogen

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Abbreviations: HSF, hepatocyte-stimulating factor; FDP, fibrinogen degradation products; fdp, fibrin degradation products; GARF, goat anti-rat fibrinogen; ELISA, enzyme-linked immunosorbent assay; P_i/NaCl, phosphate-buffered saline.

secreted by cultures over a 24-hr period after the addition of HSF. The amount of fibrinogen produced by the hepatocytes and secreted into the medium was quantified with use of an ELISA (16, 17). Total protein in the culture medium was determined according to Lowry *et al.* (18). The amount of fibrinogen produced was expressed as μg of fibrinogen secreted per mg of cell protein per 24 hr. To quantify the amount of HSF produced by the leukocytes, several dilutions of the leukocyte supernatant were added to hepatocyte cultures and the amount of fibrinogen produced was determined. This assay was standardized by defining a unit of HSF activity as that amount necessary to elicit a 50% maximal response (15). In this way, the total amount of HSF (i.e., units) produced by a known number of leukocytes in 3 hr was determined.

Measurement of Fibrinogen Secretion by Using [^{3}H]Leucine. In one set of experiments, [^{3}H]leucine (55 Ci/mmol; 20 μCi per 35-mm dish; 1 Ci = 3.7×10^{10} becquerels) was added to hepatocyte cultures during the 24-hr test period so that newly synthesized and secreted fibrinogen could be distinguished from exogenous fibrinogen and the D or E fragment added directly to the cultures. After 24 hr, medium was collected and was shaken with an excess of GARF-Sepharose at 4°C for 20 hr to ensure complete binding of the ^{3}H-labeled fibrinogen (^{3}H-fibrinogen). The medium was removed and the GARF-Sepharose was washed with five 1-ml aliquots of borate-saline (pH 8.5) to remove nonspecifically bound radioactivity. The washed GARF-Sepharose was resuspended in borate-saline, and ^{3}H radioactivity was measured in duplicate 0.5-ml aliquots added to 5.0 ml of Aquasol and counted in a Beckman model LS7000 liquid scintillation spectrometer.

Two-Dimensional Immunoelectrophoresis. Hepatocytes were incubated for 24 hr in the presence or absence of HSF. Media from duplicate 100-mm tissue culture plates (6 ml per plate) were pooled, concentrated 100-fold in a Minicon concentrator (Amicon), and subjected to crossed immunoelectrophoresis according to the methods of Clark and Freeman (19). Briefly, 4 μl of each sample was placed into a well punched from an agar-coated glass plate (1% agar in barbital buffer, pH 8.9). These samples were then electrophoresed at 10 V/cm for 1.8 hr. Agar strips, each containing a separate electrophoresed sample, were cut out and placed on separate sheets of plastic backed film (Gelbond; FMC Marine Colloids Division). Agar that contained rabbit antisera to rat plasma was then poured onto each plastic film and allowed to harden in contact with the agar strips containing the first-dimension samples. Electrophoresis in the second dimension was performed at 8.75 V/cm for 2.5 hr. The gels were soaked for 24 hr, dried, and stained with Coomassie blue.

Purification Procedures. Rat fibrinogen was purified from plasma as described (20). The purified fibrinogen was electrophoresed under both reducing and nonreducing conditions. Fibrinogen was judged pure by the presence of a single large molecular weight band (320,000) under nonreduced conditions and the presence of only Aα, Bβ, and γ chains under the reduced conditions. The electrophoresis procedure was performed according to the method of Laemmli (21).

Rat plasminogen was purified from rat plasma by single-step lysine affinity chromatography as described by Deutsch and Mertz (22). The plasminogen was activated to plasmin by treatment with 250 units of streptokinase per mg of plasminogen.

Fragments D and E were prepared by treating a 5-ml solution of fibrinogen at 20 mg/ml in Tris·HCl buffer (containing 5 mM $CaCl_2$) with 1 mg of plasmin. After 6 hr of incubation the entire digest was passed through the lysine affinity column to remove the plasmin. Fragments D and E were then separated by ion exchange chromatography on QAE-Sephadex according to the three-step gradient procedure described by Chen *et al.* (23).

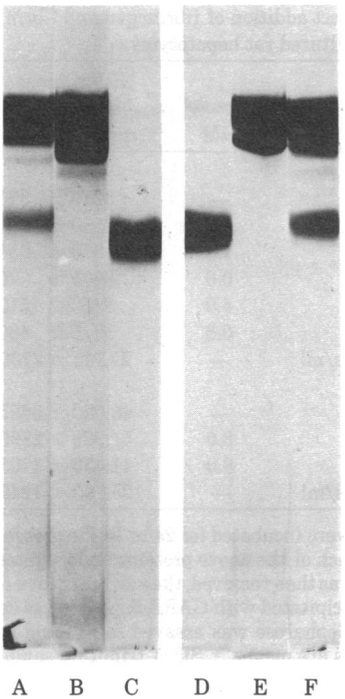

FIG. 1. $NaDodSO_4$/polyacrylamide gel of plasmin-digested fibrinogen and fibrin. Lane A, plasmin-digested fibrinogen prior to QAE-cellulose separation; lane B, fragment D from QAE; lane C, fragment E from QAE; lane D, fragment E′ from QAE; lane E, fragment D′ from QAE; lane F, plasmin-digested fibrin prior to QAE-cellulose separation.

RESULTS

Isolation of Fragments D and E. Fragments D and E were prepared from plasmin digests of fibrin or fibrinogen as described in *Materials and Methods*. When each of the isolated fragments was analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis, a clear separation between fragments D and E was apparent. Fragment D is more heterogeneous, and its constituents have been designated D_1, D_2, and D_3. However, fragment E is more homogeneous. The molecular weights of the fragments have been reported to be between 80,000 and 100,000 for the D fragments and 40,000 for fragment E. These fragments migrated in the $NaDodSO_4$ gels (24) to positions that corresponded roughly to these sizes. The separation shown in Fig. 1 quite closely parallels that reported in other studies (25, 26).

The Direct Effect of Fibrinogen and Fibrin Degradation Fragments. Several studies have suggested that fragments D and E stimulate fibrinogen synthesis. However, none of the reported studies has tested the effects of these fragments directly on hepatocytes. Therefore it was important to determine whether these act directly on hepatocytes or indirectly by activating other cells to produce stimulating factors. Two different concentrations of each fragment as well as undegraded fibrinogen were added to primary cultures together with [^{3}H]leucine (20 μCi/ml). After 24 hr of incubation, the medium was removed and the newly synthesized ^{3}H-fibrinogen was measured as described. As shown in Table 1, no significant changes were found in the amount of newly synthesized fibrinogen secreted into the medium. The large amount of incorporated radioactivity in experiment 2 (Table 1) reflects the use of a more active hepatocyte culture than in experiment 1. However, the addition of supernatants from activated leukocytes caused a 3-fold increase in ^{3}H-fibrinogen synthesis. Thus, the system is quite

Table 1. Direct addition of fibrinogen and fibrin degradative products to cultured rat hepatocytes

	μM	^{3}H-Fibrinogen secretion, cpm/mg/24 hr	% of control
Experiment 1			
Control	—	8240 ± 370	100
Fragment E	1.2	7580 ± 845	90
Fragment E	7.8	8870 ± 650	105
Fragment D	0.6	8005 ± 70	95
Fragment D	4.0	8550 ± 510	102
Fibrinogen	0.6	5975 ± 480	71
HSF, 1.3 units/ml	—	27,745 ± 2230	330
Experiment 2			
Control	—	45,020 ± 5495	100
Fibrin - E	8.0	51,960 ± 2890	115
Fibrin - D	8.0	41,435 ± 1705	92
HSF, 1.3 units/ml	—	125,890 ± 1240	280

Hepatocytes were incubated for 24 hr in the presence of [^{3}H]leucine together with each of the above proteins and fragments. The medium from each dish was then removed, the newly synthesized ^{3}H-fibrinogen was immunoprecipitated with GARF-Sepharose as described, and the labeled GARF-Sepharose was assayed in a liquid scintillation spectrometer. Values are means ± SD of triplicate samples.

responsive to agents known to affect hepatic synthesis of plasma proteins (15, 27, 28).

The Effect of Fragments D and E on Leukocytes. Because neither the FDP nor the fdp were capable of stimulating fibrinogen synthesis via direct interaction with the hepatocytes, studies were begun to determine if stimulation of fibrinogen could occur through an indirect pathway involving leukocytes. It seemed possible that fragment D or fragment E—or both—could have an effect on leukocytes because numerous studies have implicated the leukocytes as producers of mediators of the acute inflammatory reaction. To test this hypothesis, normal peripheral leukocytes were prepared as described and incubated with different concentrations of fragments D, E, or D plus E for 3 hr at 37°C in a shaking water bath. After incubation, the cells were pelleted, and GARF-Sepharose was added to each cell-free supernatant to remove any remaining FDP. The GARF-Sepharose-bound FDP were removed (as determined by antifibrinogen ELISA) by centrifugation, and the remaining samples were assayed for HSF activity by the cultured rat hepatocyte bioassay system.

The results of these experiments are shown in Fig. 2. When each sample was added to cultured hepatocytes at a final concentration of 50 μg/ml, samples containing D, E, or D plus E produced significant increases in the amount of fibrinogen secretion over that produced by a leukocyte sample incubated in the absence of FDP (i.e., P_i/NaCl/HSF). The leukocyte response to FDP appears to be specific for these fragments because leukocytes incubated in the presence of high concentrations of fibrinogen (3 μM) or albumin (5 μM) do not secrete detectable HSF (when assayed at 50 μg/ml).

Potency of Fragments D and E on HSF Production. The results shown in Fig. 2 demonstrate that fragments D and E can stimulate the synthesis of fibrinogen. The stimulation occurs by an indirect pathway that involves a leukocyte-derived factor. Leukocytes obtained from rabbits, rats, or humans secrete one or more protein mediators that cause various metabolic changes when administered to experimental animals (29–38). The protein fraction from rabbit leukocytes that contains these biological activities has collectively been termed leukocyte endogenous mediator, or LEM (34). We shall refer to the factor that stimulated hepatocytes directly as the hepatocyte-stimulating factor, or HSF. The amount of HSF produced appears to depend on the concentration of fragment D or E present in the leukocyte incubation medium.

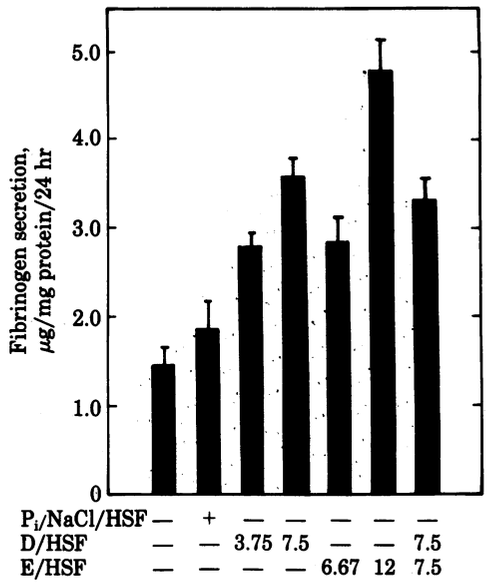

FIG. 2. Effect of leukocyte HSF (obtained from cells incubated with fragment D or E) on hepatocyte fibrinogen secretion. Six tubes containing 4×10^7 leukocytes per ml were incubated with or without fragment D or E (M_r fragment E = 40,000 and M_r fragment D = 80,000). After incubation at 37°C for 3 hr, a sufficient amount of GARF-Sepharose was added to each cell-free supernatant to remove any remaining D or E fragment. Each HSF sample (50 μg) was then added to triplicate 35-mm dishes containing hepatocytes in 1 ml of medium. The hepatocytes were incubated for 24 hr at which time the medium was collected and assayed by ELISA for fibrinogen. Indicated concentrations (μM) are those of fragments D, E, or D plus E.

To define more quantitatively the potency of each fragment on the production of HSF, a series of tubes—each containing 4×10^7 cells per ml in P_i/NaCl—was incubated with different concentrations of either fragment. The results of these experiments are shown in Fig. 3. Concentrations of either fragment between 1.0 and 10 μM increase HSF production by as much

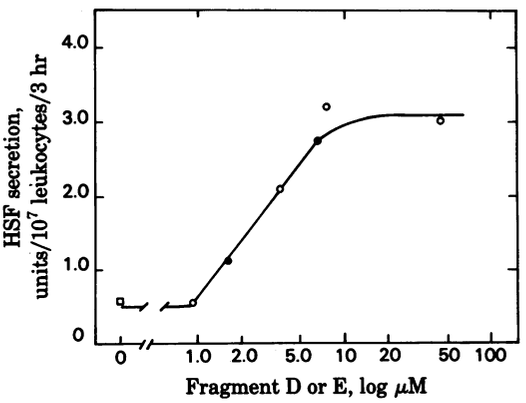

FIG. 3. Effect of fragments D and E on the rate of leukocyte HSF secretion. Seven tubes containing leukocytes (4×10^7 per ml) and various concentrations of fragment D (●) or E (○) were incubated for 3 hr at 37°C in a shaking water bath. After incubation, the cell-free supernatants were treated with GARF-Sepharose as described in the legend to Fig. 2. The specific activity (i.e., units/mg of HSF) was then determined by hepatocyte bioassay of each sample. The rate of HSF secretion for each sample was then determined from the respective specific activities and cell numbers. □, HSF secreted by cells incubated in P_i/NaCl alone.

as 6-fold over that produced by nonactivated (i.e., P_i/NaCl-incubated) leukocytes. Fibrin degradative fragments were also capable of stimulating the leukocytes to produce HSF (data not shown). It should be emphasized here that fragments of both fibrinogen and fibrin have similar potencies over the concentration range shown in Fig. 3.

Does HSF Regulate only Fibrinogen Synthesis? It has been suggested that the plasminolytic fragments of fibrinogen, specifically fragment D, only stimulated an increased synthesis of fibrinogen because no increase in either haptoglobin or albumin was detected after infusion of fragment D into rabbits (6) and rats (5). Our data demonstrate that fragment D or E has no direct effect on the hepatocyte's production of fibrinogen—rather that the stimulation comes through the action of fragment D or E or the leukocyte. This finding raises the possibility that fragment D or E may stimulate the production of a HSF specific for fibrinogen.

To test this notion, media samples taken from hepatocyte cultures that had been exposed to HSF from E-stimulated leukocytes were concentrated and analyzed by crossed-immunoelectrophoresis. Patterns shown in Fig. 4 demonstrate that several proteins in addition to fibrinogen are significantly increased, thus indicating that the stimulation produced by the HSF also involves other plasma proteins. These results agree with those of Fouad *et al.* (28). Furthermore, the crossed-immunoelectropherogram shows that haptoglobin is one of the proteins that responds to HSF. Albumin would not be expected to be increased because it is known that its synthesis is diminished during an acute inflammatory reaction (35).

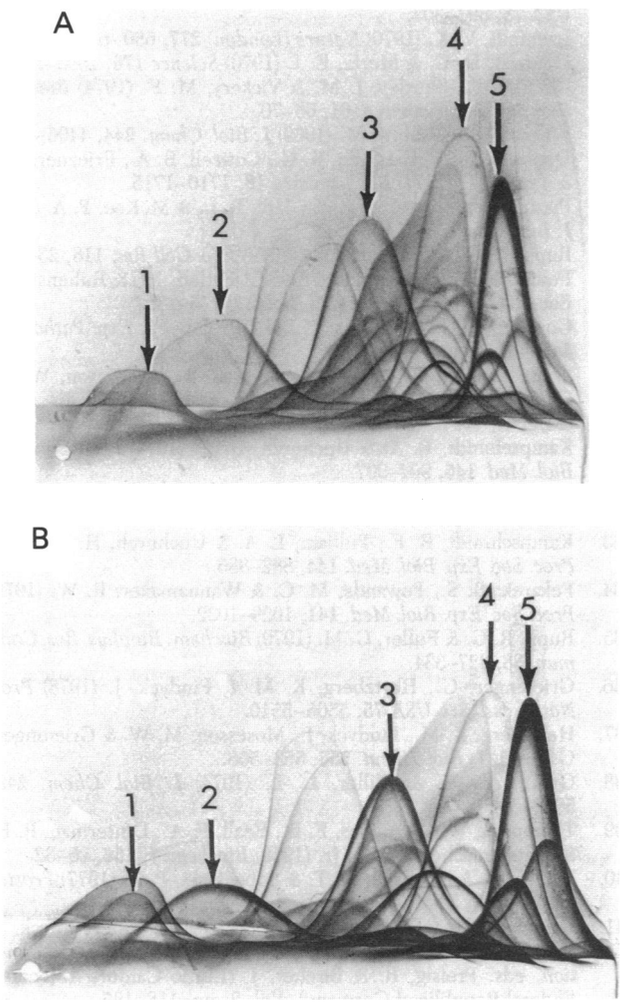

FIG. 4. Crossed-immunoelectrophoresis of media samples taken from HSF-stimulated hepatocytes. (*A*) Concentrated medium from hepatocytes that had been stimulated with HSF (the HSF was produced by leukocytes that had been stimulated by fragment E). (*B*) Concentrated medium from control (nonstimulated) hepatocytes. Note arrows show altered peak heights that reflect different concentrations. Numbered arrows correspond to: 1, fibrinogen; 2, not known; 3, haptoglobin; 4, not known; and 5, albumin.

DISCUSSION

The control mechanisms for plasma protein synthesis and secretion have been the subject of considerable interest in recent years. It appears that several different factors may be involved in regulation of fibrinogen synthesis including thrombin (9) as well as various hormones and serum factors (36–40). Of particular interest here is the suggestion that certain fragments of the parent molecule (fibrinogen) may exert some control over its own synthesis. If indeed this is correct, then it represents a truly unique feedback control mechanism for eukaryotic cells. We report here that in fact plasminolytic fragments of fibrinogen (fragments D, E, or both) are involved in the stimulation of fibrinogen synthesis, although the pathway is indirect and involves cells of the reticuloendothelial system.

Our finding that neither fragment D nor E exerts any direct regulatory role on fibrinogen synthesis was unexpected because numerous studies had implicated these peptides as regulators of fibrinogen synthesis. We reasoned that these peptides must be influencing the hepatocyte through some indirect pathway. Studies from many laboratories (30–33) as well as our own (15, 27) have demonstrated that certain leukocyte factors can stimulate an increase in selected plasma proteins including fibrinogen. It is well established that fibrinogen, fibrin, plasmin, and leukocytes (monocytes and neutrophils) would all be present at a site of inflammation. It seemed quite plausible that the leu-

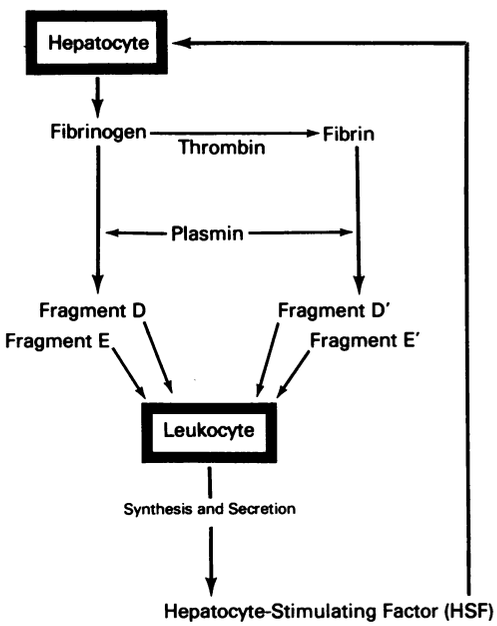

FIG. 5. Suggested indirect feedback pathway for fibrinogen and other plasma protein synthesis. The plasmin-derived fragments of fibrinogen or fibrin stimulate the production of HSF that is transported via the blood to the liver and signals the hepatocyte to increase synthesis and secretion of selected plasma proteins. Basically this scheme follows a natural physiologic pathway in which increased fibrin results in increased levels of D' and E' and thus in increased levels of acute-phase globulin.

kocyte could be a link in the pathway for fibrinogen synthesis that involves fragments D and E. When leukocytes were treated with fragment D or E they responded by producing a factor that when added to the isolated hepatocyte had a dramatic effect on fibrinogen synthesis. Neither fibrinogen nor fibrin stimulated leukocytes to product HSF; however, plasminolytic fragments D and E at the same molar concentration stimulated the production of HSF by the leukocytes.

It was important to determine the specificity of the HSF produced by the fragments because two other reports (4, 5) suggest that FDP had no apparent effect on the other plasma proteins. Several investigators have used two-dimensional immunoelectrophoresis to determine the relative amounts of a number of plasma proteins simultaneously (28, 37, 41). Our results with this technique show that the amounts of at least four proteins in addition to fibrinogen are noticeably enhanced as a result of HSF-stimulation of hepatocytes.

The data presented in this report demonstrate that a functional role for fibrinogen and fibrin degradative products is to activate leukocytes to produce a hepatocyte-stimulating factor. It is quite unlikely, however, that these peptides are the only agents capable of this activation. In fact, several reports have demonstrated that peritoneal leukocytes produce HSF in response to activating agents other than fibrinogen or fibrin fragments (15, 27). The dose-dependent response of isolated leukocytes to fragments D and E suggests that certain physiologic states in which there are high levels of the fragments in the circulation are associated with high levels of HSF. Although endogenous blood levels have not as yet been measured, we have determined in *in vitro* culture that fibrinogen synthesis can increase 3- to 4-fold when HSF reaches 1–3 units/ml.

In summary, we have presented evidence that demonstrates a unique feedback regulatory pathway for fibrinogen synthesis that involves specific plasminolytic fragments of the fibrinogen molecule itself. We have shown that the leukocytes can be stimulated in a dose-dependent manner by these fragments to produce a potent stimulatory factor that affects synthesis not only of fibrinogen but also of several other plasma proteins. Finally we suggest in a summary scheme (Fig. 5) an indirect feedback loop for control of plasma levels of fibrinogen during an inflammatory state.

We thank our colleagues, Drs. Darrell Carney and Paul Weigel, for critically reviewing the manuscript. We also thank Mrs. Kathy Albright for her efforts in preparing the manuscript. This work was supported in part by National Institutes of Health Grant HL 16445 and the James W. McLaughlin Fellowship Fund.

1. Marder, V. J. & Budzynski, A. Z. (1973) *Thromb. Diath. Haemorrh.* **33**, 199–207.
2. Budzynski, A. Z. & Marder, V. J. (1977) *Thromb. Haemostasis Gen. Info.* **38**, 793–800.
3. Barnhardt, M. I., Cress, D. C., Noonan, S. M. & Walsh, R. T. (1970) *Thromb. Diath. Haemorrh. Suppl.* **39**, 143–159.
4. Bocci, V., Conti, T., Muscetalla, M., Pacini, A. & Pessina, G. P. (1974) *Thromb. Diath. Haemorrh.* **31**, 395–402.
5. Franks, J. J., Kirsch, R. R., Frith, O. C., Purves, L. R., Franks, J. A., Mason, P. & Saunders, S. J. (1981) *J. Clin. Invest.* **67**, 575–580.
6. Kessler, C. M. & Bell, W. R. (1979) *J. Lab. Clin. Med.* **93**, 758–767.
7. Kessler, C. M. & Bell, W. R. (1979) *J. Lab. Clin. Med.* **93**, 768–782.
8. Kessler, C. M. & Bell, W. R. (1980) *Blood* **55**, 40–47.
9. Alving, B. M., Evatt, B. L. & Bell, W. R. (1977) *Am. J. Physiol.* **233**, H562–H567.
10. Alving, B. M., Bell, W. R. & Evatt, B. L. (1977) *Am. J. Physiol.* **232**, H478–H484.
11. Ittyerah, T. R., Weidner, N., Wochner, R. D. & Sherman, L. A. (1979) *Br. J. Haematol.* **43**, 661–668.
12. Otis, P. T. & Rapaport, S. I. (1973) *Proc. Soc. Exp. Biol. Med.* **144**, 124–129.
13. Seglen, P. O. (1973) *Exp. Cell Res.* **82**, 391–398.
14. Weigel, P. H., Schnaar, R. L., Kuhlenschmidt, M. S., Schmell, E., Lee, R. T., Lee, Y. C. & Roseman, S. (1979) *J. Biol. Chem.* **254**, 10830–10834.
15. Ritchie, D. G. & Fuller, G. M. (1981) *Inflammation* **5**, 287–299.
16. Kwan, S.-W., Fuller, G. M., Krautter, M. A., van Bavel, J. H. & Goldblum, R. G. (1977) *Anal. Biochem.* **83**, 589–595.
17. Ritchie, D. G., Nickerson, J. M. & Fuller, G. M. (1981) *Anal. Biochem.* **110**, 281–290.
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
19. Clark, H. M. G. & Freeman, T. (1968) *Clin. Sci.* **35**, 403–413.
20. Nickerson, J. M. & Fuller, G. M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 303–307.
21. Laemmli, V. K. (1970) *Nature (London)* **277**, 680–685.
22. Deutsch, D. G. & Mertz, E. I. (1970) *Science* **170**, 1095–1096.
23. Chen, J. P., Shurley, J. M. & Vickery, M. F. (1974) *Biochem. Biophys. Res. Commun.* **61**, 66–70.
24. Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412.
25. Doolittle, R. F., Cassman, K. G., Cottrell, B. A., Friezner, S. J. & Takagi, T. (1977) *Biochemistry* **16**, 1710–1715.
26. Pizzo, S. V., Schwartz, M. L., Hill, R. L. & McKee, P. A. (1972) *J. Biol. Chem.* **247**, 636–645.
27. Rupp, R. G. & Fuller, G. M. (1979) *Exp. Cell Res.* **118**, 23–30.
28. Fouad, F. M., Scherer, R., Abd-El-Fattah, M. & Ruhenstroth-Bauer, G. (1980) *Eur. J. Cell Biol.* **21**, 175–182.
29. Gordon, A. H. & Limaos, E. A. (1979) *Br. J. Exp. Pathol.* **60**, 441–447.
30. Wannemacher, R. W., Jr., Pekarek, R. S., Thompson, W. V., Curnow, R. T., Beall, R. A., Zenzer, T. V., DeRobertis, F. R. & Beisel, W. R. (1975) *Endocrinology* **96**, 651–661.
31. Kampschmidt, R. A. & Upchurch, H. F. (1974) *Proc. Soc. Exp. Biol. Med.* **146**, 904–907.
32. Bornstein, D. L. & Walsh, E. C. (1978) *J. Lab. Clin. Med.* **91**, 236–245.
33. Kampschmidt, R. F., Pulliam, L. A. & Upchurch, H. F. (1973) *Proc. Soc. Exp. Biol. Med.* **144**, 882–886.
34. Pekarek, R. S., Powanda, M. C. & Wannamaker, R. W. (1972) *Proc. Soc. Exp. Biol. Med.* **141**, 1029–1032.
35. Rupp, R. G. & Fuller, G. M. (1979) *Biochem. Biophys. Res. Commun.* **88**, 327–334.
36. Grieninger, G., Hertzberg, K. M. & Pindyck, J. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5506–5510.
37. Hertzberg, K. M., Pindyck, J., Mosesson, M. W. & Grieninger, G. (1981) *J. Biol. Chem.* **256**, 563–566.
38. Griffin, E. E. & Miller, L. L. (1974) *J. Biol. Chem.* **249**, 5062–5069.
39. Thompson, W. L., Abeles, F. B., Beall, F. A., Dinterman, R. E. & Wannemacher, R. W., Jr. (1976) *Biochem. J.* **156**, 25–32.
40. Mapes, C. A., George, D. T. & Sobocinski, P. Z. (1977) *Prostaglandins* **13**, 73–85.
41. Grieninger, G., Hertzberg, K. M., Kiang, T.-Y. & Pindyck, J. (1979) in *The Liver. Quantitative Aspects of Structure and Function*, eds. Preisig, R. & Bircher, J. (Editio Cantor, Aulendor, Federal Republic of Germany), Vol. 3, pp. 118–125.