Journal of Clinical Investigation
Vol. 43, No. 4, 1964

Abnormal Plasminogen-Plasmin System Activity (Fibrinolysis) in Patients with Hepatic Cirrhosis: Its Cause and Consequences *

ANTHONY P. FLETCHER, OLIVER BIEDERMAN, DON MOORE, NORMA ALKJAERSIG, AND SOL SHERRY

(From the Department of Internal Medicine, Washington University School of Medicine, and the Jewish Hospital, St. Louis, Mo.)

Patients suffering from hepatic cirrhosis of moderate or advanced degree frequently have an ill-defined coagulation disorder and may, especially if subjected to operative stress, develop a severe hemorrhagic diathesis, often associated with pathological plasma proteolysis (pathological fibrinolysis). Moreover, many such patients may exhibit, constantly or intermittently, evidence of abnormal plasminogen-plasmin system activity (fibrinolysis), documented by rapid lysis of their clotted blood and other tests.

Interest in this latter phenomenon stems from Goodpasture's paper (1), published in 1914, in which he reported that blood, drawn from four cirrhotic patients, when clotted, underwent spontaneous lysis more rapidly than did that from control subjects. Later Ratnoff (2) confirmed this finding and substantially extended study of this phenomenon. He noted that rapid plasma clot lysis was a frequent accompaniment of Laënnec's cirrhosis, was sometimes observed in patients who experienced hepatic damage during the course of some other illness, but was not observed in either acute hepatitis or obstructive jaundice without obvious associated hepatic damage. Subsequently, other authors (3–8) have reported that a high proportion of patients, suffering from advanced hepatic cirrhosis, exhibited rapid spontaneous whole blood clot lysis, shortened euglobulin lysis times, and other evidence of enhanced plasminogen-plasmin system activity. Moreover it has been reported (7, 9, 10) that the administration of drugs capable of eliciting enhanced thrombolytic activity in the normal subject produced an anomalous and exaggerated response in the cirrhotic patient.

Hitherto, despite the apparent clear-cut nature of these phenomena, their interpretation and significance have remained obscure owing to deficiencies in understanding of the function of the plasminogen-plasmin system *in vivo*. Though recent studies (11–14) have delineated the crucial role of plasminogen activator in the induction of physiological thrombolytic phenomena and in the genesis of pathological plasma proteolytic (fibrinolytic) states, neither the mechanisms underlying *in vivo* release of plasminogen activator nor those responsible for the rapid disappearance of this moiety from the circulation have been fully elucidated.

The aim of the present study has been to define the anomaly of plasminogen system function responsible for the enhanced fibrinolytic activity in the cirrhotic patient and to assess the significance of this enhanced activity in the genesis of the coagulation disorder that may complicate this condition. The first problem has been investigated primarily by determination of the respective plasma clearance rates for plasminogen activator in normal subjects and in cirrhotic patients and the second by studies designed to relate changes in the activity of the plasminogen system to the development of the recently described and distinctive coagulation disorder, defective fibrin polymerization (15–18). In this latter disorder, arising secondarily to pathological plasma proteolysis, high molecular weight fibrinogen fragments (19, 20) circulate in plasma and, by inhibiting the polymerization of fibrin monomer, produce disturbances in hemostatic function.

* Submitted for publication September 6, 1963; accepted December 4, 1963.

Presented in part at the Seventy-sixth Annual Meeting of the American Association of Physicians, Atlantic City, N. J., 1963. This work was supported by a grant from the National Heart Institute (H-3745), U. S. Public Health Service.

The investigative protocol involved the induction of enhanced plasma thrombolytic activity in patients suffering from moderately advanced or advanced hepatic cirrhosis and in other subjects without liver disease by one of two procedures. The majority of studies were performed with intravenously administered nicotinic acid used to induce enhanced plasma thrombolytic activity, but in some patients, suffering from mental disease, studies were performed after therapeutic electroshock. With both stimuli the response of the cirrhotic patients, both with respect to its degree and duration, greatly exceeded that of subjects without liver disease. The degree and persistence of the rise in plasma thrombolytic activity in the cirrhotic patients was of such magnitude as to induce coagulation alterations of the type observed in patients suffering from pathological plasma proteolysis. Moreover, the data on the clearance of plasminogen activator indicated that these findings were attributable to gross deficiency in the ability of cirrhotic patients to clear plasminogen activator from the circulation. Consequently, it was concluded that the normal liver exerts important control functions over plasma thrombolytic activity and that loss of this hepatic clearance function, as a consequence of certain hepatic diseases, increases susceptibility to the development of pathological plasma proteolysis and to the coagulation disorder of defective fibrin polymerization.

Methods

Plasma thrombolytic activity was assayed by the euglobulin lysis time as previously modified to permit expression of the results in units (11) and by the I^{131}-labeled clot lysis assay (12, 13). The plasma euglobulins were precipitated from 0.5 ml plasma according to the method of Milstone (19 vol water containing 0.32 vol 1% acetic acid added to 1 vol plasma) (21). After centrifugation the precipitate was resuspended in 0.5 ml 0.01 M Veronal buffer, pH 7.4, and clotted with 0.5 ml thrombin (2 U per ml). Clots were incubated at 37° C, and the time for complete lysis was recorded. For the I^{131} assay, clots were prepared from 0.2 ml bank blood plasma to which 0.5 casein U of plasminogen and sufficient I^{131}-labeled fibrinogen were added to yield a final count for the fully lysed clot of 25,000 cpm per clot. 0.2 ml of the patient plasma to be tested (refrigerated on drawing and assayed within a 2-hour period) was incubated with a single clot for 2 hours at 37° C. Determination of supernatant radioactivity and plasma thrombolytic activity was carried out as previously described (13).

Plasma plasminogen, antiplasmin, fibrinogen, the plasma thrombin clotting time (by a procedure utilizing thrombin titration mixture), and the plasma urokinase lysis time (a method for detecting the presence of inhibitors to plasminogen activator) were determined as previously described (12, 22).

Patient groups: electroshock series. Observations were made on 12 patients suffering from mental depression, before and after unmodified electroshock therapy. The group contained three patients, in whom the diagnosis of hepatic cirrhosis had been confirmed by hepatic needle biopsy, and nine patients without clinical or laboratory evidence, including normal bromsulfophthalein (B.S.P.) excretion tests, of hepatic disease. Venous samples, refrigerated on drawing, were collected before and 1, 5, 15, 30, 60, 120, and 180 minutes after electroshock, during which each patient experienced a typical convulsive seizure.

Nicotinic acid series. There were 32 patients in this group: eight patients with moderately advanced or advanced hepatic cirrhosis; three patients in whom hepatic cirrhosis was suspected clinically and on biochemical findings but in whom repeated hepatic biopsy failed to confirm the diagnosis; four patients with carcinomatous involvement of the liver (one primary and three metastatic from breast, colon, and stomach); and 17 subjects without clinical or biochemical evidence of hepatic disease. Each received 100 mg nicotinic acid intravenously over a 3-minute period and developed a typical flush. Though differences in intensity and duration of the flush were noted, this was not correlated with the subsequent response in plasma thrombolytic activity. One patient, suffering from hepatic cirrhosis, experienced, immediately after the injection, a brief episode of syncope and hypotension, probably of vasovagal origin. Blood samples, immediately refrigerated, were withdrawn before and 10, 15, 30, 60, 120, 180, and 240 minutes after completion of the injection.

The existence of hepatic cirrhosis was documented in all patients with this diagnosis by appropriate historical, physical, and laboratory findings, and in all patients except one, where the diagnosis was confirmed at autopsy, by positive needle biopsy histology. The initial biochemical findings in the cirrhotic group, in whom the majority of studies with nicotinic acid was performed, are shown in Table I; the findings attest to the generally advanced nature of the disorder in this patient group.

Control observations were made on convalescent middle-aged hospitalized patients without fever, hematological disorders, or other clinical or biochemical evidence of hepatic disease. Control series patients had usually been admitted for the study of chronic rather than acute medical disorders. The data shown in Figures 5 and 6 were obtained from apparently healthy laboratory personnel.

Statistical testing was by the *t* test, using the method of paired comparison (23) for determining the significance of experimental deviation from control values. Standard terminology has been used to designate components of the plasminogen-plasmin system (24).

TABLE I

*Diagnostic and biochemical findings in the group of cirrhotic patients who received nicotinic acid and in whom the majority of observations was made**

Age	Sex	Remarks	Bilirubin	Alkaline phosphatase	Albumin	Globulin	S-GOT	S-GPT	BSP retention	C-CF
			mg/100 ml	*Bodansky U/ml*	*g/100 ml*	*g/100 ml*	*U/ml*	*U/ml*	%	
72	F	Biopsy—Laënnec's cirrhosis, ascites, esophageal varices; died	4.5	6.4	2.2	3.2	116	39	33	4+
19	F	Biopsy—postnecrotic cirrhosis (pregnancy)	2.6	9.0	3.5	4.4	150	100		4+
70	M	Biopsy—Laënnec's cirrhosis, Prothrombin time, 53%	14.7	4.8	2.0	4.5	610	390	29	3+
54	F	Biopsy—Laënnec's cirrhosis, esophageal varices	21.1	10.6	2.8	3.2	80	51	28	
65	M	Biopsy—Laënnec's cirrhosis, ascites	2.6	2.4	2.4	3.7	46	6	40	4+
59	F	Biopsy—Laënnec's cirrhosis, esophageal varices	6.6	3.5	2.5	2.3	65	52	70	4+
45	M	Biopsy—Laënnec's cirrhosis	4.9	5.2	3.0	3.0	140	62	41	

* Abbreviations: S-GOT, serum glutamic oxaloacetic transaminase; S-GPT, serum glutamic pyruvic transaminase; BSP, bromsulfophthalein; C-CF, cephalin-cholesterol flocculation.

Results

Enhanced plasma thrombolytic activity after nicotinic acid or electroshock. Figure 1 illustrates serial determinations of plasma thrombolytic activity in cirrhotic patients (the two left-hand portions of Figure 1) and in normal subjects without hepatic disease (the two right-hand portions) after the iv injection of 100 mg nicotinic acid. Plasma thrombolytic activity was determined by the euglobulin lysis time (upper portion of Figure 1) and by the I^{131}-labeled fibrin clot method (lower portion). Several patients were studied by both methods, but some were studied by only one; consequently, the two series, illustrated respectively at the top and bottom of Figure 1, though comparable, are not identical.

Nicotinic acid injection produced, in both normal subjects and cirrhotic patients, a sharp increase in plasma thrombolytic activity. This response was maximal at approximately 15 minutes, but both the degree of response and its duration in the cirrhotic subject greatly exceeded that of the normal subjects. At 15 minutes, the average increase of plasma thrombolytic activity in the cirrhotic group was 2 to 3 times that of the normal subjects, and whereas plasma thrombolytic activity in the normal subjects had returned to base-line values within 1 hour, enhanced plasma thrombolytic activity persisted for 2 to 4 hours in the cirrhotic patients.

Statistical testing showed that the difference in response between the two groups was highly significant, $p < 0.001$ for each of the two methods (euglobulin lysis and isotopic assay) tested independently. The ratio of response, with respect to plasma thrombolytic activity, between the two groups was determined by measurement, using planimetry of the areas under the response curves. Comparison, in this manner, revealed that the average over-all increase of plasma thrombolytic activity in the cirrhotic patients was 5 times that of the controls, the euglobulin method showing a 6-fold difference and the isotopic method a 4-fold one. This discrepancy between the results of the two methods probably was attributable to the fact that the patient groups tested by each method, although overlapping, were not identical. Figure 1 illustrates that the results with the two methods were well correlated and exhibited no divergence between the general shape of the response.

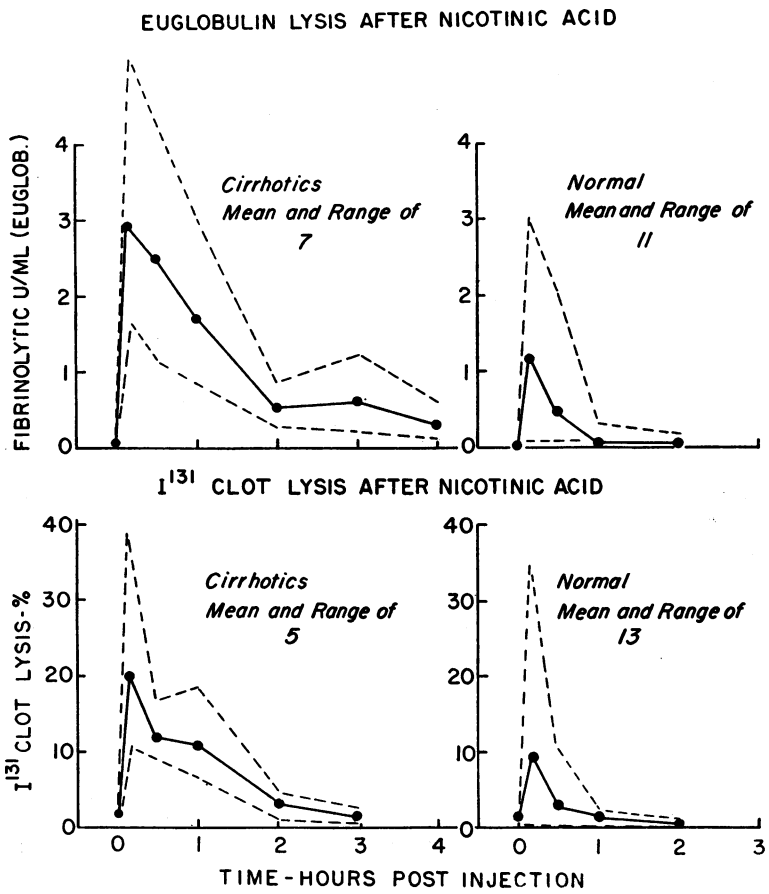

FIG. 1. PLASMA THROMBOLYTIC ACTIVITY (MEAN AND RANGE OF OBSERVATIONS, ORDINATE) AFTER NICOTINIC ACID INJECTION IN CIRRHOTIC PATIENTS AND IN NORMAL SUBJECTS AS A FUNCTION OF TIME (ABSCISSA). Plasma thrombolytic activity was determined by euglobulin lysis and by the I^{131} clot lysis technique.

The striking differences demonstrated between normal subjects and cirrhotic patients with respect to the pattern of enhanced plasma thrombolytic activity developing after nicotinic acid was also apparent after the use of a quite dissimilar stimulus, therapeutic electroshock. These data, plasma thrombolytic activity being assayed by the euglobulin lysis technique, are shown in Figure 2. Whereas, in the normal subjects, electroshock produced a mean peak elevation of plasma thrombolytic activity of 0.4 U at 1 minute with decline to virtually base-line values within 30 minutes, the corresponding observation for the three cirrhotic patients was a level of 1.6 U at 1 minute with maintenance of sustained levels for 2 to 3 hours. The difference in response between the two

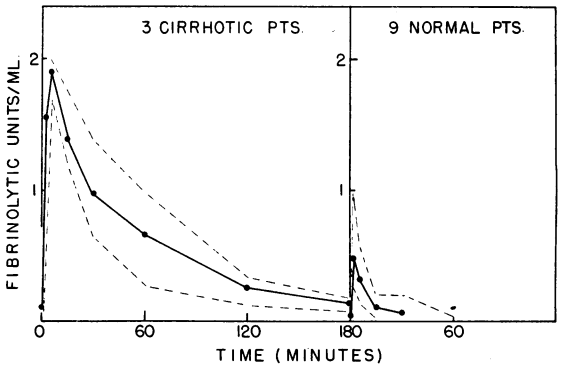

FIG. 2. PLASMA THROMBOLYTIC ACTIVITY (MEAN AND RANGE OF OBSERVATIONS) IN EUGLOBULIN UNITS (ORDINATE) AS A FUNCTION OF TIME (ABSCISSA) AFTER ELECTROSHOCK IN THREE CIRRHOTIC PATIENTS AND NINE NORMAL SUBJECTS.

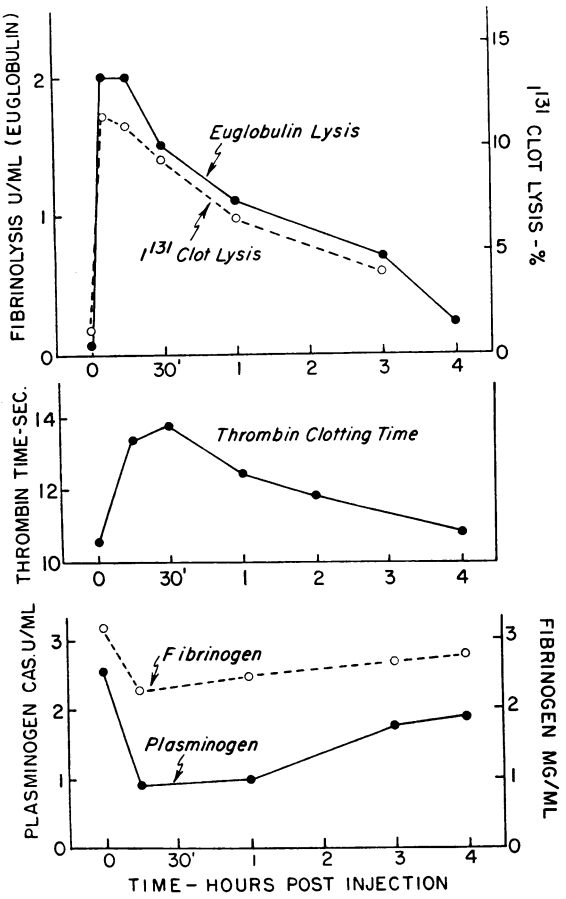

FIG. 3. OBSERVATIONS MADE IN A SINGLE CIRRHOTIC PATIENT AFTER THE IV INJECTION OF 100 MG NICOTINIC ACID. The ordinates are, respectively, from top to bottom: plasma thrombolytic activity determined by both euglobulin lysis and I^{131} lysis techniques, the thrombin clotting time, plasma fibrinogen, and plasminogen. The abscissa is time after injection.

groups, determined by planimetry of the areas under the respective response curves, was 16 times greater for the cirrhotic as compared to the normal group. Testing of this difference, by the method of paired comparison, indicated a statistical significance at a level of $p < 0.001$.

Secondary effects of plasminogen activator excess. The exaggerated and long-sustained increase in plasma plasminogen activator concentration that occurred in cirrhotic patients after nicotinic acid injection was associated with alterations in other plasminogen system components and in plasma substrates susceptible to plasmin digestion. Figure 3 illustrates such findings in a patient suffering from advanced hepatic cirrhosis who received 100 mg nicotinic acid at zero time. The upper portion of Figure 3 documents, by the use of both the euglobulin lysis and I^{131}-labeled clot assays, the substantial long-sustained rise of plasma thrombolytic activity that followed nicotinic acid injection and again illustrates the excellent correlation observed between these two methods. Concomitantly with the early maximal increase of plasma plasminogen activator, which occurred at 15 minutes, there was a sharp fall of plasma plasminogen (bottom section of Figure 3) from 2.6 to 0.9 casein U per ml plasma; the plasminogen concentration remained depressed during the period of maximal thrombolytic response, subsequently rising to 1.9 casein U per ml plasma at the end of the observation period (4 hours). The initial rate of plasmin formation, consequent to the activation of 1.7 casein U of plasminogen per ml plasma in 15 minutes, was so rapid that plasmin inhibition by antiplasmin was incomplete; as a consequence, plasma fibrinogen fell 28% (bottom of Figure 3), although by the end of the observation period it was returning towards its control value. The thrombin clotting time (middle of Figure 3) rose from 10 to 13.8 seconds, a finding attributable to the presence of fibrinogen proteolysis products in plasma, as demonstrated by the use of a specific immunochemical assay.[1]

Plasma plasminogen, fibrinogen, and thrombin clotting time determinations for all subjects receiving nicotinic acid are presented in Table II. The top line of each table section displays zero time determinations for the normal and cirrhotic groups. Plasma plasminogen and fibrinogen were significantly higher in the normal group (plasminogen, 2.71 versus 1.86 casein U per ml plasma and fibrinogen, 3.18 versus 2.58 mg per ml plasma), but the thrombin time differences, 10.0 seconds as compared to 10.7 seconds, were not statistically different.

After nicotinic acid, the normal group exhibited

[1] During *in vivo* states resulting in fibrinogen proteolysis (pathological plasma proteolytic states) high molecular weight fibrinogen fragments may be readily demonstrated in plasma by immunoelectrophoretic techniques. Such specific fibrinogen fragments were demonstrated, during the present investigation by using preliminary electrophoresis of plasma in acrylamide gel for proteolysis product separation followed by immunodiffusion against specific antisera for identification (25). The full findings will be reported separately.

only minor decreases in plasma plasminogen (maximum, 0.33 casein U per ml at 15 minutes) and in plasma fibrinogen (maximum, 0.46 mg per ml at 15 minutes) with a small increase in the plasma thrombin clotting time (1.1 seconds at 30 minutes); although the fall of plasma fibrinogen reached the customary boundary of statistical significance ($p = 0.05$), all changes were transitory, and testing of the combined determinations, obtained over the 1-hour observation period, against the control values showed only nonsignificant decreases for plasma plasminogen and fibrinogen with an increase of doubtful significance ($0.05 < p < 0.1$) for the plasma thrombin clotting time. In contrast, effects of much greater magnitude were observed in cirrhotic subjects receiving nicotinic acid. At 15 minutes plasminogen decrease averaged 0.92 casein U per ml plasma, fibrinogen fall averaged 0.83 mg per ml plasma, and the increase in the thrombin clotting time was 3.2 seconds. All these changes were statistically significant. Moreover the table shows that these changes were persistent, and testing of all values obtained during the 1-hour observation period against the control determinations revealed that the cirrhotic group developed, in response to the stimulus, highly significant decreases of plasminogen and fibrinogen with a similarly significant increase of the thrombin clotting time.

Plasma inhibitors. *In vivo,* the biological activities of the plasminogen-plasmin system are in part controlled by and dependent upon the ratio of plasma inhibitors to active enzymatic moieties. Antiplasmin concentration was assayed in the plasma of five cirrhotic patients and in 57 normal subjects. Antiplasmin assays, expressed as percentage inhibition of a standard plasmin solution, were 68% (range, 55 to 80%) for 0.1 ml cirrhotic plasma and 69.1% (range, 57 to 84%) for normal plasma, whereas for 0.05 ml cirrhotic plasma the assays were 42% (range, 25 to 55%) and 41.6% (range, 16 to 66%) for normal plasma; neither of these differences was statistically significant ($p > 0.1$). Thus, despite the abnormal plasma protein patterns of the cirrhotic group (Table I), plasma antiplasmin in cirrhotic patients was within the normal range.

Plasma activator inhibitor was assayed in normal and cirrhotic plasma by determining plasma inhibitory action on the plasminogen activator, urokinase. Figure 4 illustrates the mean lysis times and their standard deviations for four cir-

TABLE II

*Plasma plasminogen, fibrinogen, and thrombin clotting time determinations in normal and cirrhotic patients receiving nicotinic acid at zero time**

Assay	Time after injection	Normal subjects	Significance of deviation from control values	Cirrhotic patients	Significance of deviation from control values	p Values, normal versus cirrhotic subjects (zero time)
	min		*p*		*p*	
Plasminogen, *casein U/ml plasma*	0	2.71 ± 0.72		1.86 ± 0.45		0.02 < p < 0.05
	15	2.38 ± 0.66	>0.1	0.94 ± 0.17	0.001 < p < 0.01	
	30	2.45 ± 0.78	>0.1	1.00 ± 0.27	0.001 < p < 0.01	
	60	2.39 ± 0.67	>0.1	1.21 ± 0.40	0.02 < p < 0.05	
	15, 30, and 60 values combined	2.40 ± 0.69	>0.1	1.05 ± 0.32	<0.001	
Fibrinogen, *mg/ml plasma*	0	3.18 ± 0.31		2.58 ± 0.67		0.02 < p < 0.05
	15	2.72 ± 0.36	0.05	1.75 ± 0.235	0.02 < p < 0.05	
	30	2.93 ± 0.34	>0.1	2.05 ± 0.36	>0.1	
	60	3.18 ± 0.39	>0.1	2.30 ± 0.55	>0.1	
	15, 30, and 60 values combined	2.94 ± 0.40	>0.1	2.03 ± 0.45	0.02 < p < 0.05	
Plasma thrombin clotting time, *seconds*	0	10.0 ± 0.6		10.7 ± 0.6		>0.1
	15	10.7 ± 1.15	>0.1	13.9 ± 1.8	0.001 < p < 0.01	
	30	11.09 ± 1.33	>0.1	14.1 ± 1.6	0.001 < p < 0.01	
	60	10.9 ± 1.44	>0.1	12.3 ± 1.7	0.02 < p < 0.05	
	15, 30, and 60 values combined	10.9 ± 1.22	0.05 < p < 0.1	13.4 ± 1.8	0.001 < p < 0.01	

* Means and standard deviations are given for group determinations at 15, 30, and 60 minutes and for the combined readings obtained during the observation period (15, 30, and 60 minutes totaled). Significance values for each patient group were calculated by the method of paired comparison with zero time values as control figures. The right-hand column shows significance for the differences between groups at zero time.

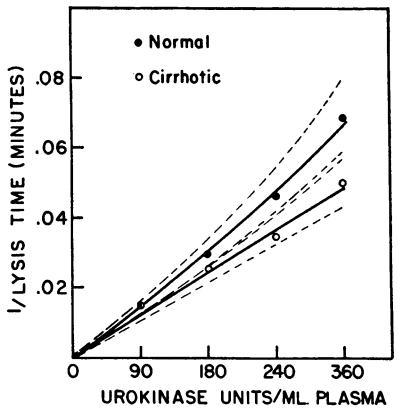

FIG. 4. MEAN AND RANGE OF UROKINASE LYSIS TIME DETERMINATIONS ON PLASMA FROM FIVE NORMAL AND FOUR CIRRHOTIC PATIENTS. The ordinate is reciprocal of lysis time, and the abscissa, urokinase units per milliliter plasma. Significant differences between the two sets of plasmas were not detected ($p > 0.1$).

rhotic and five normal sera; fibrinogen was added and the mixture clotted by thrombin after the addition of four different concentrations of urokinase. For each individual urokinase concentration the cirrhotic plasmas exhibited somewhat slower lysis times than did the normal plasmas, although the differences were not statistically significant ($p > 0.1$). The absence of a statistically significant difference between the two sets of determinations indicates that the abnormal response of the cirrhotic to nicotinic acid and electroshock could not be explained by a significant lack of activator inhibitor.

Studies in patients with hepatic disorders other than cirrhosis. Three patients, all alcoholics, in whom the diagnosis of hepatic cirrhosis had been suspected on clinical and laboratory grounds but not confirmed on biopsy, were also studied after the injection of 100 mg nicotinic acid. In these cases, hepatic biopsy, repeated in two patients, showed only fatty infiltration with mild fibrosis and no evidence of regenerative phenomena.

These patients responded to nicotinic acid in a manner indistinguishable from that of the normal subjects and unlike that of the cirrhotic group. Determinations for plasma activator, plasma plasminogen, and plasma fibrinogen and the change in thrombin clotting time all lay within the range of the normal, or the low normal, response.

Four patients with carcinoma of the liver (one with primary hepatoma and three with metastatic hepatic involvement) were studied after nicotinic acid injection. In this patient group there was a virtual failure of plasma thrombolytic activity to increase in response to nicotinic acid injection. In striking contrast to the behavior of both normal and cirrhotic groups, only one of the serial specimen euglobulin lysis tests (totaling 16) showed any alteration from control values and that only to 0.82 U. Test by the chi square procedure (i.e., detectable versus no detectable alteration of serial sample from control sample value) revealed that this failure of the neoplastic group to respond to nicotinic acid injection was highly significant when compared with the behavior of the normal group ($p < 0.001$). The technical validity of this finding was confirmed by the results of the isotopic assay method run on two lots of these serial samples; this method also showed no deviation from base-line values with

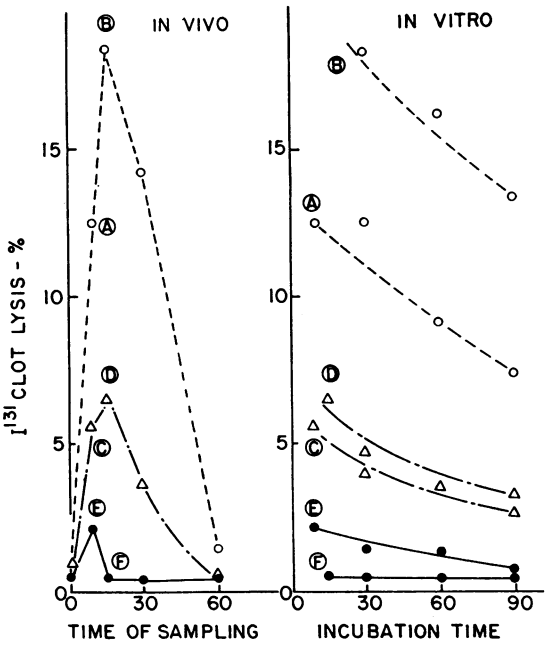

FIG. 5. RESPONSE OF PLASMA THROMBOLYTIC ACTIVITY TO THE INTRAVENOUS INJECTION OF NICOTINIC ACID (left-hand section) AND THE *in vitro* ACTIVITY DECAY CURVES, AT 37° C, OF THESE PLASMA SPECIMENS (right-hand section). The ordinates are plasma thrombolytic activity; the left-hand abscissa, time after nicotinic acid injection; and the right-hand abscissa, time of plasma specimen incubation, *in vitro*, at 37° C. Three patients, their specimens marked, respectively, A and B, C and D, and E and F, were studied (left-hand section), and the activator decay curve, *in vitro*, of each similarly labeled plasma sample is shown in the right-hand section.

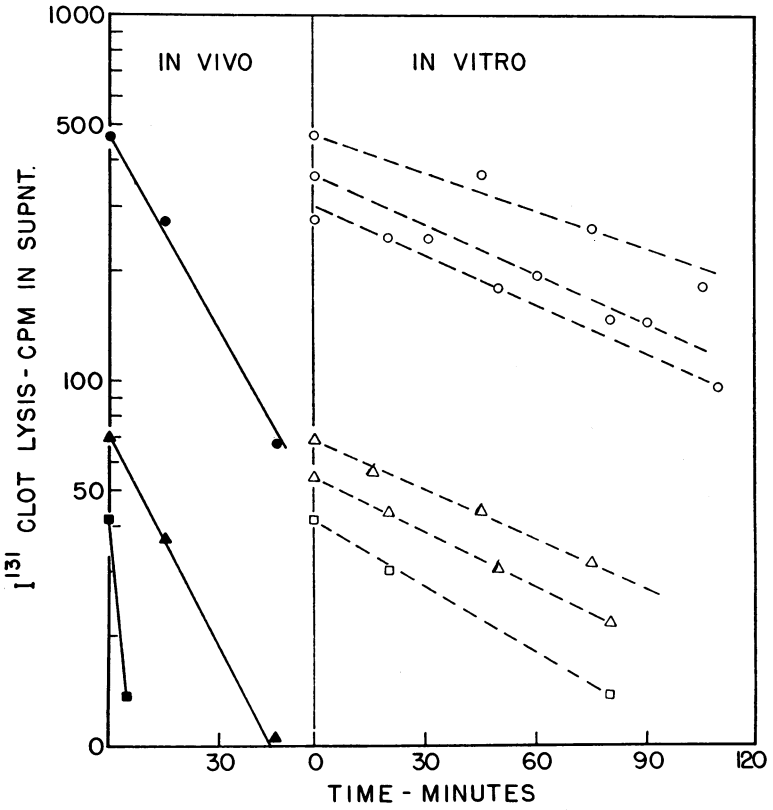

FIG. 6. THE DATA FROM FIGURE 5 IN EXPONENTIAL FORM. The ordinate is log activator concentration, and the abscissas are time. On the left, peak *in vivo* activity is defined as zero time, and on the right, zero time marks the commencement of *in vitro* plasma incubation. Both the *in vivo* disappearance rate and the *in vitro* activator decay rates at 37° C are an exponential function of activator concentration, although the former is much more rapid.

these sample sets. Moreover assays for plasminogen, on three of the sample sets, failed to reveal any alteration in this component as a result of nicotinic acid injection. One patient, who received a subcutaneous dose of 0.5 ml 1/1,000 epinephrine also failed to show any plasma thrombolytic activity response, a highly unusual finding (7, 26).

In vivo clearance of plasminogen activator. Figure 5 illustrates the response of plasma thrombolytic activity (measured by the I^{131} radiochemical assay) in three normal subjects, after the intravenous injection of 100 mg nicotinic acid. The left-hand portion of the diagram shows that, like the normal plasma thrombolytic response illustrated in Figure 1, plasma thrombolytic activity reached its peak at 10 to 15 minutes and then declined rapidly, so that by 60 minutes it had fallen to near control or control levels.

The right-hand panel of Figure 5 displays data designed to determine the *in vitro* decay rate of plasminogen activator in plasma. Plasma samples from the subjects studied in the left-hand panel, drawn during the period of enhanced thrombolytic activity, were incubated at 37° C for varying time intervals before reassay for thrombolytic activity. These decay curves, shown in the right-hand panel, are marked to indicate their identity with respect to the left-hand panel samples, i.e., the decay curve marked A on the right was derived from plasma sample A shown on the left. The decay curves for plasminogen activator in plasma at 37° C, shown on the right, indicate that decay rates are at least partially a function of plasmino-

gen activator concentration, being more rapid when it is high and lessening as concentration falls. Figure 6, an exponential plot of the data contained in Figure 5, demonstrates that both the *in vivo* plasma "clearance" data and the *in vitro* decay data for plasminogen activator, incubated in plasma at 37° C, are exponential functions of activator concentration [2] with respect to time. Consequently the data for each of these individual patient studies may be properly expressed as 50% plasma clearance or 50% plasma decay times. Moreover, the data of Figures 5 and 6 show that the *in vivo* disappearance rate of plasminogen activator (left-hand sections) is much faster than can be accounted for on the basis of *in vitro* activator decay rates at 37° C (right-hand portions).

Plasminogen activator clearance data in normal and cirrhotic patients. Analysis of the data obtained from the cirrhotic patients indicated, in line with the findings from the normal patients discussed above, that both the *in vivo* clearance of plasminogen activator and the *in vitro* decay rate of plasminogen activator in cirrhotic plasma at 37° C were exponential with time. Consequently the total averaged data for the normal and cirrhotic patients are shown in Figure 7, plotted in exponential form as log activator concentration versus time. Each individual point shown in Figure 7 represents the averaged value for all relevant determinations, and a close fit of the data is demonstrated. *In vivo* clearance data are shown on the left of the figure and *in vitro* decay data on the right, the interrupted lines representing the I^{131} isotopic assay determinations and the

[2] It has been determined by *in vitro* investigation that I^{131}-labeled clot assay values are linearly related to unbound plasma plasminogen activator concentration with streptokinase (12), with urokinase (27), and with plasminogen activator extracted from tissues (unpublished data).

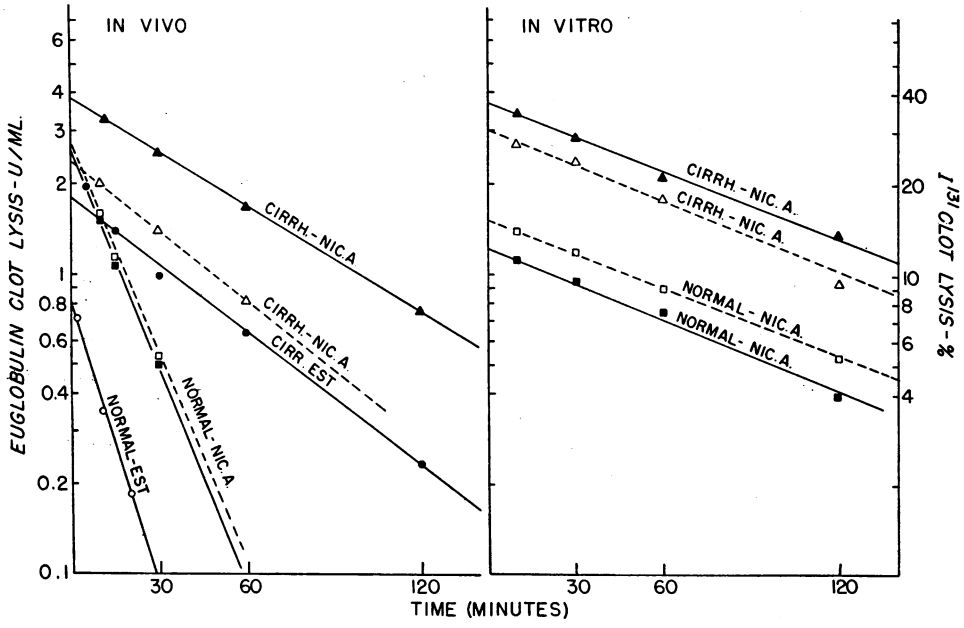

FIG. 7. TOTAL AVERAGED DATA FOR ALL *in vivo* AND *in vitro* PLASMA ACTIVATOR DETERMINATIONS. The figure is similar in design to Figure 6. On the left, all determinations from each patient class have been averaged, i.e., CIRRH-Nic.A. represents the mean data for all cirrhotic patients receiving nicotinic acid; NORMAL-EST represents all normal subjects after electroshock. The right-hand section represents the total *in vitro* decay data plotted similarly. Both ordinates are log activator concentration, and the abscissas are time. The left-hand data demonstrate that for both cirrhotic and normal patients stimulated by either nicotinic acid injection or electroshock *in vivo* activator disappearance is an exponential function of activator concentration. Similarly, the right-hand data demonstrate that, *in vitro*, plasma activator decay is also exponential for all classes of patient plasma. Solid lines designate the data obtained with the I^{131} assay, and the broken lines, the euglobulin data.

TABLE III

*In vivo 50% activator clearance rate for each class of patient and stimulus distinguished by the assay method employed and in vitro plasma activator decay data**

	No.	Assay	Stimulus	Group	Half life		p
					Range	Mean ± SD	
					min		
In vivo	8	Euglobulin	Electroshock	Normal	2–8.5	5.0 ± 2.4	<0.001
	3			Cirrhotic	40–50	45 ± 5	
	9	Euglobulin	Nicotinic acid	Normal	6–26.5	12.3 ± 6.4	<0.001
	5			Cirrhotic	30–85	59 ± 21	
	9	I^{131} assay	Nicotinic acid	Normal	6–22	13.2 ± 4.5	<0.001
	5			Cirrhotic	32–104	46.7 ± 17	
	3	Euglobulin	Nicotinic acid	Normal	45–105	81 ± 13	>0.1
	3			Cirrhotic	52–123	74±14	
In vitro	3	I^{131} assay	Nicotinic acid	Normal	53–95	74 ± 8	>0.1
	3			Cirrhotic	60–100	82 ± 9	

* In all instances differences between cirrhotic patients and normal subjects were statistically highly significant ($p < 0.001$). *In vitro* plasma activator decay data did not reveal significant differences between cirrhotic and normal plasma ($p > 0.1$). Differences between the *in vivo* data and the *in vitro* decay data were highly significant ($p < 0.001$)

solid lines those obtained by the euglobulin method.

The left-hand portion of Figure 7 demonstrates that striking differences were evident between plasminogen activator clearance from the plasma in normal subjects and in cirrhotic patients; moreover, these differences were of the same order of magnitude for both stimuli, nicotinic acid and electroshock. The right-hand portion of Figure 7 reveals that the averaged lines for activator decay in plasma incubated at 37° C were approximately parallel, indicating that decay of plasminogen activator *in vitro* proceeded at a rate independent of the type of plasma in which it was contained. Individual plots of this exponential type were used to calculate the 50% plasma clearance for each patient and the 50% plasma decay rate for each such study. The results are shown in Table III.

The upper portion of Table III lists the calculated *in vivo* half life of plasminogen activator obtained from observations employing the euglobulin lysis method and the radiochemical assay; the lower portion of Table III cites the calculated *in vitro* 50% plasma decay rates. The results obtained with the two different assay methods demonstrate very satisfactory agreement, bearing in mind that the patient groups studied with each method were overlapping rather than identical. For instance the *in vivo* 50% plasma clearance of activator in normal subjects, after nicotinic acid, was 12.3 ± 6.4 minutes determined by the euglobulin lysis method and 13.2 ± 4.5 minutes with the radiochemical assay. The findings in Table III indicate that the plasma clearance of plasminogen activator in the cirrhotic patient was greatly slowed as compared to that of the normal subject, the 50% plasma clearance time being approximately 9 times slower after electroshock therapy (euglobulin method) and 5 times (euglobulin method) or 4 times slower (radiochemical method) after nicotinic acid stimulation. All these differences were significant at $p < 0.001$. In contrast, the results with both assay methods indicated that *in vitro* decay rates for plasminogen activator in plasma at 37° C were similar in either cirrhotic or normal plasma ($p > 0.1$).

Discussion

Both the enhanced plasma thrombolytic activity, developing after nicotinic acid injection, electroshock, and other stimuli (9–11, 26), and the very

low level of thrombolytic activity detectable in plasma from resting man (13) have been shown to be due to the presence of plasminogen activator in plasma. The present work offers interesting confirmation of this conclusion, for whereas the previous evidence was necessarily indirect [study of the action of specific activators in a plasma milieu (27), the action of such plasma on different substrates (11, 12, 27), and the demonstration of plasminogen activator activity in the euglobulin fraction of plasma (11)], the present evidence is direct, resting upon the *in vivo* demonstration of plasminogen activation. In both normal and cirrhotic patients, the injection of nicotinic acid was shown to induce a fall of plasma plasminogen, although the decrease in the cirrhotic patients was of much greater magnitude ($p < 0.001$).

Since most of the conclusions derived during this study rest upon the accuracy of plasminogen activator assay in plasma, some consideration must be given to the validity of the methodological approach. Theoretically, the euglobulin lysis time assay method for activator might have yielded artifactual results in plasma from cirrhotic patients because of variation in plasma components other than plasminogen activator, i.e., the euglobulin clot formed from such plasma would have been of abnormal composition. Although differences were detected between the two subject groups with respect to the pretreatment values for plasma fibrinogen and plasma plasminogen (two moieties influencing this test), the radiochemical assay would have been unaffected by changes in plasma composition, since a standardized substrate with a defined plasminogen and fibrin concentration is employed for this assay procedure. In fact both assay procedures yielded comparable results that were excellently correlated (Table III). Calculation from the total experimental data yielded (regression of euglobulin lysis on I^{131} lysis) $r = 0.61$ ($p < 0.001$) for normal subjects, the line equation being $y = 2.85 + 5.09x$, and $r = 0.87$ ($p < 0.001$) for the cirrhotic patients, the line equation being $y = 0.32 + 5.45x$. Since the calculated regression slopes lay within 10% of each other, the influence of technical artifact in the use of the euglobulin activator assay with plasma from cirrhotic patients can be confidently excluded.

The respective responses of normal and cirrhotic subjects to a stimulus capable of eliciting enhanced plasma activator activity has been previously investigated by Kwaan, MacFadzean, and Cook (7). They studied a disease entity, common in Hong Kong, termed cryptogenic splenomegaly (28), in which idiopathic splenomegaly commonly antedates the onset of hepatic cirrhosis, the condition not infrequently terminating as primary hepatoma. They demonstrated, using single plasma samples taken 20 minutes after the subcutaneous injection of epinephrine, exercise, or other suitable stimuli that patients, in whom the manifestations of hepatic cirrhosis were of predominant importance, developed significantly greater enhancement of plasma thrombolytic activity than did patients with cryptogenic splenomegaly without cirrhosis or normal subjects. Moreover in four patients, first observed during the cirrhotic phase of the disorder (at which time enhanced thrombolytic activity occurred in response to the stimulus), the development of hepatoma resulted in virtual abolition of the patients' response to the stimulus.

The present studies, while confirming the observation that patients with hepatic carcinoma may have a decreased response to a standard thrombolytic stimulus, were primarily designed to investigate other facets of the relationship between hepatic function and plasma thrombolytic activity. The striking finding [3] that the *in vivo* 50% plasma clearance in normal subjects after nicotinic acid averaged 12.7 ± 5.4 minutes and after electroshock therapy, 5.0 ± 2.4 minutes, whereas the *in vitro* 50% decay time for plasminogen activator in either cirrhotic or normal plasma averaged 78 ± 9.2 minutes indicates that, *in vivo,* plasma clearance mechanisms for activator play, apart from the spontaneous decay or destruction of activator in plasma, a crucial role in the regulation of plasma thrombolytic activity. This rapid *in vivo* clearance, or inactivation of plasma activator, or both, explain the many dif-

[3] References here and subsequently are to the averaged results from Table III without distinction as to the assay method employed. This simplification of the data is justified by the fact that the data obtained by the separate assay procedures are virtually identical (Table III); consequently, the averaged figures used hereafter, although distinct from those of the table, are little different.

ficulties encountered during attempts to induce a therapeutic thrombolytic state by the administration of pharmacological agents that induce activator release—such states have invariably been transient and of insignificant degree. Moreover it has been reported (29) that the 50% *in vivo* plasma clearance rate of the plasminogen activator streptokinase was 83 minutes, a rate so unexpectedly rapid that reticuloendothelial system clearance was suggested as its cause.

The present data provide relatively precise evidence as to the nature of the functional anomaly responsible for the strikingly different activator clearance rates determined for normal and cirrhotic patients. The finding that both stimuli, nicotinic acid injection and electroshock, induced similar effects excludes the possibility of either abnormal nicotinic acid metabolism or aberrant response to the electroshock as being the unique cause of the anomaly. Indeed in conjunction with the previously cited data concerning the response of the cirrhotic patient to other stimuli (7), the results indicate that cirrhotic patients respond in an exaggerated fashion to all stimuli capable of inducing thrombolytic activity in the normal subject. This abnormal response of the cirrhotic patient is not related to deficient plasma inhibitory characteristics as *a*) assays for plasma antiplasmin revealed no significant differences between the normal and cirrhotic groups, and determination of the urokinase lysis time showed only a nonsignificant decrease of inhibitory activity in cirrhotic plasma (Figure 4), and significantly *b*) plasminogen activator decay rates in plasma from the two different classes of subjects were identical (Table III).

Consequently the finding of a 4-fold difference between the 50% plasma activator clearance rates for normal and cirrhotic patients after nicotinic acid stimulation (12.7 ± 5.4 versus 53 ± 19 minutes) and a 9-fold difference between these groups after electroshock therapy (5.0 ± 2.4 versus 45 ± 5.0 minutes) assumes crucial etiological significance. Whereas the 50% *in vivo* plasma clearance of activator in the normal subject was 7 to 16 times as rapid as the 50% *in vitro* plasma decay rate (i.e., 78 ± 9 minutes), the corresponding *in vivo* plasma clearance rate of activator in the cirrhotic patient was less than twice the *in vitro* decay rate of plasminogen activator in plasma (Table III). Thus while the normal subject clears plasminogen activator from the plasma with remarkable speed, this ability is almost absent in the cirrhotic patient. Since there is no reason to believe that hepatic cirrhosis induces extraneous changes in intravascular-extravascular equilibria that could retard loss of activator from the plasma, the results suggest that the almost total failure of the cirrhotic subject to actively clear this moiety from the circulation is a direct consequence of the structural or functional hepatic changes consequent upon cirrhosis. The normal liver must play an important role in the active clearance of plasminogen activator from the circulation and, in this manner, exert important regulatory functions over physiological and pathological activity of the plasminogen-plasmin system.

This hypothesis is supported by a previous report (7) that hepatic vein blood exhibited a somewhat lower level of thrombolytic activity than did that from the inferior vena cava and by the significant observations of Grossi, Rousselot, and Panke (5) made on the portal and systemic vein blood of cirrhotic patients undergoing portacaval shunt procedures. They observed, using the euglobulin lysis time assay, that although the thrombolytic activity of portal and systemic venous blood was similar in 18 patients, in 15 others (mostly those patients showing a grossly shortened euglobulin lysis time) activator activity in the portal vein blood exceeded, sometimes substantially, that found in the systemic vein blood. All the experimental evidence, including that of the present study, indicating that the liver actually clears plasminogen activator from the plasma, has been obtained during states of enhanced plasma thrombolytic activity. Nevertheless there is little reason to doubt that hepatic clearance of plasminogen activator is of significance under strictly physiological circumstances, for many of the clearance studies performed during the present investigation were made during states of only mild enhancement of plasma thrombolytic activity, and the evidence in the cirrhotic patient, in whom hepatic clearance may be grossly deficient, indicates that virtual absence of this hepatic function predisposes to the development of pathological plasma proteolysis.

The precise functional defect responsible for failure of the cirrhotic liver to clear plasminogen

activator must, at present, remain conjectural, although the evidence is sufficient to permit useful speculation. Ratnoff's evidence (2) that rapid whole plasma clot lysis, commonly detected in the cirrhotic patient, was seldom if ever observed in patients suffering from hepatitis (even of such severity to cause deep jaundice, coma, and ultimately death), and our evidence that, in three patients with hepatic failure but without biopsy evidence of cirrhosis, there was no apparent failure to clear plasminogen activator, would suggest that hepatic cellular injury or death per se was not the responsible cause. Nevertheless the observation that four patients, with carcinomatous involvement of the liver but without marked signs of hepatic failure, failed to exhibit enhanced plasma thrombolytic activity after nicotinic acid injection ($p < 0.001$) coupled with the interesting work of Kwaan, Lo, and McFadzean (30), suggesting that hepatic neoplasm might produce an inhibitor of plasminogen activation, indicates that multiple factors complicate interpretation of this problem. Moreover there is the possibility that blood shunt formation, secondary to cirrhosis, might interfere with hepatic clearance of activator. However, shunt formation is unlikely to explain completely the findings, as recent investigation (31) of extrahepatic shunt flow in patients with moderately advanced or advanced hepatic cirrhosis indicated that it averaged only 14% of total hepatic blood flow, although the calculated intrahepatic shunt flow was somewhat higher. Tentatively we suggest that clearance of plasminogen activator is probably a function of hepatic cell mass and that reduction of this function may occur during various disease states, its effects being particularly evident as a result of hepatic cirrhosis. Such hepatic functional defect might be masked *in vivo* by a capacity of hepatic cells, as a result of injury or neoplastic involvement, to produce an inhibitor, probably nonspecific, to plasminogen activation.

Patients suffering from hepatic cirrhosis and other chronic forms of nonobstructive hepatic disease frequently exhibit a coagulation disorder of usually ill-defined etiology, and the present observations may bear upon its pathogenesis. Despite the attention of many investigators (3, 5, 8, 32–35), these disorders are relatively poorly understood. The patients usually display thrombocytopenia of mild or moderate degree, and coagulation assays usually indicate the presence of a multifactorial defect involving chiefly Factors II (prothrombin), V (accelerator globulin), VII (proconvertin), IX (Christmas factor), and possibly other thromboplastic factors. Conventional coagulation assays tend to correlate poorly with hemorrhagic phenomena and sometimes fail to provide an adequate explanation for the clinical findings.

Although many investigators (1–8, 33–35) have reported the presence of rapid whole blood clot lysis, an accelerated euglobulin lysis time, or other evidence of abnormal plasminogen-plasmin system activity in from 20 to 60% of patients with advanced cirrhosis, these phenomena were not considered relevant to the hemorrhagic diathesis (except in rare instances where they were extreme in nature), because plasma fibrinogen levels usually remained within the low normal range. The recent description of a unique coagulation defect, termed defective fibrin polymerization (15–18), in which the polymerization of fibrin monomer is inhibited through the presence in plasma of fibrinogen proteolysis products suggests that the high incidence of abnormal fibrinolytic activity in cirrhotic patients may play an important role in the genesis of the coagulation abnormalities and accompanying hemorrhagic diathesis.

In this connection the present demonstration of the exquisite sensitivity of cirrhotic patients to stimuli, capable of inducing release of activator in the normal subject, assumes unusual importance. Whereas the injection of nicotinic acid in the normal subject caused only enhanced plasma thrombolytic activity of transient degree without other significant findings, the same procedure in the cirrhotic patient caused greatly increased plasma activator activity of prolonged degree, sufficient to induce a substantial fall in plasma plasminogen ($p < 0.001$), a substantial fall of plasma fibrinogen ($0.001 < p < 0.01$), and a significant increase in plasma thrombin clotting time ($0.001 < p < 0.01$). These findings in the cirrhotic patients, although in this instance clinically inconsequential, differ only in degree from those observed in patients suffering from defective fibrin polymerization of clinical significance. Indeed Ratnoff (36) suggested that the raised plasma

thrombin clotting time, commonly found in the cirrhotic patient, might reflect a defect in the coagulation of fibrinogen by thrombin, and others (37–38) have reported a depression of plasma plasminogen and fibrinogen in the cirrhotic patient of comparable degree to our own findings.

The present data suggest that the coagulation disorder, defective fibrin polymerization, resulting from the failure of the cirrhotic patient to clear plasminogen activator, may play a hitherto unsuspected role in the coagulation disorders that complicate this condition. Confirmation of this hypothesis would have important therapeutic implications, since treatment with specific inhibitors might prove advantageous (14).

Summary

1) Enhanced plasma thrombolytic activity of short duration developed in control subjects receiving 100 mg nicotinic acid intravenously, but patients with hepatic cirrhosis exhibited a grossly exaggerated response with greatly enhanced peak activity of much prolonged duration. Similar differences were observed after therapeutic electroshock in normal and cirrhotic patients. Nicotinic acid injection induced in cirrhotic, but not in normal subjects, significant decreases in plasma plasminogen and fibrinogen and a rise in the thrombin clotting time, although plasma inhibitors did not differ significantly between the two groups.

2) The *in vivo* 50% plasma clearance rate for plasminogen activator in normal subjects after nicotinic acid injection was 13 ± 5 minutes compared to 53 ± 19 minutes in the cirrhotics. Differences were not detected in the *in vitro* decay rates of plasminogen activator in normal and cirrhotic plasmas, the mean 50% time being 78 ± 9 minutes for both. Essentially similar findings were obtained with normal and cirrhotic patients who received therapeutic electroshock.

3) Abnormal fibrinolysis in the cirrhotic patient may be due to a failure of hepatic clearance mechanisms for plasminogen activator. The evidence also suggested that the normal liver exerts an important control function over plasminogen-plasmin system activity.

Acknowledgments

We are greatly indebted to the staff of the Jewish Hospital, particularly to Dr. Albert Eisenstein, for their interest and for the opportunity to study patients with cirrhosis and a normal population. Dr. Walter Moore at St. Mary's Hospital and Dr. Franz Hornung at the Malcolm Bliss Hospital kindly provided facilities for the study of patients undergoing electroshock therapy.

References

1. Goodpasture, E. W. Fibrinolysis in chronic hepatic insufficiency. Bull. Johns Hopk. Hosp. 1914, **25**, 330.
2. Ratnoff, O. D. Studies on a proteolytic enzyme in human plasma IV. The rate of lysis of plasma clots in normal and diseased individuals, with particular reference to hepatic disease. Bull. Johns Hopk. Hosp. 1949, **84**, 29.
3. Finkbiner, R. B., J. J. McGovern, R. Goldstein, and J. P. Bunker. Coagulation defects in liver disease and response to transfusion during surgery. Amer. J. Med. 1959, **26**, 199.
4. Grossi, C. E., A. H. Moreno, and L. M. Rousselot. Studies on spontaneous fibrinolytic activity in patients with cirrhosis of the liver and its inhibition by epsilon amino caproic acid. Ann. Surg. 1961, **153**, 383.
5. Grossi, C. E., L. M. Rousselot, and W. F. Panke. Coagulation defects in patients with cirrhosis of the liver undergoing portasystemic shunts. Amer. J. Surg. 1962, **104**, 512.
6. Kwaan, H. C., A. J. S. McFadzean, and J. Cook. Plasma fibrinolytic activity in cirrhosis of the liver. Lancet 1956, **1**, 132.
7. Kwaan, H. C., A. J. S. McFadzean, and J. Cook. On plasma fibrinolytic activity in cryptogenic splenomegaly. Scot. med. J. 1957, **2**, 137.
8. Bergström, K., B. Blombäck, and G. Kleen. Studies on the plasma fibrinolytic activity in a case of liver cirrhosis. Acta med. scand. 1960, **168**, 291.
9. Weiner, M., K. de Crinis, W. Redisch, and J. M. Steele. Influence of some vasoactive drugs on fibrinolytic activity. Circulation 1959, **19**, 845.
10. Weiner, M. The fibrinolytic response to nicotinic acid in abnormal liver states. Amer. J. med. Sci. 1963, **246**, 294.
11. Sherry, S., R. I. Lindemeyer, A. P. Fletcher, and N. Alkjaersig. Studies on enhanced fibrinolytic activity in man. J. clin. Invest. 1959, **38**, 810.
12. Alkjaersig, N., A. P. Fletcher, and S. Sherry. The mechanism of clot dissolution by plasmin. J. clin. Invest. 1959, **38**, 1086.
13. Sawyer, W. D., A. P. Fletcher, N. Alkjaersig, and S. Sherry. Studies on the thrombolytic activity of human plasma. J. clin. Invest. 1960, **39**, 426.
14. Fletcher, A. P., N. Alkjaersig, and S. Sherry. Fibrinolytic mechanisms and the development of

thrombolytic therapy. Amer. J. Med. 1962, **33**, 738.

15. Fletcher, A. P., N. Alkjaersig, and S. Sherry. Pathogenesis of the coagulation defect developing during pathological plasma proteolytic ("fibrinolytic") states. I. The significance of fibrinogen proteolysis and circulating fibrinogen breakdown products. J. clin. Invest. 1962, **41**, 896.
16. Alkjaersig, N., A. P. Fletcher, and S. Sherry. Pathogenesis of the coagulation defect developing during pathological plasma proteolytic ("fibrinolytic") states. II. The significance, mechanism and consequences of defective fibrin polymerization. J. clin. Invest. 1962, **41**, 917.
17. Bang, N. U., A. P. Fletcher, N. Alkjaersig, and S. Sherry. Pathogenesis of the coagulation defect developing during pathological plasma proteolytic ("fibrinolytic") states. III. Demonstration of abnormal clot structure by electron microscopy. J. clin. Invest. 1962, **41**, 935.
18. Latallo, Z. S., A. P. Fletcher, N. Alkjaersig, and S. Sherry. Inhibition of fibrin polymerization by fibrinogen proteolysis products. Amer. J. Physiol. 1962, **202**, 681.
19. Niewiarowski, S., and E. Kowalski. Un nouvel anticoagulant dérive du fibrinogene. Rev. Hémat. 1958, **13**, 320.
20. Triantaphyllopoulos, D. C. Effects of intravenous injections of the anticoagulant fraction of incubated fibrinogen on blood coagulation. Canad. J. Biochem. 1960, **38**, 909.
21. Milstone, H. A factor in normal human blood which participates in streptococcal fibrinolysis. J. Immunol. 1941, **42**, 109.
22. Fletcher, A. P., N. Alkjaersig, and S. Sherry. The maintenance of a sustained thrombolytic state in man. I. Induction and effects. J. clin. Invest. 1959, **38**, 1096.
23. Ostle, B. Statistics in Research. Ames, Iowa, Iowa State College Press, 1954.
24. Sherry, S., A. P. Fletcher, and N. Alkjaersig. Fibrinolysis and fibrinolytic activity in man. Physiol. Rev. 1959, **39**, 343.
25. Fischer, S., A. P. Fletcher, N. Alkjaersig, and S. Sherry. Fibrinogenolysis *in vivo:* identification, occurrence, and characterization in man (abstract). J. clin. Invest. 1963, **42**, 931.
26. Genton, E., F. Kern, and K. von Kaulla. Fibrinolysis induced by pressor amines. Amer. J. Med. 1961, **31**, 564.
27. Sawyer, W. D., N. Alkjaersig, A. P. Fletcher, and S. Sherry. A comparison of the fibrinolytic and fibrinogenolytic effects of plasminogen activators and proteolytic enzymes in plasma. Thrombos. Diathes. haemorrh. (Stuttg.) 1960, **5**, 149.
28. McIntosh, J. F. Cryptogenic splenomegaly (Banti's disease). China med. J. 1932, **46**, 992.
29. Fletcher, A. P., N. Alkjaersig, and S. Sherry. The clearance of heterologous protein from the circulation of normal and immunized man. J. clin. Invest. 1958, **37**, 1306.
30. Kwaan, H. C., R. Lo, and A. J. S. McFadzean. Antifibrinolytic activity in primary carcinoma of the liver. Clin. Sci. 1959, **18**, 251.
31. Nakamura, T., S. Nakamura, T. Kaneko, T. Suzuki, K. Tokita, and S. Abe. Measurement of extrahepatic shunted blood flow in liver cirrhosis. J. Lab. clin. Med. 1962, **60**, 889.
32. Rapaport, S. I., S. B. Ames, S. Mikkelsen, and J. R. Goodman. Plasma clotting factors in chronic hepatocellular disease. New Engl. J. Med. 1960, **263**, 278.
33. Zucker, M. B., M. Siegel, E. E. Cliffton, J. W. Bellville, W. S. Howland, and C. E. Grossi. The effect of hepatic lobectomy on some blood clotting factors and on fibrinolysis. Ann. Surg. 1957, **146**, 772.
34. Zucker, M. B., M. Siegel, E. E. Cliffton, J. W. Bellville, W. S. Howland, and C. E. Grossi. Generalized excessive oozing in patients undergoing major surgery and receiving multiple blood transfusions. J. Lab. clin. Med. 1957, **50**, 849.
35. Zetterqvist, E., and I. von Francken. Coagulation disturbances with manifest bleeding in extrahepatic portal hypertension and liver cirrhosis. Acta med. scand. 1963, **173**, 753.
36. Ratnoff, O. D. An accelerating property of plasma for coagulation of fibrinogen by thrombin. J. clin. Invest. 1954, **33**, 1175.
37. Blix, S. The proactivator of the fibrinolytic system in human plasma. Acta med. scand. 1962, **171**, 83.
38. Purcell, G., and L. L. Phillips. Fibrinolytic activity in cirrhosis of the liver. Surg. Gynec. Obstet. **1963, 117, 139.**