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Research Article

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Human Prothrombin Metabolism in Normal Man and in Hypocoagulable Subjects

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ABSTRACT The metabolism of human prothrombin labeled with radioactive iodine was studied in seven normal subjects and four hemophilic patients. Results in the normal subjects were: plasma volume, 37.6 ±3.8 ml/kg; plasma prothrombin concentration, 303 ±40 U/ml (0.153 ± 0.02 mg/ml); prothrombin half-life, 2.81 ±0.51 days; total plasma prothrombin pool, 5.72 ±0.62 mg/kg, representing 64.1 ±9.1% of total body prothrombin; fractional catabolic rate, 42.5 ±12.4% of the plasma pool per day; prothrombin synthesis rate, 2.43 ± 0.76 mg/kg per day. Results in the hemophilic patients did not differ significantly from normal. Circulating products of prothrombin activation could not be demonstrated in normal individuals or hemophilic subjects. The data suggest that continuous physiologic activation of the blood coagulation mechanism plays only a small part, if any, in the normal catabolism of prothrombin.

INTRODUCTION

The central event in blood coagulation is the conversion of prothrombin to the enzyme thrombin. Studies of the *in vivo* behavior of this zymogen in man have been limited to measurements of the decay of biological activity after infusions of large amounts of plasma or prothrombin concentrates (1–5), or after the administration of large doses of a hypoprothrombinemic agent (6–10). Such experimental approaches, by their very nature, involve some degree of perturbation of the physiological steady state, and their validity, therefore, is open to question. Perhaps, on this account, the observed biological half-life of human prothrombin has varied between less than 20 (5) and more than 120 (3, 4) hr. The availability of highly purified human prothrombin (11) has made possible the application of radioactive tracer techniques to the study of prothrombin metabolism. The present investigation was undertaken to delineate the parameters of prothrombin turnover in normal subjects and in patients with severe hemorrhagic disease, and to determine whether products of prothrombin activation, observed in vitro (12, 13), are normally detectable in the circulation.

METHODS

Prothrombin-^{131}I. Human prothrombin was purified from fresh acid citrate dextrose (ACD) plasma by a previously published method (11), involving successive adsorptions on diethylaminoethyl (DEAE)-cellulose and barium citrate, followed by precipitation with ammonium sulfate. This material ("step 3" prothrombin), which is approximately 95% purified, was labeled with iodine-131 by the iodine monochloride technique of McFarlane (14), except that reagents were mixed in a small plastic beaker and allowed to react for 30 min. Unbound iodine was removed by passage through a 1.5 × 3 cm column of Amberlite MB1 (Rohm & Haas Co., Philadelphia, Pa.). A macromolecular impurity in the preparation, which also reacts with the radioiodine, was removed by gel filtration on 2 × 90 cm columns of Sephadex G-100 equilibrated with sterile, pyrogen-free 0.25 M NaCl–0.01 M sodium citrate buffer, pH 7.0. The labeled, gel-filtered zymogen was free of nonprotein-bound radioactivity and contained an average of less than 0.5 atoms of protein-bound iodine per molecule of prothrombin. Labeling efficiency was 30–40%, and specific activity of the preparations was 2–10 μc/mg of protein. No change in the biological activity of prothrombin could be detected after iodination. Acrylamide-gel electrophoresis of the iodinated material (Fig. 1) showed a single protein band, with a mobility identical with that of unlabeled prothrombin, containing more than 98% of the total radioactivity applied to the gel. Gel filtration of a mixture of labeled prothrombin and normal human plasma (Fig. 2) showed a single radioactive peak, emerging some-

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what earlier than the third (albumin) optical density peak. The elution characteristics of this labeled prothrombin, of unlabeled prothrombin, and of plasma prothrombin activity are identical. For some experiments prothrombin was further purified by ion-exchange chromatography on Biorex 70 (11) before iodination. The chromatographed material ("step 5" prothrombin) contained no detectable protein impurities but was nevertheless gel filtered after iodination.

Metabolic studies. Seven healthy male volunteers, two patients with severe factor VIII deficiency (factor VIII < 1.5%), and two patients with severe factor IX deficiency (factor IX < 1.5%) were studied. The hemophilic subjects all had severe disease by clinical criteria as well, each patient having a history of multiple hemarthroses. In all subjects routine hematologic tests and complete coagulation evaluations were performed before the experimental procedure. No subjects had taken any medications regularly or had received blood or plasma within 4 wk of the experimental procedure. Save for the single congenital deficiencies in the four patients with hemophilia, all other coagulation parameters were normal.

In many experiments subjects received their own prothrombin, prepared on the previous day from a 2 unit plasmapheresis. In such cases care was taken to replace volume lost during plasmapheresis with physiologic saline. In several experiments autologous, single donor prothrombin was utilized, and in two studies prothrombin prepared from a pool of donors was infused. All subjects took 10 drops of a saturated solution of potassium iodide three times a day on the day preceding and the day of the radioactive prothrombin infusion, and 10 drops once daily for the remaining 6–8 days of the experiment. Immediately before injection, the prothrombin-^{131}I was passed through a type GS, 0.22 μ Millipore filter, precoated with 0.5–1.0 ml of the recipient's plasma. Such filtered materials were shown to be sterile and pyrogen free. 20–50 μc of labeled prothrombin, in a volume of 5–10 ml, were injected intravenously through a disposable plastic syringe. No significant side effects were noted in any of the subjects during these studies. 5-ml blood samples were

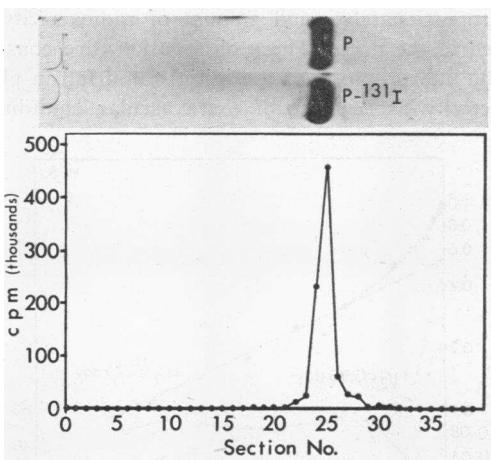

FIGURE 1 Electrophoretic homogeneity of prothrombin-^{131}I. Labeled prothrombin was electrophoresed in acrylamide gel (15) by use of a discontinuous Tris citrate–borate buffer system (16). After it was stained, the strip was cut into 1 cm sections for radioactivity measurement. Unlabeled prothrombin (upper strip) and labeled prothrombin show identical electrophoretic patterns. P: prothrombin.

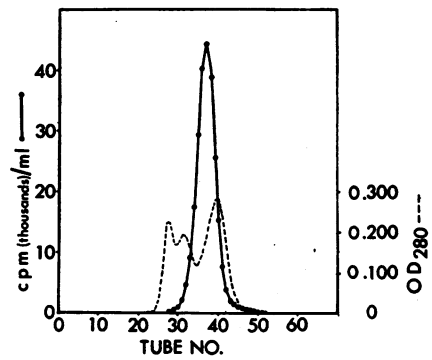

FIGURE 2 Gel filtration pattern of prothrombin-^{131}I. Labeled prothrombin was mixed with a small amount of normal plasma and gel filtered on a 2 × 85 cm column of Sephadex G-100. Fractions of 4.3 ml were collected. Radioactivity was present only in the prothrombin peak.

collected into ⅙ volume of a refrigerated stock solution of 0.109 M trisodium citrate before, and at 15 min, 2 hr, 4 hr, and 18 hr after injection. Daily samples were drawn thereafter for 6–8 days. All samples were collected through an 18 gauge disposable needle by a two-syringe technique. Venipuncture was performed within ½ min of application of the tourniquet, and the tourniquet was then removed. The 1st 2–3 ml of each venipuncture was discarded, after which the requisite amount of blood was collected into an iced plastic syringe (Pharmaseal Laboratories, Glendale, Calif.) containing a measured volume of citrate anticoagulant. Plasma prothrombin concentration was measured several times during each study by the two-stage method of Ware and Seegers (17), slightly modified (11). Hematocrits also were determined several times during the experiment and were stable. In all but two studies 24-hr urine collections were made throughout the observation period. At the conclusion of the experiment urine and plasma aliquots were assayed for radioactivity in a Nuclear-Chicago Autogamma spectrometer with a counting efficiency of 33% and background of 25 cpm.

In order to determine the distribution of plasma radioactivity after infusion, additional blood samples were withdrawn at intervals during the experiment, centrifuged at 4°C, and the plasma immediately gel filtered at 4°C on columns of Sephadex G-100 or Sephadex G-150 equilibrated with 0.25 M NaCl–0.01 M sodium citrate buffer, pH 7.0. The possibility that some prothrombin was being excreted unchanged in the urine was investigated by concentration of urine by dialysis against polyethylene glycol and gel filtration of the concentrate on columns of Sephadex G-150. No radioactive prothrombin was detectable in the urine by this procedure.

Analysis of data. Calculations of catabolic and synthetic rates and the intravascular-extravascular distribution of prothrombin were made from the plasma disappearance curves by the method of Matthews (18). Fractional catabolic rates were also determined from the ratio of daily urinary excretion of radioactivity to the mean total plasma radioactivity during the same period of time (19).

The plasma volume was obtained from the total radioactivity injected divided by the concentration of plasma radioactivity in the 15 min sample. Both radioactivity and prothrombin concentration of plasma samples were corrected for the dilution caused by the added citrate anticoagulant. Prothrombin concentrations were converted from units per mil-

TABLE I
Summary of Clinical Data

Subject	Clinical status	Age	Height	Weight	Plasma volume	Plasma prothrombin concentration		Hematocrit
		yr	*cm*	*kg*	*ml/kg*	*U/ml*	*mg/ml*	%
S.S.	Normal	35	169	71.0	38.4	290	0.147	43
J.M.	Normal	33	168	68.0	34.3	330	0.167	44
R.F.	Normal	28	188	88.9	35.8	300	0.152	44
W.S.	Normal	29	168	66.1	38.7	314	0.159	42
C.P.	Normal	26	173	78.6	38.4	284	0.144	44
L.S.	Normal	30	173	73.0	37.5	324	0.164	38
C.S.	Normal	27	165	66.0	39.9	278	0.141	43
Mean					37.6	303	0.153	43
2 SD					±3.8	±40	±0.020	±4
J.R.	Factor VIII deficient	47	164	70.0	33.5	288	0.146	47
V.C.	Factor VIII deficient	24	167	65.0	35.4	292	0.148	42
R.M.	Factor IX deficient	31	177	56.5	52.2	206	0.105	44
F.A.	Factor IX deficient	30	165	56.8	31.6	242	0.123	45

liliter to milligrams per milliliter by using a conversion factor of 1970 units per milligram of prothrombin. This figure was obtained from dry weight measurements of purified human prothrombin (11) and should be related to the normal range of plasma prothrombin concentration in our laboratory, 270–330 U/ml (corrected for the presence of anticoagulant).

The assumption that each subject was in a steady state with respect to prothrombin metabolism seemed justified on the basis of the stability of hematocrit and prothrombin concentration throughout the experimental period. Daily fractional catabolic rates calculated from urinary radioactivity were constant after the 1st day, a finding consistent with the concept that catabolism of prothrombin occurs in the plasma or in a pool in rapid equilibrium with the plasma (18).

RESULTS

Table I summarizes the pertinent clinical data for all the experimental subjects. All 11 subjects were males, ranging in age from 24 to 47 yr. Mean plasma volume for the normal subjects was 37.6 ±3.8 mg/kg (mean ±2 SD). The mean normal plasma prothrombin concentration was 303 ±40 U/ml (0.153 ±0.020 mg/ml), in good agreement with the normal range for this laboratory, established from a much larger normal population, of 270–330 U/ml.

The four hemophilic patients were stable with respect to their hemorrhagic disease at the time of study and had normal hematocrits. However, one patient with factor IX deficiency (R.M.) was noted to have mild liver disease, with hepatomegaly, and elevated alkaline phosphatase and transaminases, a slightly increased plasma volume, and a moderately depressed plasma prothrombin concentration. The second patient with factor IX deficiency (F.A.), without demonstrable hepatic dysfunction, had a slight decrease in plasma volume and prothrombin concentration, the reasons for which are unknown.

A typical turnover study is illustrated in Fig. 3. Results of all studies, both in normals and in patients, were qualitatively similar. The plasma radioactivity curves resolved into two exponential components, the second taken to represent the catabolism of prothrombin. The half-time for disappearance of the first component in all subjects was between 6 and 12 hr. Daily urinary excretion of radioactivity was a constant fraction of the corresponding mean circulating plasma radioactivity after the 1st day of each study. During the initial 12 hr a disproportionately small amount of radioactivity was excreted in the urine. These observations are consistent with the interpretation of the initial rapid fall in plasma radioactivity as a phase of extravascular equilibration

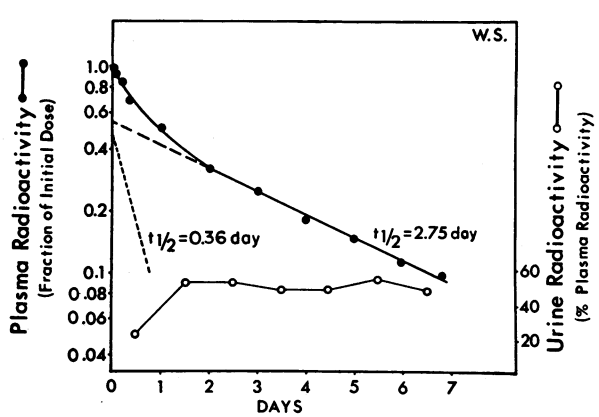

FIGURE 3 Plasma disappearance and urinary excretion of radioactivity in a normal subject (W.S.) after intravenous administration of prothrombin-^{131}I.

rather than rapid catabolism of a partially denatured fraction of the injected prothrombin preparation.

The distribution of plasma radioactivity was investigated by Sephadex gel filtration. In the early part of these studies blood samples were drawn with a single syringe technique, and gel filtration patterns of the type illustrated in Fig. 4 were always obtained. Three radioactive peaks are present: a major peak with the characteristic elution volume of plasma prothrombin, a small peak (tubes Nos. 65–75) of low molecular weight radioactivity, presumably inorganic iodide, and a radioactive peak (tube Nos. 19–23) emerging in the void volume of the column. In vitro studies of human prothrombin activation in plasma (13) have shown the latter peak to be due to the complexing of a radioactive product of prothrombin activation, presumably thrombin, with α_2-macroglobulin, a physiologic thrombin inactivator (20, 21). The presence of such a complex in the plasma of normal individuals would be strong presumptive evidence for the existence of a process of continuous physiologic coagulation. When a two-syringe technique of blood drawing was employed, however, the gel filtration pattern depicted in Fig. 5 was always obtained. Only the single prothrombin peak is present. Further investigation has shown that patterns similar to that of Fig. 4 are produced even when normal plasma is drawn directly into a plastic syringe containing a mixture of radioactive prothrombin and citrate anticoagulant. Thus, in order to avoid any activation of the blood coagulation system during venipuncture, it is absolutely necessary to utilize a two-syringe technique. With this technique, no radioactive products of prothrombin activation could be detected in the circulation of any subjects. Attempts to identify circulating prothrombin activation products by immunologic means (12) were similarly unfruitful.

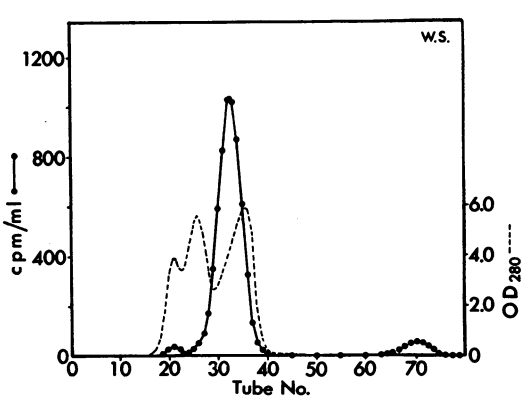

FIGURE 4 Distribution of plasma radioactivity in a normal subject (W.S.) 24 hr after administration of 50 μc of prothrombin-^{131}I. Plasma sample was obtained by single syringe technique and gel filtered on a 2 × 85 cm column of Sephadex G-100. Fractions of 4.8 ml were collected.

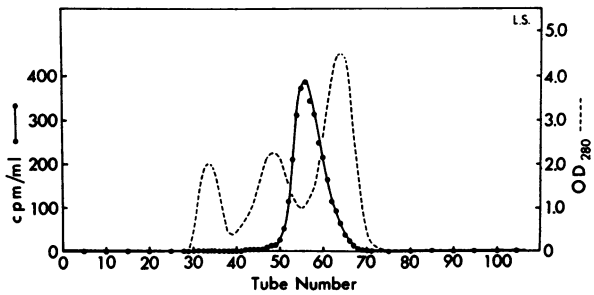

FIGURE 5 Distribution of plasma radioactivity in a normal subject (L.S.) 22 hr after administration of 25 μc of prothrombin-^{131}I. Plasma sample was obtained by two syringe technique and gel filtered on a 2.5 × 88 cm column of Sephadex G-150. Fractions of 4.5 ml were collected.

Table II presents the observed and calculated parameters of prothrombin metabolism in normal and hypocoagulable subjects. Five of the normals received their own prothrombin, and two of these five (C.S. and L.S.) also served as donors of prothrombin for other studies (R.F., R.M., and V.C.). Chromatographed prothrombin from a single donor was used in one normal (C.P.), while chromatographed prothrombin prepared from pooled fresh plasma was given to two of the hemophilic subjects (J.R. and F.A.). No differences could be detected in the behavior of these preparations with respect to plasma disappearance curves, distribution of radioactivity on Sephadex columns, or urinary excretion of radioactivity. Mean biological half-life of prothrombin in normal subjects was 2.81 ±0.51 days. The mean total plasma prothrombin pool in normal subjects was 5.72 ± 0.62 mg/kg, representing 64.1 ±9.1% of the total body pool of prothrombin. From these figures, the normal total body prothrombin pool can be calculated to be 8.96 ±1.46 mg/kg. The normal fractional catabolic rate derived from plasma radioactivity data was 42.5 ±12.4% of the plasma pool per day, in good agreement with the catabolic rate calculated from urinary radioactivity excretion of 45.4 ±11.2% of the plasma pool per day. The mean rate of prothrombin synthesis in normal males was 2.43 ±0.76 mg/kg per day.

Of the four hemophilic patients, only R. M., with mild liver disease, had abnormal turnover parameters. Both his rates of prothrombin synthesis and catabolism were slightly increased. Even these values, however, fall within 2.5 SD of their respective normal means and probably do not represent significant deviations from normal. The depressed plasma prothrombin concentration in R.M. apparently is secondary to an expanded plasma volume, since his total plasma prothrombin pool was normal. The other three patients had normal rates of prothrombin catabolism and synthesis and normal total body pools of prothrombin, although patient F.A. had a somewhat low plasma pool with a slightly increased ex-

TABLE II

Human Prothrombin Metabolic Data

Subject	Prothrombin preparation	$t_{\frac{1}{2}}$ prothrombin	Total plasma prothrombin pool	Intravascular distribution	Fractional catabolic rate: Data derived from: Plasma	Fractional catabolic rate: Data derived from: Urine	Prothrombin synthesis rate
		days	*mg/kg*	*% of total pool*	*% of plasma*	*pool/day*	*mg/kg per day*
S.S.	Isologous step 3	2.68	5.54	64.6	45.5	45.4	2.52
J.M.	Isologous step 3	2.41	5.72	60.8	51.6	52.7	2.95
R.F.	Autologous (C.S.) step 3	3.10	5.44	72.1	34.0	41.5	1.85
W.S.	Isologous step 3	2.75	6.15	63.9	43.1	50.9	2.65
C.P.	Autologous step 5	3.08	5.53	66.2	36.1	36.3	2.00
L.S.	Isologous step 3	3.00	6.15	63.8	40.0	46.0	2.46
C.S.	Isologous step 3	2.66	5.49	57.5	47.1	44.9	2.58
	Mean	2.81	5.72	64.1	42.5	45.4	2.43
	2 SD	±0.51	±0.62	±9.1	±12.4	±11.2	±0.76
J.R.	Pooled step 5	3.00	4.89	56.8	48.0	48.9	2.35
V.C.	Autologous (L.S.) step 3	2.66	5.24	57.1	50.0	60.1	2.62
R.M.	Autologous (C.S.) step 3	2.29	5.48	71.4	59.4	*	3.26
F.A.	Pooled step 5	3.04	3.89	53.3	50.3	*	1.96

* Catabolic rates were not calculated from urine excretion of radioactivity in subjects R. M. and F. A. due to inadequate urine collections.

travascular distribution. It is noteworthy that the existence of a severe hemorrhagic diathesis and an obviously impaired blood coagulation mechanism in these patients was not reflected in a slower turnover of prothrombin.

DISCUSSION

The purity of the prothrombin preparation used in the present study has been demonstrated, both before and after iodination, by acrylamide-gel electrophoresis, Sephadex gel filtration, and immunologic methods (11, 12). No change in biologic activity in vitro could be detected after the labeling procedure. The absence in all experimental subjects of any early increased urinary excretion of radioactivity, together with the presence of only two logarithmic components in the plasma disappearance curves, are further in vivo evidence that the injected material was in the native state.

Previous studies of the in vivo behavior of prothrombin in man have been limited to measurement of plasma biological activity after infusion of prothrombin-containing materials or after administration of large amounts of a drug presumed to block prothrombin synthesis. By neither method can a complete set of metabolic parameters be determined. However, it is possible to compare the reported biologic half-lives of prothrombin with the normal range of 55–80 hr found in the present investigation. In general, results of infusion studies have been variable and difficult to interpret. Thus, after administration of plasma to patients congenitally deficient in prothrombin, van Creveld (1) obtained a half-life of less than 24 hr, while Borchgrevink et al. (7) estimated a figure of 48–60 hr. In similar patients, Soulier, Prou-Wartelle, and Josso (3) and Biggs and Denson (4) reported values of 123 hr and 72 hr, respectively, after infusion of a prothrombin-rich plasma protein fraction. Didisheim, Loeb, Blatrix, and Soulier (2) injected a prothrombin-rich fraction into a cirrhotic patient and obtained a half-life of approximately 40 hr, while the data of Tullis, Melin, and Jurigian (5), apparently in similar patients, suggest values of less than 24 hr. In two patients with factor IX deficiency given a prothrombin-rich plasma fraction, half-lives of 42 and 120 hr were calculated by Biggs and Denson (4). Since none of these patients was totally lacking in prothrombin, plasma disappearance curves should have been corrected for concurrent prothrombin synthesis in order to arrive at a true biologic half-life for prothrombin; only Biggs and Denson (4) considered such a correction. It is noteworthy that no studies have been reported in normal individuals by infusion techniques.

Studies performed with large doses of hypoprothrombinemic agents have given more uniform results. After administration of warfarin to normal subjects, half-lives of approximately 80, 85, and 72 hr were obtained by Frick (6), Hasselback and Hjort (8), and Hjort, Egeberg, and Mikkelson (9), respectively. Loeliger, van der Esch, Mattern, and Hamper (10) found a half-life of 68 hr after the administration of acenocoumarol. Using phenindione, Borchgrevink et al. (7) reported a half-

life of 60 hr. Thus the prothrombin half-life values obtained in normal individuals with this technique are in good agreement with the results of the present study.

Comparison may also be made of the turnover parameters of prothrombin in normal subjects with those of other plasma proteins. The mean plasma volume of 37.6 ±3.8 ml/kg is in excellent agreement with the value of 35.6 ±7.0 ml/kg obtained by Takeda (22) with fibrinogen and the figure of 38.3 ±6.8 ml/kg obtained by Takeda and Reeve (23) with albumin, but is somewhat lower than the mean values of 40.2 ml/kg determined by Andersen (24) with IgG and 42.0 ml/kg reported by Petz et al (25) with the C'3 component of complement. The fractional catabolic rate of prothrombin in normal subjects, 30–55% of the plasma pool per day, is several times greater than that for albumin (23) or IgG (24, 26), but is similar to the rates for fibrinogen (22), haptoglobin (27), and the C'3 component of complement (25, 28). The latter three proteins, like prothrombin, are known to possess specific metabolic functions, which may contribute to their rapid catabolism. Nearly two-thirds of the total body pool of prothrombin (molecular weight Ca. 70,000 [29]) is present in the intravascular compartment. This figure is considerably higher than those reported for other plasma proteins of comparable size. Thus, only 42% of the total pool of albumin (23) and 50% of the body pools of transferrin (30) and haptoglobin (27) are intravascular. That size alone does not determine the intravascular-extravascular distribution of plasma proteins is illustrated by the behavior of immunoglobulins G and D. Although both have molecular weights of approximately 160,000, only 50% of the former (24, 26) but 74% of the latter (31) are present in the intravascular compartment. In this connection it is interesting that, although small amounts of albumin and transferrin may be found in normal urine, there is no detectable urinary excretion of prothrombin.

Studies in four hemophilic patients do not demonstrate any abnormalities in prothrombin metabolism. Were a large part of prothrombin catabolism actually due to coagulation turnover, one might reasonably expect that these severely hypocoagulable subjects, with classical histories of hemorrhage and with nearly total lack of factors VIII or IX, would show a reduced fractional catabolic rate. Analysis of the results of the present study indicate that a difference in the mean catabolic rates of the normal subjects, and the hemophilic patients of less than 19% would not be statistically significant at the 5% level. Thus, if only a small part of prothrombin catabolism were due to coagulation turnover, then the uncertainties inherent in the present techniques would not allow differentiation of the normal state from that of severe hypocoagulability. This conclusion is in agreement with earlier studies with hypoprothrombinemic agents in a hemophilic patient (9) and in normal subjects given heparin (8), in which normal prothrombin half-lives were found. Takeda and Chen (32) have reported hypercatabolism of fibrinogen in patients with hemophilia A. In view of the results of the present study, it seems likely that such a finding reflects increased direct breakdown of fibrinogen, perhaps related to the patients' multiple previous exposures to fibrinogen in plasma and factor VIII preparations, rather than to an increased rate of fibrin formation.

The concept of a continuous hemostatic balance between fibrin formation and breakdown has been advanced by many authors. Review of the pertinent literature, however, indicates that no direct evidence is available to support this concept (33, 34). In the present study no evidence of radioactive prothrombin breakdown products could be detected in the normal circulation, and no differences could be measured in prothrombin metabolism between normal and hypocoagulable subjects.

Such considerations lead to the conclusion that if continuous coagulation, in the sense of a continuous fluid-phase activation of prothrombin, indeed occurs, it accounts for less than 19% of the normal prothrombin degradation rate.

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