P. R., *Am. J. Syph., Gonorrh. and Ven. Dis.*, 1951, v35, 361.

4. Smith, P. F., and Morton, H. E., *J. Bact.*, 1951, v61, 395.

5. Cohn, E. G., McMeekin, T. L., Oncley, J. L., Newell, J. M., and Hughes, W. L., *J.A.C.S.*, 1940, v62, 3386.

6. Beveridge, W. I. B., Campbell, A. D., and Lind, P. E., *Med. J. Aust.*, 1946, v1, 179.

Received March 24, 1953. P.S.E.B.M., 1953, v83.

Plasma Thromboplastin Component (PTC) Potency of Plasma Fractions.* (20268)

SIDNEY G. WHITE,† PAUL M. AGGELER, AND BYRON E. EMERY.
(Introduced by G. S. Gordan.)

From the Department of Medicine, University of California School of Medicine, San Francisco, and the Cutter Laboratories, Berkeley, Calif.

In plasma thromboplastin component (PTC) deficiency disease, little or no shortening of the prolonged blood coagulation time or improvement in deficient prothrombin utilization follows the *in vivo* or *in vitro* addition of fraction I of Cohn(1,2). Other differential characteristics of the anti-hemophilic factor (AHF) and PTC are shown in Table I. A purified preparation of PTC, free of AHF,

TABLE I. Differential Characteristics of Plasma Thromboplastin Component (PTC) and Anti-Hemophilic Factor (AHF).

Source of material	PTC	AHF
Normal serum	P*	A*
Hemophilic plasma	P	A
PTC deficient plasma	A	P
45-50% saturated $(NH_4)_2SO_4$ fraction of normal plasma	P	A
0-33% saturated $(NH_4)_2SO_4$ fraction of normal plasma	A	P
Pseudoglobulin-albumin fraction of normal serum†	P	A
$BaSO_4$ adsorbed normal plasma	A	P
Citrate eluate from $BaSO_4$ which was mixed with normal plasma or serum	P	A

* P = Present; A = Absent.
† Supernate after removal of euglobulin by dilution and acidification.

* This work was supported in part by a research grant from the National Cancer Institute, of the National Institutes of Health, Public Health Service, and by grants from the Adalynn Herndon Memorial Fund and the Henry Schussler Memorial Fund. Opinions expressed are those solely of the authors and in no way reflect the official views of the U. S. Air Force.

† Major, M. C., U. S. Air Force.

TABLE II. *In Vitro* Effect of Plasma Fractions on Residual Serum Prothrombin in PTC Deficiency.

Substance added (0.1 cc to 2. cc of patient's blood)	Concentration, mg/cc*	Residual serum prothrombin† (units/cc)
0.32% sodium citrate in 0.9% NaCl		180
Normal plasma		<10
Fraction I	3.80	105
II	5.10	255
II + III	18.50	<10
IV	9.52	15
IV-1	4.09	<10
IV-4	5.43	100
V	29.8	200

* Each fraction was reconstituted to its original plasma concentration and dialysed against 0.32% sodium citrate in 0.9% NaCl overnight. The dialysis fluid was then changed and the fractions dialysed another 4 hr.
† Two-stage method of Ware and Seegers(4), after incubation of blood for 1 hr at 37°C.

prothrombin and prothrombin conversion accelerators has been made by acidification of normal serum, adsorption of the serum with barium sulfate, and elution of the PTC from the barium sulfate with sodium citrate(2). This preparation is highly potent *in vitro*, but is unsuitable for intravenous administration in the human subject. The present investigation was conducted in an effort to find a potent PTC preparation suitable for intravenous administration among the fractions precipitated from plasma by varying the alcohol concentration and pH at low temperatures according to Cohn's methods(3).

Results. The PTC potency of the various fractions was determined by testing their

TABLE III. Effect on Coagulation Time and Residual Serum Prothrombin of Addition of Fraction IV* to the Blood in Hemophilia and PTC Deficiency.

Substance added (0.1 cc to 2. cc of patient's blood)	Coagulation time† (min.)		Residual serum prothrombin‡ (% of normal)	
	Hemophilia	PTC deficiency	Hemophilia	PTC deficiency
0.9% NaCl	540	22	100	100
Undiluted fraction IV	35	13	100	10
1:10 fraction IV	—	13	—	14
1:100 "	—	13	—	66
1:1000 "	—	16	—	43

* Containing 6.5% protein.
† Done in duplicate in 13 mm tubes at 37°C.
‡ Two-stage method of Ware and Seegers(4), after incubation of blood for 1 hr at 37°C.

effect *in vitro* on the PTC deficient patient's defective prothrombin utilization. The PTC potency of fractions II + III, IV and IV-1 was high, that of I and IV-4 slight, and that of II and V none (Table II). When fraction III was separated from fraction II + III, it was found to contain thrombin, and therefore could not be assayed separately for PTC nor administered intravenously to the patient. None of the other fractions contained thrombin, as shown by the failure of coagulation to occur when 0.05 cc of the fraction was added to 0.5 cc of bovine fibrinogen (Armour) and allowed to stand at room temperature for 4 hours. Fraction IV-1, although potent, was not sufficiently soluble for intravenous use.

It is obvious from the *in vitro* tests that either fraction II + III or fraction IV might be suitable for intravenous administration, but since fraction II + III is used in the preparation of immune globulin, whereas fraction IV is discarded, it was decided to limit the *in vivo* investigations to fraction IV.

A preparation of fraction IV for intravenous use was made as follows: an 11% concentration of the fresh fraction was mixed in a Waring blendor, passed through a sterilizing porcelain filter, and frozen. After filtration it contained 9.2% total solids, 6.5% protein, 190 units of prothrombin per cc(4), and 69% of the proconvertin of an equivalent volume of plasma(5). *In vitro* tests with this preparation showed it to be active when added in a proportion of 1:20 of a 1:1000 dilution to the patient's blood (Table III). Tests for sterility, pyrogenicity, toxicity, and vasodepressor effects in rabbits were negative. No untoward effects on pulse, blood pressure, temperature or respiration were observed after intravenous administration of the fraction to a PTC deficient patient. Although the concentrated solution produced some shortening of the whole blood clotting time in hemophilic blood, it had no effect on prothrombin utilization. The results of two successive doses of 85 cc each of the fraction given intravenously to a PTC deficient patient at weekly intervals are shown in Table IV. The effect of each dose, derived from approximately 550 cc of plasma, was roughly comparable to that of 250 cc of plasma (Table V). Fraction IV therefore appears to contain approximately one-half of the PTC potency of plasma. The best preparations of fraction I contain only

TABLE IV. Effect on Coagulation Time and Residual Serum Prothrombin of Intravenous Administration of Fraction IV* in PTC Deficiency.

	Coagulation time,† min.	Residual serum prothrombin,‡ % of normal
Before 1st intrav. admin. of 85 cc of fraction IV	240	100
15 min. after 1st dose	11	31
3 hr " " "	22	62
24 hr " " "	18	57
2 days " " "	22	56
4 days " " "	21	61
7 days " " "	22	100
Before 2nd intrav. admin. of 85 cc of fraction IV		
15 min. after 2nd dose	12	17
4 hr " " "	12	24
24 hr " " "	16	45
4 days " " "	23	88

* Containing 6.5% protein.
† Done in duplicate in 13 mm tubes at 37°C.
‡ Two-stage method of Ware and Seegers(4), after incubation of blood for 1 hr at 37°C.

TABLE V. Comparative Effects of Intravenous Administration of 250 cc of Normal Plasma and 85 cc of Fraction IV* in PTC Deficiency.

	Coagulation time,† min.		Residual serum prothrombin,‡ units/cc	
	Plasma	Fraction IV	Plasma	Fraction IV
Before	>120	>120	260	260
15 min. after	15	11	85	82
4 days "	17	21	134	164
7 " "	16	22	220	260

* Containing 6.5% protein.
† Done in duplicate in 13 mm tubes at 37°C.
‡ Two-stage method of Ware and Seegers(4), after incubation of blood for 1 hr at 37°C.

about one-tenth of the AHF potency of plasma(6). Fraction IV is present in the plasma in a concentration of approximately 1 g per 100 cc, while fraction I is present in a concentration of only 0.38 g per 100 cc. It would, therefore, require almost twice the volume of an equivalent protein concentration of fraction I derived from 5 times the original quantity of plasma to produce similar results in hemophilia.

Conclusions. 1. The PTC factor is concentrated in fractions III and IV-1 of the plasma proteins. Small quantities are found in fractions I and IV-4. None is found in fractions II and V. 2. Since fraction III contains thrombin and fraction IV-1 is poorly soluble, neither is suitable for intravenous administration. 3. The intravenous administration of fraction IV produced marked shortening of the whole blood coagulation time and reduction in residual serum prothrombin in PTC deficiency. The results appear superior to those which can be achieved by the intravenous administration of fraction I in hemophilia.

1. Aggeler, P. M., White, S. G., Glendening, M. B., Page, E. P., Leake, T. B., and Bates, G., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 692.
2. White, S. G., Aggeler, P. M., and Glendening, M. B., *Blood*, 1953, v8, 101.
3. Cohn, E. J., Strong, L. E., Hughes, W. L., Jr., Mulford, D. J., Ashworth, J. H., Melin, M., and Taylor, H. L., *J. Am. Chem. Soc.*, 1946, v68, 459.
4. Ware, A. G., and Seegers, W. H., *Am. J. Clin. Path.*, 1949, v19, 471.
5. Owren, P. A., and Aas, K., *Scandinav. J. Clin. and Lab. Invest.*, 1951, v3, 201.
6. Spaet, T., Personal communication.

Received March 26, 1953. P.S.E.B.M., 1953, v83.

Metabolic Products of Cholesterol in Bile and Feces of Rat.* Steroids and Bile Acids. (20269)

SUNE BERGSTRÖM AND ARNE NORMAN. (Introduced by O. Wintersteiner.)
From the Department of Physiological Chemistry, University of Lund, Lund, Sweden.

The transformation of cholesterol into cholic acid was first demonstrated in the dog with deuterated cholesterol(1). The same reaction has recently been shown to occur in the rat with cholesterol-4-C^{14}(2,3). In the latter work we further showed that cholic acid was the main excretory end product of cholesterol metabolism constituting more than 80% of the labelled acidic products of the bile. This agrees with observations(4,5) that most of the isotope of administered cholesterol-4-C^{14} that was excreted came via the bile and feces as acidic products and that only very small amounts appeared in the expired carbon dioxide. That the labelled acidic products in the bile and feces behaved like bile acids in fractionations has subsequently been shown in the same laboratory(6).

In this paper some further results will be presented on the fractionation of the acidic products in bile and feces of rats that had received cholesterol-4-C^{14} by intraperitoneal administration.

Experimental. One mg of cholesterol-4-C^{14}

* This work is part of an investigation supported by "Statens Medicinska Forskningsrad" and "Knut och Alice Wallenbergs Stiftelse".