TABLE I.
Effect of Various Compounds Upon Pancreatic and Hepatic Changes Produced by Ethionine.

No. animals	Sex	Duration of exp., hr	Administered		Pancreas		Total liver lipids* % (wet wt) ± Standard dev.
			Ethionine*	Additional	Loss of basophilia	Necrosis	
3	f	24	—	—	0	0	5.1 ± 0.5
3	m	24	—	—	0	0	5.2 ± 0.7
3	f	24	+	—	+	0	14.6 ± 0.1
3	m	24	+	—	+	0	5.0 ± 1.2
3	f	24	+	Methionine‡	0	0	4.9 ± 0.1
3	f	24	+	Cysteine‡	+	0	14.4 ± 2.3
6	f	24	+	Glucose§	+	0	5.3 ± 0.3
3	f	24	+	Choline chloride‖	+	0	13.5 ± 2.5
6	f & m	48	—	—	0	0	5.2 ± 0.2
6	f	48	+	—	+	+	18.2 ± 5.8
3	f	48	+	Methionine‡	0	0	4.8 ± 0.3

* 1 mg per g body weight intraperitoneally.
† Determined as described previously (11).
‡ Administered intraperitoneally in amounts equimolar to and simultaneously with ethionine. In addition, one-half molar quantities were administered every 12 hr until time of sacrifice.
§ 2.5 g by stomach tube, in 3 divided doses, simultaneously with ethionine and 1.5 g 12 hr later.
‖ 42 mg intraperitoneally in 3 divided doses, simultaneously with ethionine and 10.8 mg 12 hr later.

ethionine administration. The lesion is interpreted as a result of interference with methionine (and possibly protein) metabolism.

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Plasminogen Purification by Acid Extraction.* (18063)

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Several methods have been reported for the partial purification of the active form of serum protease (see Tagnon *et al.*(1) and Rocha e Silva and Rimington(2) for references). Relatively few attempts have been made to isolate the naturally-occurring inactive form (plasminogen, profibrinolysin, serum tryptogen, etc.) in a state of high purity. The preparations commonly used have consisted essentially of the euglobulin fraction of serum or plasma, obtained by salting out, dialysis, or dilution and acidification (Milstone(3), Christensen(4,5), Christensen and MacLeod(6), Kaplan(7), Holmberg(8), MacFarlane and Pilling(9), Ratnoff (10).) In these preparations the enzyme

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represents only a small portion of the total proteins, and in addition, such preparations are contaminated by variable amounts of inhibitor and antistreptokinase. Oncley and associates(11) obtained subfractions of human plasma containing plasminogen and prothrombin by ethanol fractionation. However, their method of fractionation involved spontaneous activation of a portion of the plasminogen in the presence of fibrin and other proteins, resulting in contamination of the preparation by active plasmin and by the split products of fibrin. Recently Cohn and associates(12) have reported a newer method of plasma fractionation, but information regarding purity and yield of the plasminogen-containing fraction has not been published. The most highly purified preparations described up to the present are those of Remmert and Cohen(13), who reported purification of 135-165 fold as compared with whole serum. Other procedures have been published, Loomis, George and Ryder(14); Richert (15), but insufficient data on original and final purity have been given to evaluate the amount of purification obtained. Remmert and Cohen experienced difficulty with their procedures because of the inconsistent behavior of different preparations during purification.

The present paper details a simple procedure involving a hitherto undescribed property of plasminogen, solubility in dilute mineral acids, by means of which purification of as much as 250 fold or more may be obtained.

Materials and methods. 1. Streptokinase prepared by modification of methods described previously (Christensen, 1950) was obtained from Lederle Laboratories.† The preparation contained about 550 streptokinase units per microgram of nitrogen. For use as activator in fibrinolytic assays, it was diluted to contain 3000 units per assay tube, since preliminary titration showed this to be the optimum quantity to give maximum plasminogen activation under the conditions of this assay. In determination of casein proteolysis by the method of Remmert and Cohen(1), aliquots of the plasminogen preparations were activated with 10-fold dilutions of streptokinase. In the majority of cases maximum activation was obtained with 150 to 1500 units of streptokinase in the digestion mixture.

2. Fibrin: Armour's Bovine Fibrinogen, clotted with Lederle Hemostatic Globulin† in the presence of plasminogen and streptokinase was used as substrate for fibrinolytic assays.

3. Casein: Several preparations, including one obtained from Dr. Philip Cohen, were employed. Results with different preparations were of the same order of magnitude, but not identical. In order that our results might be comparable with those of Remmert and Cohen, assays reported in the present paper were carried out with their sample of casein.

4. Several preparations containing plasminogen activity were available. Two lots of plasma fraction III,‡ prepared by Harvard method 9 (Oncley *et al.*)(4) were available in the lyophilized state. In addition a fraction of human placental extract†, a by-product of the commercial preparation of placental immune globulin, was available as a frozen paste and as a lyophilized powder.

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† We are indebted to Mr. Frank Ablondi of Lederle Laboratories for generous gifts of Hemostatic Globulin and placental fractions as well as preparation and purification of special lots of streptokinase for these studies.

‡ One lot of plasma fraction III was obtained through the kindness of Dr. Dwight Mulford, Division of Biologic Laboratories, Massachusetts State Department of Health. The second lot of plasma fraction III was obtained from the American National Red Cross through the courtesy of Dr. L. E. Strong.

TABLE I.
Comparison of Acid and Buffer Extraction of Plasminogen from Plasma Fraction III and Placental Fraction III.

Extracting medium	Total activity*	Total nitrogen	Purity (units/mg N)
	Plasma fraction III		
0.2 N H_2SO_4	2.0×10^6	37 mg	53,000
Saline phosphate buffer pH 7.5	2.5×10^6	280 mg	8,930
	Placental fraction III		
0.2 N H_2SO_4	0.8×10^6	265 mg	3,020
Saline phosphate buffer pH 7.5	0.8×10^6	750 mg	1,066

* Activity in fibrinolytic units.

TABLE II.
Purification of Acid Extracts by Precipitation and Reextraction with Acid.

Extract No.	Total activity*	Total nitrogen	Purity (units/mg N)
	Plasma fraction III		
1	2.88×10^6	85.56 mg	33,600
2	0.768×10^6	2.88 mg	266,000
	Placental fraction III		
1	0.7×10^6	116.5 mg	6,000
2	0.3×10^6	7.0 mg	42,900
3	0.18×10^6	2.76 mg	65,200

* Activity in fibrinolytic units.

This material, on the basis of its method of preparation, is probably equivalent to plasma fraction III.

5 Fibrinolytic protease assays were performed by the method described previously (Christensen, 1950). One unit of plasmin is that amount, in a digest mixture of 1.0 ml, which will lyse the standard fibrin clot in 30 minutes at 35°C. For greater accuracy, the lysis times of serial dilutions of the protease are plotted, and the dilution lysing in 30 minutes obtained by interpolation on the curve.

6. Proteolysis of casein was determined as described by Remmert and Cohen(1), using a sample of casein prepared by them.

Experimental. Preliminary experiments with various procedures for protein fractionation, as well as with the procedures described by Remmert and Cohen(1) confirmed their view that the precipitation behavior of plasminogen preparations is inconsistent, and that there appears to be a marked tendency for plasminogen to co-precipitate with other proteins. It was decided, therefore, to attempt selective extraction rather than precipitation of the enzyme. A variety of extraction procedures, including extraction at varying pH levels with dilute solutions of alcohols and salts, were tried without notable success. Dilute solutions of mineral acids, however, were found to extract all plasminogen activity from the starting materials. The procedure is based on extraction of the starting material with H_2SO_4 or HCl for a period of 15-30 minutes at room temperature. The normality of the acid is not critical, the usual concentration employed being 0.2N H_2SO_4. However, satisfactory results have been obtained with concentrations between 0.1N and 0.05N. The ratio of dry weight of crude plasminogen to acid does not appear to be critical. Satisfactory preparations have been obtained with concentrations (dry weight) of crude plasminogen of 4-10% in the extracting medium. The usual practice has been to extract 200-500 g of frozen placental extract (about 20-50 g dry weight) in 500 ml 0.2N acid, or 10 g dried plasma fraction III in 200 ml acid. Following acid extraction, the insoluble debris is removed in an angle centrifuge. The acid supernatant is brought to 0.05M phos-

phate and the pH adjusted to about 7.5 with NaOH. If desired, the sulfate ions may be removed before neutralization by treatment of the acid extract with sufficient Amberlite IR-4-B (Rohm and Haas Company) to raise the pH to about 7. Phosphate is added as above and the pH adjusted with acid or base. A slight, finely-divided precipitate usually forms on neutralization. If this precipitate is removed, a portion of the activity is lost. The purity of the first acid extract is variable, even from aliquots of the same lot of crude plasminogen. The cause of this variability has not been ascertained. The minimum purity obtained has been of the order of 3000 fibrinolytic units per mg. In general, the preparations from plasma fraction III average about 50,000 units per mg of nitrogen, while preparations from placental fraction III are less pure. In Table I are presented the results of typical extractions of placental and plasma fractions with acid and with saline phosphate buffer at pH 7.5.

The crude plasminogen preparations tested have been characterized by the presence of variable amounts of lipoid material in the extracts, greater in the case of the placental preparations than in the plasma fractions. The lipoidal material can be removed by a preliminary extraction of the crude preparations with alcohol or acetone, followed by ether.

The initial acid extracts may be further purified by the following procedure. The acid or neutralized extract is adjusted to pH 5.2 with alkali or acetic acid. The precipitate which forms is removed by centrifugation and reextracted with 0.2N H_2SO_4. If necessary this precipitation at pH 5.2 and reextraction with acid may be repeated. Table II illustrates typical results obtained by this procedure with placental fraction III and plasma fraction III.

Plasminogen preparations obtained in the above manner show little development of spontaneous activity, at most a fraction of 1% of the total.

The sulfuric acid extracts are less stable than the neutralized extracts. Stability was determined by storing acid and neutralized extracts of placental and plasma fractions in the refrigerator at 3°C and in a water bath at 35°C. At intervals, aliquots were removed and the residual fibrinolytic activity determined. The results of this experiment are presented in Fig. 1.

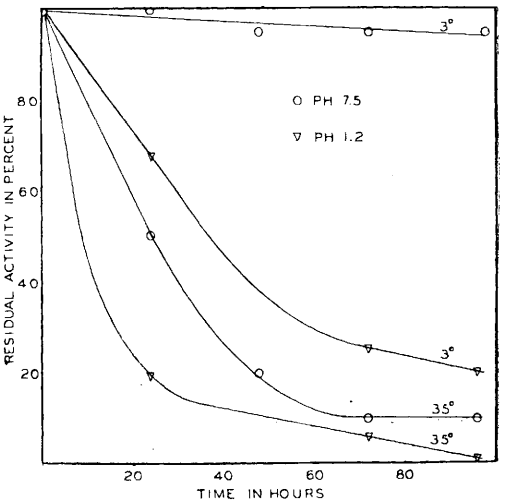

FIG. 1.

Decrease in activity of acid-extracted plasminogen at 3°C and 35°C. One sample (pH 1.2) is the original acid extract. The second sample (pH 7.5) was adjusted with NaOH after adding phosphate to 0.05 M.

As may be seen from the data in Fig. 1, the acid extracts at pH 1.2 are quite unstable at both 35° and 3°C, practically all activity being lost in 4 days. On the other hand, the neutralized extract at 3°C showed only slight loss of activity during this period. However, at 35°C, the neutralized extract was also unstable. Spontaneous activity did not develop in these preparations as indicated by their inability to lyse fibrin clots in the absence of streptokinase.

Remmert and Cohen(13) have reported purification of about 165-fold over serum, representing a purity of 19 casein proteolytic units per mg nitrogen. Analysis of plasminogen preparations, purified by acid extraction, by the casein proteolysis method indicates that purities of 20-30 casein units per mg nitrogen are frequently obtained, indicating a purification of more than 250-fold, a figure which is in agreement with the purification factor calculated from fibrinolytic data.

Summary. The procedures described above

utilize a previously undescribed property of plasminogen, solubility in dilute mineral acid, to extract it from placental and plasma fractions. Purification of over 250-fold has been obtained by successive extractions with 0.2N H_2SO_4 and precipitations at pH 5.2. The total yield has ranged from a minimum of about 10% to almost complete recovery of the enzyme present in the starting material, with an average recovery of about 30%.

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Observations on Pain and Temperature Perception within the Sternal Marrow Cavity. (18064)

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It is generally known that during the performance of a sternal marrow aspiration, the subject usually experiences an aching pain of one to five dol† intensity at the moment of actual aspiration of marrow contents. This pain is separate and distinct from any discomfort that the patient may experience during the introduction of the aspiration needle and it is not abolished by procainization of the skin and periosteum. In most areas of the body specifically studied, both pain and temperature can be perceived. Many workers (1,2) have shown that distension of the stomach with sufficient force results in pain. Wolf and Wolff(2) have demonstrated that a sense of warmth or cold is experienced when either water warmer than 40°C or colder than 18°C is circulated within the stomach. It is common experience that either pain or temperature sensation may be elicited by suitable stimulation of the skin. The normal bladder possesses both pain and temperature sensation(3).

Therefore, we wished to determine the presence or absence of temperature perception within the sternal cavity.

Sternal marrow aspiration was performed in 3 healthy, young adult males. The procedure was preceded by anesthetization with 4 cc of 2% procaine of the skin, deep tissue and periosteum overlying the site of the puncture. Great care was taken to ensure complete anesthesia of these structures before introducing the needle. An 18 gauge needle was employed. The gross appearance and the stained smears of the material aspirated gave proof that the needle was in the marrow cavity. In each subject warm or cold isotonic saline was injected in random order using a 2 ml capacity Luer glass syringe. Care was taken that the subject received no cue as to the temperature of the saline either from comments of the examiners or from spillage on the skin.

During the entire procedure, the first subject noted, in addition to the pain incident to withdrawal of the marrow sample, a deep burning pain of one to 2 dols intensity. Following marrow aspiration, while the needle remained in the cavity, 4 injections of 1 ml each of isotonic saline were given. The temperature of the 2 injections of cold saline solution was approximately 18°C and of the

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† Hardy, Wolff and Goodell(4,5) define the "dol" as a unit of painfulness equal to 2 just noticeable differences in pain intensity of pain. Ten dols equal the maximum pain.

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