onstrated a 30% reduction in GAA methylation and a 45% reduction in total synthesis of creatine from glycine. It is concluded that chronic renal disease causes reduced creatine (and creatinine) synthesis both by loss of the renal mechanism and by interference with methylation. It is suggested that extra-renal (pancreatic?) synthesis may be relatively increased to partially compensate for these 2 inhibitory factors.

1. Bloch, K., Schoenheimer, R., *J. Biol. Chem.*, 1941, v138, 167.

2. Du Vigneaud, V., Chandler, J. P., Cohn, M., Brown, G. B., *ibid.*, 1940, v134, 787.

3. Borsook, H., Dubnoff, J. W., *ibid.*, 1941, v138, 389.

4. Walker, J. B., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 7.

5. Goldman, R., Moss, J. X., *Am. J. Physiol.*, 1959, v197, 865.

6. Horner, W. H., *J. Biol. Chem.*, 1959, v234, 2386.

7. Foster, G. L., Schoenheimer, R., Rittenberg, D., *ibid.*, 1939, v127, 319.

8. Goldman, R., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 446.

9. Sandburg, A. A., Hecht, H. H., Tyler, F. H., *Metabolism*, 1953, v2, 22.

10. Walker, J. B., Walker, M. S., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 807.

11. Walker, J. B., *Biochim., et Biophys. Acta.*, 1959, v36, 574.

12. ———, *J. Biol. Chem.*, 1960, v235, 2357.

13. Fitch, C. D., Hsu, C., Dinning, J. S., *ibid.*, 1960, v235, 2362.

Received October 24, 1960. P.S.E.B.M., 1960, v105.

Separation of Plasma Thromboplastin Antecedent (PTA) and Hageman Factor (HF) from Human Plasma.* (26138)

SANDRA SCHIFFMAN, SAMUEL I. RAPAPORT, ARNOLD G. WARE, JOHN W. MEHL
Department of Biochemistry and Nutrition, and Dept. of Medicine, University of Southern California School of Medicine.

Plasma acquires increased coagulability after contact with glass or certain other charged surfaces such as kaolin or celite. Following the discovery of patients deficient in plasma thromboplastin antecedent (PTA) (1) or Hageman Factor (HF)(2), these plasma clotting factors were shown to be involved in development of this increased coagulability. It has been postulated(3) that the glass surface "activates" HF enabling it to react with PTA and so initiate coagulation.

Waaler(4) found that clotting time of plasma lengthens markedly when it is treated with glass powder, the powder removed, and the plasma allowed to stand for several hours at 37°C. He called the product exhausted plasma (EP) and believed it was depleted of HF and PTA.

Others have attempted to purify HF and PTA, but these factors have not been separated. This communication describes the separation of HF and PTA by ion exchange chromatography. As shown below, each separated factor corrects the defect of its corresponding deficiency plasma. However, the combined purified factors fail to shorten clotting time of EP unless another as yet unidentified component of plasma is also present.

Methods. Our assays were based upon the kaolin clotting time technic of Margolis(5). The clotting system consisted of 0.1 ml of a suspension of cephalin-kaolin, 0.1 ml of a substrate plasma, 0.1 ml of the test substance, and 0.1 ml of 30 mM $CaCl_2$. The cephalin-kaolin suspension was prepared by combining equal parts of a 1/35 (v/v) suspension of cephalin in veronal buffer(6) and a 40 mg/ml kaolin suspension in physiological saline. The first 3 reagents were incubated together for 3 minutes at 37°C before the pre-warmed calcium was added and clotting time determined. The following substrate plasmas were used: PTA deficiency plasma for assay of PTA, HF deficiency plasma for the assay HF, or EP for assay of the com-

* Supported by grants from the Attending Staff Assn., Los Angeles County Hosp. and Gustavus and Louise Pfeiffer Research Foundation.

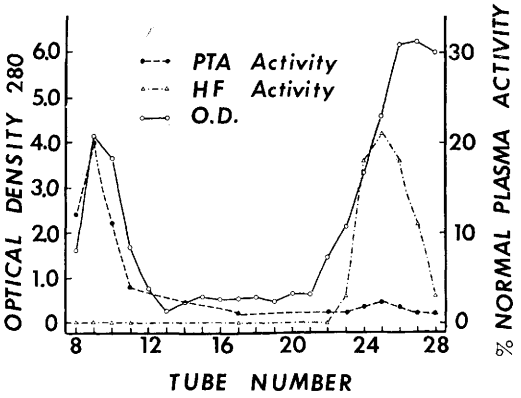

FIG. 1. PTA activity, HF activity, and protein concentration (OD at 280 mμ) of consecutive elution fractions from a DEAE cellulose column.

bined effects of HF and PTA.† The same systems were used with all contact surface removed (*i.e.*, without kaolin and with silicone-coated clotting tubes) to estimate prior glass activation. A dilution curve was obtained by substituting different concentrations of normal plasma into each assay system. The results when plotted on log-log paper produced a straight line graph from which relative concentration of unknown solutions could be estimated in % of normal plasma activity.

Separation of PTA and HF from intact‡ normal plasma was carried out by using the ion exchange resin diethylaminoethyl cellulose (DEAE, California Corp. for Biochemical Research, Los Angeles). A constant pH of 7.12 was used; salt concentration was increased continuously from 0.05 M to 0.3 M by varying NaCl concentration in presence of 0.02 M sodium diethyl barbiturate buffer. The column was lucite with an ash free filter paper support. Neither lucite nor filter paper activated normal intact plasma. Fractions were collected in polyethylene tubes in a cold room.

Results. The results of a typical separation on DEAE are shown in Fig. 1. The maximum PTA activity coincided with the first protein peak as measured by ultraviolet absorption at 280 mμ, while the greatest HF activity immediately preceded the second protein maximum. All activities were corrected for the difference in volume between the plasma sample applied and the resulting column fractions. Fractions containing either PTA or HF were tested in specific assays for AHG, PTC, proconvertin, prothrombin-Stuart and proaccelerin. Less than 1% of normal plasma activity was found in each case.

TABLE I. Correction of EP by PTA and HF Deficiency Plasmas.

Test substance	Clotting time (sec.)
Citrate saline (in silicone)	195
PTA deficiency plasma	120
HF " "	63
Normal plasma	44

A study was made of the effect of PTA and HF fractions on clotting time of EP. Our EP, prepared by Waaler's method using celite(4), was shown by specific assays to contain adequate amounts of prothrombin, proconvertin, proaccelerin, Stuart factor, AHG and PTC. Its long clotting time was partly corrected by HF deficiency plasma but not by PTA deficiency plasma (Table I). Therefore, our EP was depleted primarily of PTA.

The prolonged clotting time of EP was

TABLE II. Failure of a Mixture of Purified PTA and HF to Correct EP.

Test substance*	Clotting time (sec.)
PTA deficiency plasma + HF deficiency plasma	61
PTA fraction + PTA deficiency plasma	68
PTA " + citrate-saline	162
HF " + HF deficiency plasma	68
HF " + citrate-saline	181
PTA " + HF fraction	158

* Quantities combined in equal parts in polyethylene tubes and 0.1 ml of the mixture used as test substance.

† PTA deficiency plasma was obtained from patients whose coagulation studies have been described(7). HF deficiency plasma was taken from a patient with classical clinical and laboratory findings. Dr. Oscar Ratnoff kindly substantiated the deficiency by cross correction experiments with this plasma in his laboratory. Mixing of HF and PTA deficiency plasma in our laboratory resulted in complete correction of their individual defects.

‡ Intact plasma—plasma prepared in silicone-coated glassware or in plastic and therefore protected from contact with glass.

shortened either by a combination of PTA and HF deficiency plasmas or by each purified activity combined with its deficiency plasma (Table II). In contrast, addition of the combined purified activities did not shorten clotting time. These data suggest that plasma contains an additional activity which is missing from EP.

Summary. 1) Hageman factor and plasma thromboplastin antecedent have been separated from human plasma and each other by ion exchange chromatography. 2) The defect in "exhausted plasma" is not corrected by purified PTA, purified HF, or a combination of the two.

1. Rosenthal, R. L., Dreskin, O. H., Rosenthal, N., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 171.
2. Ratnoff, O. D., Colopy, J. E., *J. Clin. Invest.*, 1955, v34, 602.
3. Soulier, J. P., Wartelle, O., *Brit. J. Haem.*, 1960, v6, 88.
4. Waaler, B. A., *Scand. J. Clin. Lab. Invest.*, 1958, v9, Suppl. 37.
5. Margolis, J., *J. Clin. Path.*, 1958, v11, 406.
6. Rapaport, S. I., Aas, K., Owren, P. A., *J. Lab. and Clin. Med.*, 1954, v44, 364.
7. Rapaport, S. I., *ibid.*, 1958, v52, 624.

Received Sept. 12, 1960. P.S.E.B.M., 1960, v105.

Early Metabolic Effects of Human Growth Hormone.* (26139)

GASTON R. ZAHND,† JURGEN STEINKE,‡ ALBERT E. RENOLD
(Introduced by A. Marble)
Baker Clinic Research Laboratory, Dept. of Medicine, Harvard Medical School, and Peter Bent Brigham and New England Deaconess Hospitals, Boston, Mass.

The diabetogenic action of chronic administration of growth hormone is well established, both in experimental animals and in man. Insulin-like effects of the hormone have also been described, especially as a result of its acute administration(1) or of its presence *in vitro*(2,3). These effects have been related either to an insulin-like activity of the hormone itself or, *in vivo,* to a tropic effect of growth hormone upon the islets of Langerhans(4,5). In recent years, the physiologic importance of species differences in structures and function of growth hormone have become apparent(6,7,8), suggesting that previously observed effects should be reevaluated using growth hormone obtained from the same species. Although the metabolic effects of chronic administration of human growth hormone to human subjects with and without diabetes mellitus have been extensively described(9,10), the early metabolic effects have not been outlined as clearly.

In this report, an acute hypoglycemic effect of human growth in man will be described and related to changes in plasma free fatty acids described by Raben(11) and to measurements of serum insulin-like activity.

Materials and methods. Human growth hormone was prepared from pituitaries collected at autopsy according to the technic outlined by Raben(9). The potency of the preparation was tested against a sample obtained from Dr. Raben and exhibited approximately the same activity, mg per mg, using its effect upon plasma free fatty acids in man as activity index. When tested for contamination with ACTH, using the bioassay procedure of Nelson and Hume(12), 100 μg of the preparation contained less than 40 $\mu\mu$g purified ACTH.§

Growth hormone administration was carried out in 6 male human volunteers in apparent good health, ranging in age from 25 to 37 years. They were kept fasting over-

* Supported in part by grants-in-aid from Rear Admiral W. L. Capps Fund, Harvard Med. School, Massachusetts Lions Eye Research Fund and Eli Lilly & Co., Indianapolis, Ind.

† Trainee, U. S. Public Health Service.

‡ Research Fellow of Am. Diabetes Assn.

§ The authors are indebted to Dr. M. S. Raben for help and advice, and to Dr. K. Vance for assaying ACTH content of the growth hormone preparation used.