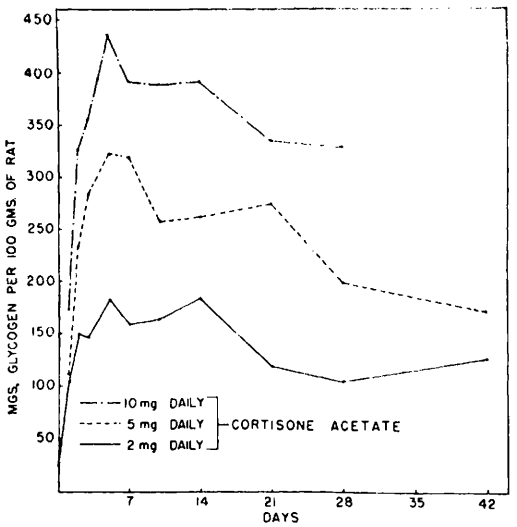

FIG. 1. Values for liver glycogen in rats treated with cortisone acetate. Each point in the curves represents avg of 6 to 8 rats. Expressed as mg of liver glycogen per 100 g of rat.

adaptation to hypercorticalism. There is a similar decline in the time-response curves showing the effect of high cortisone dosage upon urinary non-protein nitrogen and the glycosuria of rats having steroid diabetes induced by cortisone(3). This is not the only interpretation which can be made of the data. It was noted that the livers of these animals became progressively fatty especially at cortisone dosage of 5 and 10 mg daily. Baker *et al.* (4) studied similar animals having hypercorticalism induced by corticotropin and suggested that sufficient fat accumulated in the liver so that some of its functions might have been impaired. Partial impairment of hepatic participation in gluconeogenesis from protein might explain the reduction in the level of liver glycogen and also the decline in urinary nitrogen and glucose from their initial peak levels.

Summary. Male rats having an initial weight of approximately 300 g were given single daily injections of cortisone acetate in doses of 2, 5, and 10 mg/rat/day. At each of the 3 dosage levels 6 to 8 rats were killed at intervals ranging from 1 to 42 days and the amount of liver glycogen determined in each rat. The peak level was reached within 5 days and was followed by a significant decline towards, but not approaching, normal values.

1. Ingle, D. J., *J. Am. Pharm. Assn.*, (Scientific Edition), 1953, v42, 247.

2. Peterson, R., and Rose, D., *Canad. J. Technol*, 1951, v29, 317.

3. Ingle, D. J., Prestrud, M. C., and Nezamis, J. E., *Am. J. Physiol.*, 1951, v166, 171.

4. Baker, B. L., Ingle, D. J., Li, C. H., and Evans, H. M., *Am. J. Anat.*, 1948, v82, 75.

Received September 17, 1953. P.S.E.B.M., 1953, v84.

Single Dimension Chromatographic Separation of Thyroxin and Triiodothyronine.* (20603)

EDWIN C. ALBRIGHT, FRANK C. LARSON, AND WILLIAM P. DEISS.†

From the Department of Medicine, University of Wisconsin Medical School and Veterans Administration Hospital, Madison, Wis.

The presence of the amino acid 3:5:3′ triiodothyronine in the plasma of normal and hyperthyroid subjects was recently demonstrated by Gross and Pitt-Rivers(1). These workers separated triiodothyronine from thyroxine by means of two dimentional chromatography. We have found this procedure time-consuming, and the spots produced tend to be diffuse and lack sharp definition. Described below is a method by which thyroxin‡ and triiodothyronine§ can be conveniently sepa-

* This work was supported in part by the Wisconsin Alumni Research Foundation.

† Research Fellow of the Arthritis and Rheumatism Foundation.

‡ Kindly supplied by E. R. Squibb and Sons.

§ Kindly supplied by Smith, Kline and French Laboratories.

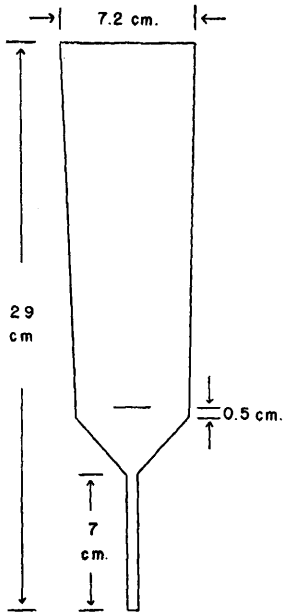

FIG. 1. Diagram of paper chromatogram.

rated by single dimension chromatography.

Method. An ascending type of chromatography was used. Whatman No. 3mm filter paper cut into a tapered form (Fig. 1), was employed(2). This has proved helpful in maintaining discrete spots; hence materially aids the separation of two amino acids with Rf values in the same range. A 4″ x 6″ x 14″ glass jar was used as the chromatographic chamber. The strips were suspended by stainless steel clips attached to brackets on the underside of the lucite lid. The tip of the paper wick was submerged 1-2 mm below the surface of the developing solvent. The jars were placed in a well insulated chamber to avoid variations in temperature. Several solvent systems were tried. In our hands butanol-primary amyl alcohol-ammonium hydroxide as employed by Robbins(3), and butanol-isoamyl alcohol-ammonium hydroxide as reported by

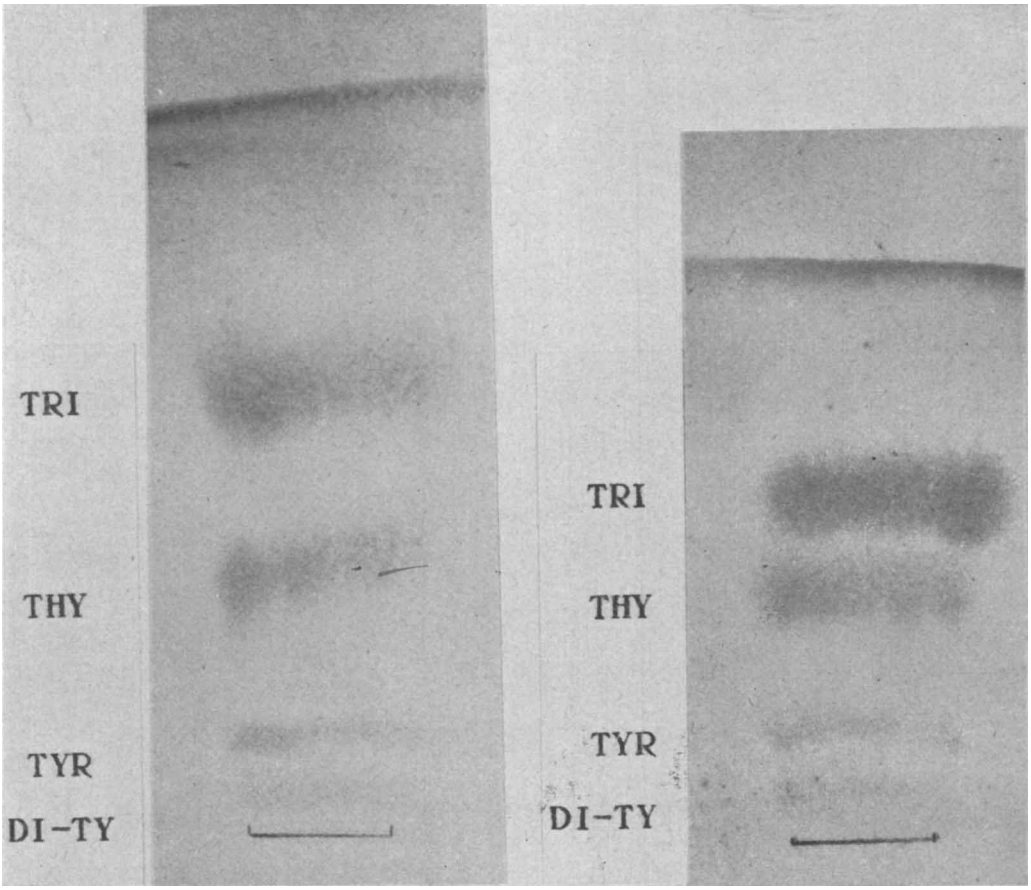

FIG. 2. Chromatograms of synthetic samples: *Left*, in butanol-dioxane-ammonia developer; *right*, in collidine-water developer. THY—thyroxin, TRI—3:5:3′-L-triiodothyronine, TYR—tyrosine, DI-TY—diiodotyrosine.

Hird(4) gave less satisfactory separation of the amino acids than the following solvents. One consisted of 44 ml of water dissolved in 125 ml of 2-4-6 collidine. The other was a mixture of 80 ml of butanol, 20 ml of dioxane, and 100 ml of 2 N ammonium hydroxide. This mixture was shaken in a separatory funnel and the aqueous phase discarded. A small beaker of concentrated ammonium hydroxide was placed in the chamber containing the collidine-water solution to saturate the atmosphere with ammonia. The *amino acids* tyrosine, diiodotyrosine, diiodothyronine, triiodothyronine and thyroxin were dissolved in 0.5 N sodium hydroxide. Solutions containing 1 mg per ml of these amino acids were prepared. These were chromatographed singly and in combination. In the serum study I^{131} labeled triiodothyronine‖ and radioactive thyroxin‖ were added to serum at a concentration of 1 μg per ml. Two ml of this serum was extracted 3 times with twice the volume of butanol. Colorimetrically identifiable quantities of the amino acid were added and the extract was concentrated to 1 ml. The solution to be chromatographed was deposited on the paper in a horizontal line 2 cm long, 9 cm from the end of the wick. Care was taken to limit the

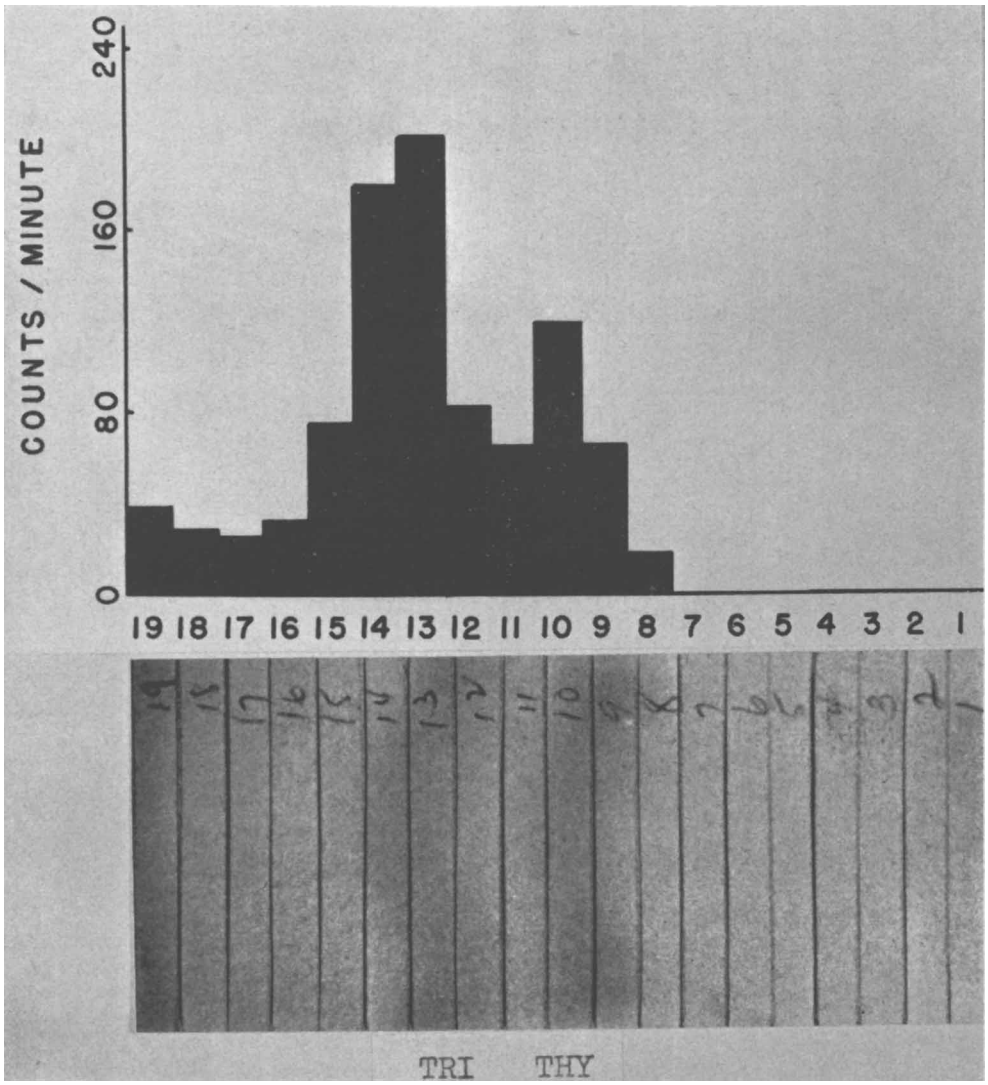

Fig. 3A

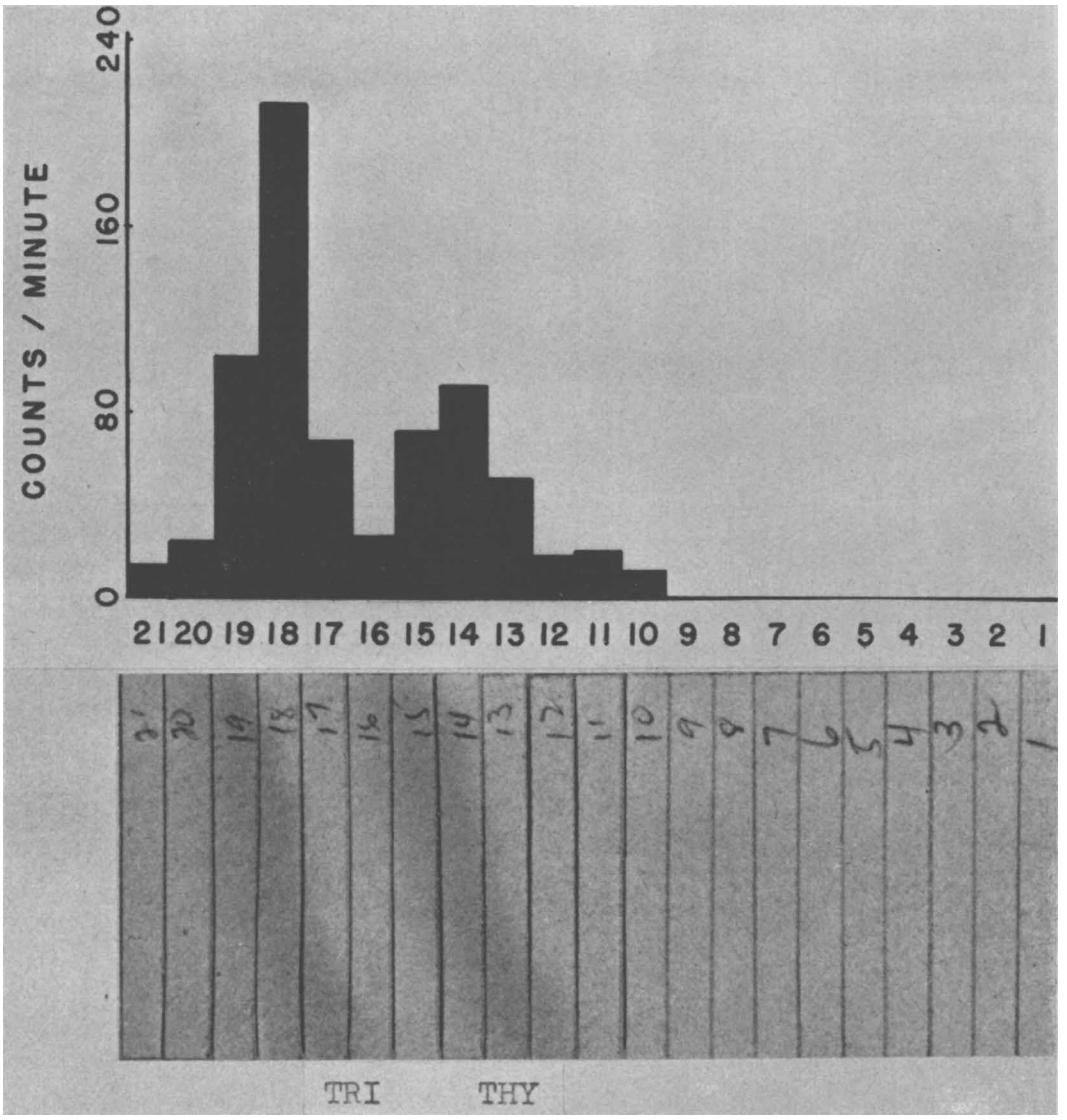

Fig. 3B

FIG. 3. Distribution of radioactivity on the chromatograms of butanol extracts of serum to which radiothyroxin and radiotriiodothyronine were added. A—collidine-water developer, B—butanol-dioxane-ammonia developer. Position of the amino acid is indicated by legend, THY—thyroxin, TRI—triiodothyronine.

width of the line to 2-3 mm. If the strip is warmed during the application of the solution, 50 microliters can be conveniently applied on this area. Eighteen hours were allowed for development. At the end of this period the strips were removed and dried at approximately 80°C. They were then sprayed with a 2.5% sodium carbonate solution and again dried. Finally, they were sprayed with a diazo reagent prepared according to Koessler and Hanke(5).¶ Approximately 50 μg of each amino acid are required for adequate intensity of color using this reaction.

In the serum studies in which the radioactive amino acids were used the paper strips were cut transversely into segments 5 mm in

¶ Obtained from Abbott Laboratories, Oak Ridge, Tenn.

TABLE I. Rf Values of Several Iodine Containing Amino Acids, Tyrosine and Iodide in Butanol-Dioxane-Ammonia and Collidine Solvents at 18 Hr at Room Temperature.

	BDA	Collidine
Thyroxin	.37	.45
3:5:3'-L-triiodothyronine	.64	.60
3:5-Diiodothyronine	.52	
Diiodotyrosine	.05	.08
Tyrosine	.12	.25
Iodide	.33	1.0

width and the radioactivity of the segments determined in an end window GM counter.

Results. Typical chromatograms of pure amino acid solutions are seen in Fig. 2 which demonstrates separation of thyroxin and triiodothyronine in both solvent systems. Thyroxin, triiodothyronine and diiodothyronine appear as pink lines with the diazo reagent while tyrosine and diiodotyrosine produce an orange color. Table I lists the Rf values observed in each solvent system.

¶ Prepared as follows: To a 50 ml flask immersed in an ice bath add (1) 1.5 ml of a solution containing 9 g sulfanilic acid and 90 ml of concentrated HCl per liter and (2) 1.5 ml of a 5% sodium nitrite solution. Allow to stand 5 min. and add an additional 6 ml of sodium nitrite solution. After 5 more min. dilute to volume with water. This reagent may be used for several days if refrigerated.

Fig. 3 shows the distribution of radioactivity on the chromatograms of butanol extracts of serum to which radioactive thyroxin and radioactive triiodothyronine have been added. Coincidence of radioactivity with the amino acid spot is shown by the accompanying bar graph.

Summary. A method is described by which iodine-containing amino acids, including thyroxin and triiodothyronine can be adequately separated by single dimension chromatography. Physiological quantities of labelled thyroxin and triiodothyronine have been added to serum, extracted with butanol and separated by this method.

The authors wish to acknowledge the invaluable assistance of Miss Sue Ames, Medical Technician.

1. Gross, J., and Pitt-Rivers, R., *Lancet,* 1952, v1, 439.

2. Kowkabany, G. N., and Cassidy, H. G., *Anal. Chem.,* 1952, v24, 643.

3. Robbins, J., and Rall, J. E., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 530.

4. Hird, F. R., and Trikojus, V. M., *Aust. J. Sci.,* 1948, v10, 185.

5. Koessler, K. K., and Hanke, M. T., *J. Biol. Chem.,* 1919, v39, 497.

Received September 22, 1953. P.S.E.B.M., 1953, v84.

Lack of Growth-Promoting Potency and of Toxicity of Glucagon (Hyperglycemic-Glycogenolytic Factor) in Hypophysectomized Rats. (20604)

I. I. GESCHWIND AND A. STAUB. (Introduced by Choh Hao Li.)

From the Department of Biochemistry (Hormone Research), University of California, Berkeley, and the Lilly Research Laboratories, Indianapolis, Ind.

The hypothesis that pituitary growth hormone stimulates the release of glucagon from the islet cells of the pancreas(1,2) has recently received experimental support from several laboratories(3-5). Elrick(6) has attempted to adduce further evidence for this claim by determining whether the growth promoting action of growth hormone is mediated through glucagon. A slight increase in the width of the proximal epiphyseal cartilage of the tibia was reported following glucagon administration to hypophysectomized rats. Since these results seem to add further weight to the aforementioned hypothesis, the experiments were deemed worthy of repetition with a highly purified glucagon preparation.

Experimental. The glucagon preparation (lot No. 208-108 B-234) employed in this study was a highly active preparation in which insulin had been destroyed by treatment with cysteine. Administration of 0.5 μg/kg intravenously into cats caused a 30 mg % rise in