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.

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

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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

TABLE I. Effect of Glucagon on Tibial Cartilage Width of Hypophysectomized Rat.

Exp. group	No. of animals	Daily dose (μg)	Cartilage disc width ($\mu \pm$ S.E.)
Control	12	0	147 ± 1.6
Glucagon*	6	5	147 ± 2.5
" *	7	50	146 ± 2.2
" †	7	100	155 ± 2.3
" †	5	250	156 ± .9

* Solution for inj. for whole experiment prepared in advance.

† Sol. for inj. prepared daily from freshly weighed powder.

blood sugar. Insulin assay indicated that the preparation was virtually free of hypoglycemic activity. The insulin employed was a crystalline preparation (lot No. 538, 155) with a potency of 26 units per mg. Growth hormone assays were performed in female rats of the Long-Evans strain, hypophysectomized at 28 days of age. Injections were begun 2 weeks post-operatively, and were continued for 4 days. All injections were of 0.5 ml volume and were given intraperitoneally, with the exception of one experiment in which the glucagon was suspended in a peanut oil-beeswax mixture (7), and injected subcutaneously in a daily dose of 0.1 ml. The animals were sacrificed 24 hours after the final injection and the tibial cartilage widths were determined after staining with silver nitrate according to the method of Greenspan *et al.*(8).

Results and discussion. The results presented in Table I demonstrate that doses of glucagon as great as 250 μg per day for 4 days are completely incapable of causing any increase in the disc width; that is, glucagon shows no evidence of any growth promoting action by this test. In the 3 experiments reported by Elrick(6), 3 different preparations of glucagon in doses of from 10 to 100 μg per day caused increases in the tibia widths. However, in 2 of the 3 experiments no dose-response relationship was observed, and in none of the experiments was a cartilage width found which was outside the range of non-specific response(8). It should be borne in mind, additionally, that none of the preparations of glucagon used in these earlier experiments was as pure as the one herein employed. This is evident from the increased hyperglycemic activity of our preparation, its freedom from insulin contamination, and its complete lack of toxicity in the doses employed. The last point is of special interest, since it has been claimed that doses of glucagon greater than 25 μg per day are toxic to 20 to 60% of the animals during a 4-day test(6). The present experiments, employing much higher doses of glucagon than those employed by Elrick(6), demonstrated that the toxicity is neither an inherent property of the glucagon, nor is it a consequence of its physiological action.

The possibility existed that the cruder preparations previously employed(6) may have contained contaminants which delayed the absorption of the injected glucagon, and hence increased its duration of action. In order to test this alternative, glucagon was administered subcutaneously in beeswax for 4 days, but without any apparent toxicity. Comparison of the control data and the experimental data in line 2 of Table II, demonstrate that the administration of glucagon in beeswax is of no assistance in promoting a growth response.

A final remaining possibility was that both the increase in tibia cartilage width and the toxicity previously reported(6) were caused by contamination of the earlier preparations of glucagon with insulin. Thus Salter and Best(9,10) have reported that injection of the hypophysectomized rat with protamine zinc insulin results in a stimulation of body and skeletal growth and that by the 15th day of injection the tibia cartilage width is twice the control value. On the other hand, the extreme

TABLE II. Effects of Glucagon in Beeswax, of Insulin, and of Glucagon in Combination with Insulin on Tibial Cartilage Width of Hypophysectomized Rat.

Exp. group	No. of animals	Daily dose (μg)	Cartilage disc width ($\mu \pm$ S.E.)
Control	8	0	170 ± 4.7
Glucagon*	6	100	171 ± 1.8
Insulin	5	.8†	172 ± 2.3
Insulin + glucagon	4	.8† 100	170 ± 5.0

* Daily subcut. inj. of 0.1 cc beeswax-peanut oil suspension for 4 days.

† Equivalent to .02 unit.

sensitivity of the hypophysectomized animal to regular insulin is well known. This possibility was investigated in 2 groups of animals, one of which was injected intraperitoneally with 0.02 unit of insulin daily for 4 days, whereas the second group received the same dose of insulin together with 100 μg of glucagon per day. The results of these experiments given in lines 3 and 4 of Table II, demonstrate complete lack of growth activity on the part of insulin alone in this dosage, or of insulin together with glucagon. Insofar as toxicity is concerned, one animal from each of these 2 groups died after the first injection, and all the other animals appeared in poor condition. The practice was therefore instituted of injecting one ml of a 20% glucose solution intraperitoneally one-half hour after the hormone injection. Under this regime, only one animal, from the combined hormone group, died in the following 3 days, and the general appearance of all animals appeared greatly improved. Although the desire to obtain more information about toxicity had to be relinquished in favor of obtaining a sufficient number of surviving animals for growth assay, it appeared, nonetheless, that insulin, at 0.02 unit per day, could be quite toxic to the hypophysectomized animal, and thus could possibly explain the toxicity of cruder glucagon preparations.

Summary. A highly purified preparation of glucagon in doses as high as 0.25 mg/day, has no effect on the width of the epiphyseal cartilage of the hypophysectomized rat as determined by the tibia test for growth hormone. Such a preparation possesses no toxicity when injected into these test animals. Possible reasons for the previously claimed growth promoting activity and toxicity of glucagon fractions are discussed.

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Effects of Interfering Virus and RDE upon Development of Toxic Reactions.* (20605)

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The occurrence of toxic reactions following intracerebral, intraperitoneal or intravenous injection of influenza viruses into certain species of animals was first described by Henle and Henle(1,2,3) and subsequently confirmed and elaborated upon by various other workers (4-7). Despite the approximately 9 years which have elapsed since the first description of the phenomenon, however, little is known about the nature of the toxins or the mechanisms of their action. Earlier reports(1-5) based on passage and cultivation experiments have shown that viral multiplication was not an essential factor in the induction of toxic episodes. These findings might possibly pose a question concerning the necessity for intimate contact between virus and susceptible cells in animals which exhibit toxic symptoms.

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