Metabolic Effects of Glucagon in the Wistar Rat

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SHORTLY after the discovery of insulin by Banting and Best, it was noted by Murlin and Kimball[1] in 1923 that the hypoglycemia produced by extracts of pancreas was preceded by a transient but significant elevation in the blood sugar concentration. Murlin postulated that this initial hyperglycemia was due to a second hormone in the pancreas which he named glucagon. Despite this early recognition glucagon awakened the interest of few investigators and knowledge of its biological properties progressed slowly. Through the efforts of Burger and Kramer[2] and Sutherland et al.,[3,4] glucagon was partially purified and its was shown that, like epinephrine, it acted directly on liver tissue (but not on muscle) to stimulate the breakdown of glycogen. Because of this effect, glucagon was called the pancreatic "hyperglycemic glycogenolytic factor," a term used rarely today.

For many years the glycogenolytic effect appeared to be the only significant action of glucagon. However, its purification and eventual crystallization by Staub and co-workers[5] at the Lilly Research Laboratories in 1953 greatly facilitated experimental studies. It has been shown in many laboratories that under experimental conditions glucagon produces an array of metabolic changes. In addition to stimulating hepatic glycogenolysis, glucagon increases amino acid catabolism, urea synthesis,[6,7] ketone body production[8,9] and electrolyte excretion.[10]

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The primary purpose of our investigation was to obtain information on the effects these metabolic alterations would have on growth and body composition. The unexpected results of the study indicate that glucagon can significantly influence energy balance in the rat.

MATERIALS AND METHODS

Male Wistar rats, weighing approximately 142 gm., were used. The animals were housed in individual metabolism cages in a room maintained at a constant temperature of 28 ± 1°c. Their diet consisted of the following ingredients: casein, 50 gm.; Drackett soya protein, 130 gm.; cystine, 1 gm.; starch, 150 gm.; sucrose, 496 gm.; lard, 100 gm.; Cellu flour, 20 gm.; choline chloride, 3 gm.; B-complex vitamin mixture, 10 gm.[11]; corn oil solution of vitamins A, D and E,[11] 10 gm.; and salt mixture,[11] 10 gm. (total weight, 1 kg.).

During the experimental period the food intake and weight of each animal was determined daily. The urine and feces of each rat were collected separately every twenty-four hours and their nitrogen contents determined by the Kjeldahl technic. The sodium concentrations of urine were determined by flame photometry. Blood glucose and cholesterol concentrations were estimated according to the methods of Somogyi[12] and Zlatkis,[13] respectively. Crystalline glucagon (Lilly) was suspended in 0.9 per cent saline at a concentration of 200 μg. per ml. and injected subcutaneously.

Organs were removed at necropsy, weighed, and after a small piece was taken for histologic examination, returned to the carcass. The analyses of the carcasses for total body water, fat and protein were performed by technics previously described.[14] The amount of insulin

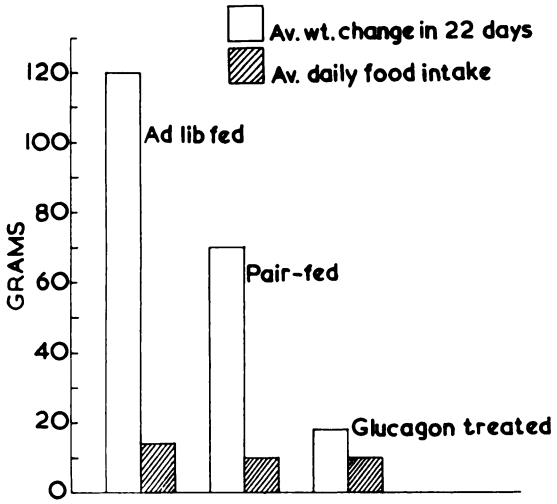

FIG. 1. Body weight changes and food intake of glucagon-treated rats and control animals.

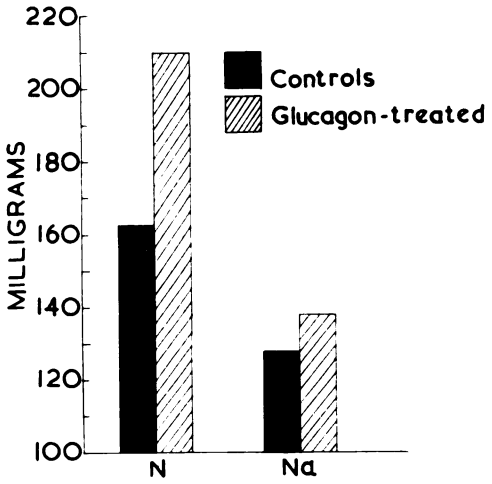

FIG. 2. Average amount of nitrogen and sodium excreted daily in the urine of glucagon-treated rats and their pair-fed controls.

extracted from the pancreas was determined by the method of Scott and Fisher,[15] and concentrations of liver glycogen by the procedure of Good et al.[16]

EXPERIMENTAL PROCEDURE AND RESULTS

Forty rats were divided into three groups. The fifteen animals in group 1 were each given subcutaneous injections at roughly eight hour intervals of 40 μg. of glucagon suspended in saline. The fifteen rats in group 2 were used as controls. They were given injections of saline, and limited to the same amount of

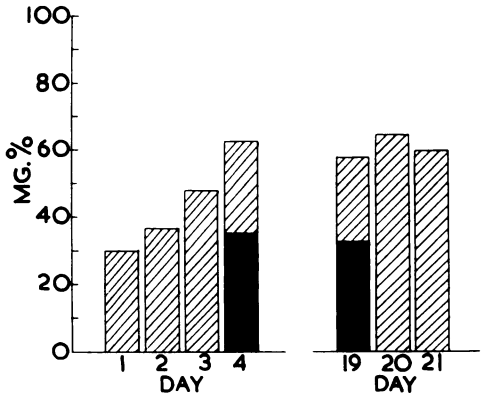

FIG. 3. The diagonally lined bars show the average change in the blood sugar concentration thirty minutes after the morning injection of 40 μg. of glucagon in rats treated for twenty-two days with this substance. The black portions of the bars show the change in the rats treated with a single injection of glucagon on the fourth and the nineteenth day.

food consumed by the treated rats in the first group. Group 3 consisted of ten rats which were used to show normal standards. They were given injections of saline and ate *ad libitum*. All animals were sacrificed on the twenty-third day. The results are presented in Tables I to III and in Figures 1, 2 and 3.

Body Weights and Food Intake. The glucagon-treated rats gained only 18 gm. during the experimental period while the rats in the control group, which were limited to identical amounts of food (an average of 10 gm. daily), gained 71 gm. (Fig. 1).

The second group of rats which ate *ad libitum* consumed an average of 4 gm. more food than the pair-fed control animals and gained 49 gm. more weight (a total of 120 gm.).

The data presented in Table I show that the glucagon-treated rats gained less weight than the untreated pair-fed animals because they retained less water and synthesized less fat and protein.

With the exception of the liver, which was abnormally heavy, the visceral and endocrine organs of the glucagon-treated animals were lighter than those of the pair-fed control animals and reflect the general inhibitory effect of glucagon on growth (Table II).

Urine. Although large doses of glucagon produce symptoms of diabetes in many species,[7,8,17] neither glucosuria nor ketonuria

TABLE I
Carcass Composition

Treatment	Animals in Group (no.)	Average Body Weight (gm.)	Average Weight Change	Average Composition (%)				Average Weight (gm.*)		
				Water	Fat	Protein	Undetermined	Water	Fat	Protein
Glucagon	15	164	18	65.30	12.37	18.26	4.07	107.40 ± 1.52	20.60 ± 0.28	30.10 ± 0.60
Control animals pair-fed	15	212	71	67.20	11.40	18.28	3.22	142.44 ± 1.67	24.13 ± 0.25	38.76 ± 0.53
Control animals, fed *ad libitum*	10	261	120	64.77	14.28	17.63	3.32	169.32 ± 1.92	37.66 ± 0.31	46.08 ± 0.56

* ± Standard error of the mean.

TABLE II
Organ Weights*

Treatment	Rats in Group (no.)	Average Body Weight (gm.)	Liver (gm.)	Kidneys (gm.)	Heart (gm.)	Adrenals (mg.)	Thyroid (mg.)	Thymus (mg.)	Testes (gm.)	Pancreas (mg.)
Glucagon	15	164	9.84 ± 0.25	1.30 ± 0.010	0.56 ± 0.015	25.1 ± 0.91	12.1 ± 0.83	160 ± 12.3	2.45 ± 0.11	525 ± 22.0
Control animals pair-fed	15	212	6.75 ± 0.17	1.59 ± 0.015	0.65 ± 0.016	30.1 ± 0.80	14.8 ± 0.67	406 ± 13.6	2.60 ± 0.08	694 ± 17.3
Control animals fed *ad libitum*	10	261	11.46 ± 0.39	1.94 ± 0.019	0.81 ± 0.016	35.0 ± 0.88	20.4 ± 0.95	494 ± 18.7	2.93 ± 0.13	839 ± 26.4

* ± Standard error of the mean.

TABLE III

Treatment	Average Insulin in Pancreas (units/gm.)	Average Liver Glycogen		Average Blood Cholesterol (mg./100 ml.)	Average Blood Sugar (mg./100 ml.)	Average Fecal Nitrogen/Day (mg.)
		mg.*	%			
Glucagon	1.34	492 ± 42	5.0	78.3 ± 3.3	86 ± 3.9	14.0
Control animals, pair-fed	1.82	209 ± 33	3.1	113.0 ± 3.7	91 ± 4.2	15.5
Control animals, fed *ad libitum*	1.68	504 ± 53	4.4	114.0 ± 4.1	104 ± 3.1	...

* ± Standard error of the mean.

occurred during these experiments. The treated rats consistently excreted about 60 mg. more urine nitrogen daily than the pair-fed control animals (Fig. 2). The nitrogen content of the feces was unaffected (Table III).

The administration of glucagon temporarily increased the sodium in the urine. During the first two days, the rats in the control groups excreted an average of 142 ± 6* mg. sodium daily, while the treated animals excreted 176 ± 9 mg. sodium. The excretion of this metabolite fell to values that averaged slightly higher than those of the pair-fed control animals although the difference was not significant.

* Standard error of the mean.

Blood. Estimation made on blood sugar at 8.30 A.M. (before the morning injection of glucagon) on days 1, 2, 3, 4, 19, 20 and 21 failed to reveal any difference between the blood glucose concentration of the pair-fed control animals and the glucagon-treated rats (Table III). However, the hyperglycemic response in the glucagon-treated rats increased during the first four days of treatment and then remained constant through the remainder of the experimental period (Fig. 3).

Blood cholesterol levels were determined on the last day only, and showed that the concentration of cholesterol was significantly less than that found in the control animals (Table III).

The concentration of glycogen in the livers of the glucagon-treated rats was more than twice that found in the control groups (Table III). Estimation of the insulin extractable from the pancreas indicates that the concentration of this hormone was significantly reduced by the administration of glucagon.

COMMENTS

It has been reported that the administration of glucagon will produce temporary, but severe diabetes in force-fed rats, and in rabbits and human subjects on normal dietary regimens.[7,8,17] Diabetes did not develop in the experiments described here because (1) the dose of glucagon (120 μg. daily) was only one-tenth of that used in earlier investigations and (2) the rats ate approximately 30 per cent less food than they did prior to treatment. The reduction of insulin content in the pancreas and the elevation in glycogen concentration of liver suggests, however, that carbohydrate metabolism was altered appreciably.

It has been reported by Costa et al.[18] and by Root[19] that although an injection of glucagon causes an immediate decrease of liver glycogen this is followed by a rapid increase to levels greater than normal. The high level of liver glycogen found in our experimental animals was in accord with these observations, and probably explains the potentiation of the hyperglycemic effect of the glucagon that occurred during the early phases of this investigation.

The most striking change was the effect of growth suppression by glucagon. The treated rats gained 70 per cent less weight than the control rats while consuming identical amounts of food. Although the increase in amino acid catabolism and nitrogen excretion induced by glucagon[7] explains the suppression of protein synthesis, it would be expected that the accompanying increase in gluconeogenesis would in turn potentiate lipogenesis. This did not occur; fat synthesis was definitely reduced.

This observation, i.e., that the potential caloric value of the carcasses of the treated rats was much less than that of the control rats despite the equality of their caloric intakes, suggested that the production of heat in the rats treated with glucagon may have been elevated. This led to an investigation of the effects of glucagon on oxygen consumption. The results of the latter studies are presented in another paper.[20]

SUMMARY

Male Wistar rats, weighing 145 gm., were given injections of 40 μg. of glucagon every eight hours for twenty-two days. They gained 75 per cent less weight, synthesized much less protein and fat and retained less water than control animals restricted to the same amounts of food. The glycogen content of the livers from the treated animals was more than twice that found in the control animals. In glucagon-treated rats, the hyperglycemic response increased during the early phases of the investigation, but glucosuria and ketonuria never occurred. The treatment produced a transient increase in the urinary excretion of sodium and a sustained increase in the urinary excretion of nitrogen. Fecal nitrogen was not affected. It was also observed that glucagon reduced the insulin content of the pancreas.

The fact that glucagon-treated rats synthesize less fat and protein than untreated control animals, consuming identical amounts of food, indicates that this hormone exerts a significant influence on caloric balance.

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