with significant reduction of the lipoprotein fractions, particularly the larger aggregates, and a fall in total blood cholesterol level. Whether this difference in results represents a species difference or is due to different amounts of cholesterol ingested will have to be explained. The dietary cholesterol in our experiment was proportionally far higher than that in Walker's observations.

Summary. 1. Rabbits on severe caloric restriction were fed cholesterol and showed significantly increased blood cholesterol, fatty acids, lipoproteins and phospholipids. The degree of these biochemical changes and of the atherosclerosis was greater than that of a control group fed similar amounts of cholesterol in addition to a normal diet. 2. Therefore, severe caloric restriction does not protect, but enhances cholesterol induced atherosclerosis in rabbits. This effect of weight loss should be taken into consideration in experiments on atherosclerosis.

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Effect of Chronic Administration of Glucagon to Rats and Rabbits.* (21302)

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Glucagon, the hyperglycemic-glycogenolytic factor of the pancreas, causes a transient increase in blood sugar when it is administered parenterally. Until Staub(1) developed methods suitable for the preparation of highly purified material of standard potency, it was not possible to study the effect of chronic administration of glucagon. This study was undertaken in an attempt to investigate the question: Will glucagon produce diabetes? Daily administration of large doses of glucagon to rats and rabbits for 6 months did not produce a diabetic state, but it did exert an interesting effect on liver glycogen stores.

* I am indebted to Dr. Paul N. Harris for performing the autopsies and carrying out the studies on pathology of the animals. My thanks are due Messers. Thomas Lenahan, Zane Frank, and Darrel Caldwell for their invaluable assistance in these experiments.

Method. Rats and rabbits were injected with glucagon daily for 6 months. Thirty-eight rats, housed in individual cages in air-conditioned animal quarters, received a commercial rat food and water *ad libitum.* Thirty of the rats were injected intraperitoneally each morning with 1 mg of a highly purified, noncrystalline, preparation of glucagon (lot 208-108B-284) in a volume of 0.25 ml. The hyperglycemic potency of this lot is such that 1 mg corresponds to approximately 0.14 mg of crystalline glucagon. This preparation contains less than 0.005 unit of insulin per mg of glucagon. The remaining 8 rats were maintained as control animals and received no treatment. Sixteen rabbits housed in air-conditioned quarters were fed rabbit pellets and water *ad libitum.* Six of these animals were injected intravenously twice a day (7:30 a.m. and 4:00 p.m.) 7 days a week with 1 mg of

TABLE I. Effect of Chronic Administration of Glucagon, 1.0 mg per Day Intraperitoneally for 6 Months on Liver Glycogen of Rats.

	Control	Glucagon treated	P
No. of rats	8	29	
Liver wt, g	14.94± .97*	16.41 ± .49*	>.05
Liver glycogen concentration, mg/g	49.01±2.87	66.66 ±3.41	.04
Total liver glycogen, g	.74± .077	1.059± .070	.03

* Mean ± stand. error of the mean.

glucagon and the other 10 received only the morning injection. All the rats and rabbits were weighed once a week. Blood sugar values were determined on the rabbits once a week and on the rats once a month. Nonfasting blood samples were taken before the morning glucagon injection, and blood sugar was determined by the Hagedorn-Jensen method(2). At the end of 6 months all the animals still alive were killed by the administration of secobarbital sodium.† The abdomen was opened and duplicate samples of liver were quickly removed, weighed, and placed in tubes containing hot 30% KOH solution for glycogen determination by the method of Good, Kramer, and Somogyi(3). The rest of the liver was removed and weighed. The animals were examined for gross pathological changes, and material was taken for histological examination of liver, kidney, adrenal, pancreas, heart, lung, thyroid, and pituitary.

Results. 1. *Rats.* During the 6-month test one rat died with severe pulmonary congestion in the 19th week of treatment with glucagon. The other 37 survived the entire test period. There were no significant differences in weight gains between treated and control groups. The average monthly blood sugar values for all the treated animals ranged from 97 to 120 mg %, and for the control animals from 84 to 137 mg %. There was no detectable difference between the 2 groups. At the conclusion of the experiment all the rats were healthy and all contained large fat pads within the abdominal cavity. Neither gross nor histological examination revealed any organic lesions. Table I presents a comparison of liver weights, liver glycogen concentration, and total liver glycogen of the treated and control animals. Glucagon caused an increase in the concentration of glycogen in the livers of the treated animals without a significant increase in liver weight.

2. *Rabbits.* None of the 6 rabbits injected with glucagon twice daily survived a full 6 months. Two died during the 6th week and one each during the 7th, 8th, 9th, and 10th week of treatment. The blood sugar values for all these rabbits were within normal range throughout the experimental period. No changes in the organs of these animals were observed either grossly or microscopically. The cause of death was ascertained in only one of this group, that one animal showing marked pulmonary edema. Of the 10 rabbits injected once daily, 3 died during the 6 months, one each during the 5th, 7th, and 8th weeks of treatment. At autopsy all 3 animals showed evidence of marked pulmonary infection. The 7 animals that lived to the end of the 6-month period gained weight at a normal rate except for the 3- or 4-week period during which all of the deaths occurred. At that time a severe respiratory infection was present in the rabbit colony. The average weight of the group at the start of the experiment was 3.236 kg and at the conclusion 4.204 kg. Post-mortem examination at the end of 6 months showed the animals to be fat and healthy. Gross examination of the aortae did not reveal any evidence of thickening or atherosclerotic plaques. All tissues studied were normal.

In Table II the liver glycogen values of the

TABLE II. Effect of Daily Intravenous Administration of Glucagon on the Liver Glycogen in Rabbits.

	Control	Glucagon treated	P
No. of rabbits	5	7	
Body wt, kg	5.02	4.204	
Liver wt, g	90.70± 5.91*	110.91±7.18*	>.05
Liver glycogen concentration, mg/g	64.42±13.23	96.09±6.98	.04
Total liver glycogen, g	6.19± 1.69	10.94±1.55	.04

* Mean ± stand. error of the mean.

† 'Seconal Sodium' (Secobarbital Sodium, Lilly).

glucagon-treated rabbits are compared with those of a group of untreated rabbits. The untreated animals had been in the laboratory quarters only a week before being killed. The concentration of glycogen in the livers of the glucagon-treated rabbits, calculated as mg/g of liver, was one and one-half times that of the untreated animals. Although comparisons between groups of animals not treated in parallel series cannot be conclusive, the results in rabbits combined with those in rats indicate that one effect of chronic administration of glucagon is to increase the concentration of glycogen in liver tissue. Further experiments in rats to be reported confirm this effect.

Discussion. Daily administration of glucagon to rats and rabbits for a period of 6 months did not alter the rate of growth of the animals, nor cause pathological changes in any of the organs or tissues studied. There was no permanent or lasting alteration of blood sugar and the animals did not have any symptoms of diabetes. The animals that died during the 6-month period, succumbed to infectious processes and not to drug action.

The only effect that could be ascribed to glucagon administration was an increase in the concentration of glycogen in the livers of both rats and rabbits. This surprising finding is the opposite of that expected on the basis of the known action of glucagon. Sutherland and Cori(5) demonstrated that glucagon causes an increase in active phosphorylase in liver and thereby increases glycogenolysis. This mechanism explains the rise in blood sugar and decrease in liver glycogen found after single injections of glucagon(6).

If active phosphorylase can also catalyze the glucose-l-phosphate → glycogen reaction, it is possible that chronic administration of large doses of glucagon will favor glycogen formation.

It is unlikely that this increase in liver glycogen is a secondary effect mediated via adrenal cortical hormones, since administration of ACTH increases liver glycogen stores by an increase in liver weight without altering the concentration of glycogen in the liver tissue (4). After glucagon administration there was an increase in liver glycogen with no significant increase in liver weight.

Summary. Daily administration of large doses of glucagon to rats and rabbits for 6 months did not produce any toxic reactions. The rate of weight gain of treated animals was the same as that of control animals. Diabetes was not produced and there was no evidence of chronic hyperglycemia. The liver glycogen concentration of the treated animals was significantly greater than that of the untreated animals.

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