Glycogen Stores in Glucagon-Treated Rats. I. Time Factors.* (22246)

ERMINIO COSTA,† GIORGIO GALANSINO,‡ AND PIERO P. FOÀ.

Department of Physiology and Pharmacology, Chicago Medical School, Chicago 12, Ill.

Glucagon is believed to cause hyperglycemia by promoting glycogenolysis in the liver. This is based on the following evidence which has been reviewed in detail recently (1) and which is largely indirect: 1) the hyperglycemic response to glucagon is decreased or absent in fasting, untreated diabetes, severe liver disease(2) and after evisceration (3) when the amount of liver glycogen is presumably low or absent; 2) glucagon is more effective when injected into the portal vein than by any other route of administration and is ineffective after occlusion of the liver circulation(2); 3) glucagon causes a sharp increase in splanchnic glucose production, as measured by venous catheterization in man (4); 4) destruction of the pancreatic alpha cells, presumably accompanied by the liberation of preformed glucagon, causes an immediate hyperglycemia with a decrease in liver glycogen(5); 5) glucagon promotes liver glycogenolysis *in vitro* by activating the phosphorylase system(6). Although these findings would lead one to expect a decrease of liver glycogen in intact animals treated with glucagon, this effect has not been clearly demonstrated. Bürger and Kramer(2) first reported a decrease in liver glycogen in dogs treated with glucagon. However, these pioneering results were obtained with crude preparations of glucagon heavily contaminated with insulin. Furthermore liver glycogen was determined in samples obtained by means of repeated biopsies, a traumatizing technic which may cause glycogenolysis in itself. Similarly, using an impure preparation of glucagon, Heard *et al.*(7) obtained a decrease in liver glycogen in normal rats, while Milman *et al.* (8) obtained similar results in vit. E-deficient rats. In contrast with these findings, the liver glycogen was found normal in rats chronically treated with small doses of glucagon(9), and elevated in animals treated with much larger doses(10).

The reasons for these unexpected results are not clear. It has been suggested(9) that since the glycogenolytic effect of small doses of glucagon is of short duration, the glucose liberated can be redeposited rapidly as liver glycogen, perhaps as a result of increased insulin secretion(11) or through a reversal of the phosphorylase reaction(10). Reports on the effect of glucagon on muscle glycogen are also contradictory and although glucagon does not stimulate muscle phosphorylase activity(6), it appears to inhibit the glycostatic effect of insulin in normal muscles(12,13) and to have a glycostatic effect of its own in the muscles of vit. E-deficient rats(8).

The purpose of this work was to investigate the effects of glucagon and of epinephrine *in vivo* on liver and muscle glycogen and on adrenal ascorbic acid. The latter was determined in order to investigate the possibility that glucagon may stimulate the pituitary-adrenal system as suggested by measurement of corticoid excretion(14) and by analogy with the effects of epinephrine(15).

Methods. Two hundred thirty-one adult Sprague-Dawley albino rats of both sexes were used.§ The animals, kept on a stock diet of Purina checkers, were allowed to eat *ad libitum* or were fasted for 24 hours, as indicated in the tables. Glucagon (Eli Lilly and Co., lot no. 208-158B-197‖; 0.5, 2.5 or 5.0 mg/kg) and epinephrine (0.15 mg/kg) were injected intraperitoneally. In addition, some of the fasted animals received simultaneous intraperitoneal injection of glucose (1 g/kg). After 2, 6, 12, or 24 hours, the animals were decapitated, both adrenal

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† Fulbright Research Fellow. Permanent address: Istituto di Farmacologia, Università di Cagliari, Italy.

‡ Research Fellow. Permanent address: Istituto di Anatomia Patologica, Università di Milano, Italy.

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TABLE I. Effect of Glucagon on Liver and Muscle Glycogen and on Adrenal Ascorbic Acid (mg/100 g of Fresh Tissue).

No. of rats	Time after inj., hr	Fasting time, hr	Glucagon, mg/kg	Glucose, g/kg	Glycogen				Adrenal ascorbic acid	
					Liver		Muscle			
					Avg ± S.E.	% change	Avg ± S.E.	% change	Avg ± S.E.	% change
6	2		—		1884 ± 162		—		431 ± 24	
6	"		0.5		1697 ± 270	− 6	—		342 ± 32	− 21
6	"		2.5		624 ± 119	− 67*	—		361 ± 39	− 16
6	"		5		591 ± 114	− 69*	—		401 ± 15	− 7
9	"		—		4139 ± 541		467 ± 28		378 ± 17	
20	"		5		1349 ± 188	− 68*	530 ± 22	+13*	358 ± 9	− 6
6	"	24	—		—		—		379 ± 15	
6	"	"	0.1		—		—		312 ± 14	− 18
6	"	"	5		—		—		260 ± 34	− 31*
6	"	"	—		—		742 ± 63		—	
6	"	"	5		—		753 ± 47	+1.5	—	
5	"	"	—		579 ± 80		—		391 ± 11	
5	"	"	2.5		190 ± 35	− 67*	—		389 ± 16	− 1
6	"	"	—		459 ± 16		395 ± 30		359 ± 5	
6	"	"	5		30 ± 3	− 94*	408 ± 24	+3	262 ± 12	− 34*
6	"	"	—		438 ± 85		320 ± 25		346 ± 6	
6	"	"	5		47 ± 7	− 90*	409 ± 9	+27*	256 ± 13	− 26*
8	6	"	—	1	517 ± 47		487 ± 47		366 ± 19	
8	"	"	5	1	288 ± 55	− 56*	485 ± 12	− 0.4	320 ± 6	− 13
5	12	"	—	1	317 ± 34		—		374 ± 21	
5	"	"	2.5	1	164 ± 15	− 48*	—		376 ± 11	+ 1
8	"	"	—	1	1086 ± 26		496 ± 42		308 ± 26	
8	"	"	5	1	822 ± 14	− 25*	552 ± 29	+11	350 ± 11	+13
8	24	"	—	1	54 ± 9		419 ± 35		376 ± 29	
8	"	"	2.5	1	196 ± 33	+264*	387 ± 22	− 8	388 ± 10	+ 3
8	"	"	—	1	175 ± 31		349 ± 23		335 ± 15	
8	"	"	5	1	366 ± 25	+108*	487 ± 40	+39*	353 ± 12	+ 5
8	"	"	—	1	125 ± 34		290 ± 39			
8	"	"	5	1	405 ± 64	+224*	345 ± 6	+18		
6	"	"	—	1			909 ± 56			
6	"	"	5	1			879 ±111	− 3.3		

* $P \leqq 0.05$.

TABLE II. Effect of Epinephrine on Liver and Muscle Glycogen and on Adrenal Ascorbic Acid (mg/100 g of Fresh Tissue).

No. of rats	Time after inj.	Fasting time, hr	Epinephrine, mg/kg	Glycogen: Liver Avg ± S.E.	Glycogen: Liver % change	Glycogen: Muscle Avg ± S.E.	Glycogen: Muscle % change	Adrenal ascorbic acid Avg ± S.E.	Adrenal ascorbic acid % change
6	20 min.	24	—	506 ± 27		377 ± 21		397 ± 37	
6	"	"	.15	144 ± 23	− 72*	267 ± 17	−29*	252 ± 18	−37*
6	2 hr	"	—	211 ± 12		301 ± 26		351 ± 25	
6	"	"	.15	538 ± 36	+153*	162 ± 27	−47*	233 ± 20	−49*
6	"	—	—	1082 ±121		339 ± 20		395 ± 20	
6	"	—	.15	1565 ±244	+ 44	247 ± 29	−28	301 ± 28	−24*

* $P \leq 0.05$.

glands and samples of liver and of gastrocnemius muscle were removed as rapidly as possible. Liver and muscle were dropped in tared centrifuge tubes containing 30% KOH and their glycogen content was determined according to the method of Good, Kramer, and Somogyi(16) supplemented by the method of Nelson(17). The adrenal ascorbic acid was determined according to the method of Roe and Kuether(18). Each group of rats treated with glucagon or epinephrine had its own control group of rats treated with saline. The statistical significance of the difference between the mean experimental and control values was calculated according to Fisher(19).

Results. The results are presented in Tables I and II. Table I indicates that: 1) 2 and 6 hours after the injection of glucagon there was a marked decrease in liver glycogen which was still noticeable 12 hours thereafter. This decrease appeared to have been greater in animals receiving larger doses of glucagon, and occurred in fed, as well as in fasted animals, whether receiving glucose or not. The lowest value reached was 28 mg/100 g of liver. Similar values of about 30 mg/100 g of fresh tissue were found in 12 animals. 2) Twenty-four hours after the injection of glucagon (2.5 or 5.0 mg/kg) there was a marked and significant increase in liver glycogen. 3) The injection of glucagon was followed by a small and inconstant increase in muscle glycogen. This was statistically significant only in three cases. 4) Two hours after the injection of the largest dose of glucagon (5.0 mg/kg), there was a decrease in adrenal ascorbic acid. This decrease occurred only in fasted animals in which liver glycogen reached very low levels and not in animals which had been fed or had received an injection of glucose. No decrease in adrenal ascorbic acid was found in animals killed 6, 12, or 24 hours after glucagon injection. Table II indicates that 20 minutes after the injection of epinephrine, liver glycogen, muscle glycogen and adrenal ascorbic acid were significantly reduced. Two hours after the injection, muscle glycogen and adrenal ascorbic acid were still below normal, while liver glycogen had increased to a value greater than that of the controls.

Discussion. The results demonstrate that the first effect of glucagon in all doses used is liver glycogenolysis. The absolute magnitude of this effect appears to be greater in animals receiving larger doses of glucagon and in fed animals having more liver glycogen, although the largest per cent decrease (about 90%) was found in animals the liver glycogen of which had been depleted by fasting. This decrease in liver glycogen tends to disappear after 12 hours and is followed 24 hours later by a marked increase. The reasons for this phenomenon are not clear. It has been suggested that, after a period of marked glycogenolysis, glucagon may cause an inversion of the phosphorylase reaction, however, glycostatic effect of glucagon never has been observed *in vivo*. In addition, much smaller doses of glucagon which cause hyperglycemia and therefore presumably stimulate the phosphorylase reaction *in vivo*, do not promote secondary glycogenesis(9). It may be suggested that the primary effect of glucagon, regardless of the dose, is an acceleration

of liver glycogenolysis and that the secondary increase in liver glycogen is the result of an increase in insulin secretion due to the accompanying hyperglycemia(20). In addition, the increase of liver glycogen may be favored by the release of ACTH and/or adrenal cortical hormone due to a stress-like effect of the primary glycogen depletion or of large nonphysiologic doses of glucagon. This hypothesis is supported by the following observations: 1) glucagon hyperglycemia stimulates insulin secretion(11), and 2) large doses of glucagon cause a marked decrease in adrenal ascorbic acid (Table I). The effect of the adrenals appears to be a permissive one only, since glucagon (2.5 mg/kg) may cause increase in liver glycogen without significant changes in adrenal ascorbic acid. In this respect, the action of glucagon would be similar to that of epinephrine which also causes: 1) hyperglycemia and, presumably, insulin secretion, 2) decrease in adrenal ascorbic acid, and 3) secondary increase of liver glycogen(15). In the case of epinephrine this compensatory response is very rapid and its effects are seen after 2 hours. In the case of glucagon the glycogenolytic effect disappears only after 12 to 24 hours, perhaps because large doses of glucagon are destroyed less rapidly than epinephrine, or perhaps because the effect of epinephrine on the adrenal cortex is more specific. In agreement with the observation that glucagon does not influence muscle phosphorylase, glucagon did not cause a decrease in muscle glycogen. The slight increase in muscle glycogen observed in these experiments and in those of others(8), may be not a direct and specific effect of glucagon, but a result of the secondary secretion of insulin. This, apparently, cannot overcome the glycogenolytic effect of epinephrine in muscle (Table II). Further experiments are in progress to investigate the role of adrenals, pancreas and pituitary in the glycostatic effects of glucagon.

Summary and conclusions. 1. Effects of epinephrine and various doses of glucagon on liver and muscle glycogen and on adrenal ascorbic acid were studied. 2. Twenty minutes after injection of epinephrine a decrease in liver and muscle glycogen, and in adrenal ascorbic acid were observed. Two hours after injection, liver glycogen was significantly higher than in controls, while muscle glycogen and adrenal ascorbic acid were still below normal. 3. Glucagon caused immediate decrease in liver glycogen. When large doses were used this decrease was followed by secondary increase to values greater than normal. 4. The glycostatic effect of large doses of glucagon in the liver was probably associated with an earlier decrease in adrenal ascorbic acid. 5. Glucagon caused an occasional slight increase of muscle glycogen. 6. It is suggested that the primary effect of epinephrine and of glucagon in all doses is an acceleration of liver glycogenolysis and that their glycostatic effects are due to secondary increases in secretion of insulin and/or adrenal cortical hormones.

1. Foà, P. P., *Adv. Int. Med.*, 1954, v6, 29.

2. Bürger, M., and Kramer, H., *Ztschr. ges. exper. Med.*, 1929, v65, 487 and v67, 441.

3. Ingle, D. J., Nezamis, J. E., and Humphrey, L. M., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 232.

4. Kibler, R. F., and Myers, J. D., *Am. J. Med.*, 1952, v13, 647, and 1953, v14, 753.

5. Weisberg, H. F., and Kaplan, C. M., *Fed. Proc.*, 1953, v12, 151.

6. Sutherland, E. W., and Cori, C. F., *J. Biol. Chem.*, 1951, v188, 531.

7. Heard, R. D. H., Lozinski, E., Stewart, L., and Stewart, R. D., *J. Biol. Chem.*, 1949, v172, 856.

8. Milman, A. E., Treacy, A. M., and Milhorat, A. T., *Fed. Proc.*, 1954, v13, 100.

9. Galansino, G., Weinstein, H. R., Magill, A. M., and Foà, P. P., *Amer. J. Physiol.*, 1955, v180, 27.

10. Root, M. A., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 108.

11. Foà, P. P., Santamaria, L., Weinstein, H. R, Berger, S., and Smith, J. A., *Amer. J. Physiol.*, 1952, v171, 32.

12. Snedecor, J. G., DeMeio, R. H., and Pincus, I. J., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 396.

13. Rodriguez-Candela, J. L., *Ciba Found. Colloquia, Endocrin.*, 1953, v4, 233.

14. Kirtley, W. R., Waife, S. O., Helmer, O. M., and Peck, F. B., *Diabetes*, 1953, v2, 345.

15. Long, C. N. H., *Ciba Found. Colloquia Endocrin.*, 1953, v6, 136.

16. Good, C. A., Kramer, H., and Somogyi, M.,

J. Biol. Chem., 1933, v100, 485.
17. Nelson, N., *J. Biol. Chem.*, 1944, v153, 375.
18. Roe, J. H., and Kuether, C. A., *J. Biol. Chem.*, 1943, v147, 399.
19. Fisher, R. A., *Statistical Methods for Research Workers*. Oliver and Boyd, Edinburgh, 1950.
20. Foà, P. P., Weinstein, H. R., and Smith, J. A., *Am. J. Physiol.*, 1949, v157, 197.

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Abnormalities of Urogenital System in Strain A x C Line 9935 Rats. (22247)

MARGARET K. DERINGER AND W. E. HESTON.
National Cancer Institute, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Md.

At autopsy congenital absence of one kidney was observed in a rat of the strain A x C line 9935 colony maintained at the National Cancer Institute. Subsequently, a number of rats of this strain were observed to have one kidney missing, and a number were observed to have other urogenital abnormalities. Observations on the extent and incidence of these abnormal conditions are presented herein.

Materials and methods. The strain A x C line 9935 (black agouti irish) rats were obtained from Dr. W. F. Dunning* of the College of Medicine, Wayne University in October 1945. At that time the strain had been inbred by brother x sister matings for 30 generations. The strain A x C line 9935 was started in 1926 by Doctors Dunning and M. R. Curtis from a cross between the August line 1561 and the Copenhagen line 2331. Neither of these 2 strains has ever been observed to have any urogenital abnormality. Dr. Dunning indicated that an abnormality was first observed in March 1937 in animals of the 16th brother by sister generation of the subline (which is the one on which the present observations were made). The right testicle was absent in 5 of a group of 50 males in which methylcholanthrene was being injected. She has observed urogenital abnormalities in the hybrid progeny of a male of the 12th generation of this same subline outcrossed to an August line female. In 1946, she briefly described the urogenital anomaly as occurring with about equal frequency in the 2 sexes and varying in extent in them. Either the right or the left side might be involved. In affected females, an adrenal and an ovary were absent occasionally and in affected males, the testis was absent or atrophic more frequently(1). Following the initial observation in this laboratory a routine search for similar abnormalities was made in young animals (one to 18 days of age) and in adult animals (2 to 17 months of age) of inbred generations 30 through 34. The majority of the adult animals were breeding females and for them data on the size and number of litters produced were available for comparison in the normal and abnormal animals. When the strain A x C line 9935 rats, in which the abnormalities had been observed, were outcrossed to strain M-520, the abnormalities were observed in the F_1 progeny(2).

Results. The incidence of urogenital abnormalities in young and adult rats in shown in Table I. It will be observed that the incidence of urogenital abnormalities in older animals was slightly higher than that in younger animals. However, when the data were analyzed statistically this difference was not found to be significant in either the females or the males (for the females, $\chi^2 = 3.39$; $P = 0.07$, and for the males, $\chi^2 = 0.26$; P is between 0.5 and 0.7).

Animals with urogenital abnormalities always showed either the absence of one kidney or the presence of one cystic kidney. Each of the two manifestations was often associated with various other abnormal conditions. There was no significant difference in the frequency

*Present address, University of Miami, Coral Gables, Fla.