Glycogen Stores in Glucagon-Treated Rats. III. Effect of Adrenalectomy and Hypophysectomy.* (22963)

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Intraperitoneal injections of glucagon and of epinephrine cause an immediate and marked reduction in liver glycogen(1,2), due to an increase in phosphorylase activity(3,4). This glycogenolytic phase is followed, 24 hours after glucagon and 2 hours after epinephrine, by marked increase in liver glycogen (1,5,6) and is accompanied by increase in adrenal weight and decrease in adrenal ascorbic acid. In alloxan diabetic rats, the glycogenolytic effect of glucagon and epinephrine appears to be unimpaired, but no secondary increase in liver glycogen can be obtained(7). These results suggest that the rebound phenomenon requires the presence of 1) an intact insulin-secreting system capable of being stimulated by glucagon or epinephrine hyperglycemia and 2) the adrenal glands, possibly participating in a permissive manner. This paper describes the effects of glucagon and epinephrine on liver and muscle glycogen in adrenalectomized and in hypophysectomized rats with and without cortisone or insulin treatment.

Materials and methods. Two hundred sixteen male Sprague-Dawley albino rats weighing 150-200 g were used. Of these, 139 were adrenalectomized,§ 77 were hypophysectomized.‖ All animals were kept on diet of Purina checkers *ad libitum*. During the first postoperative week the diet of hypophysectomized rats was ground and moistened with water to help overcome anorexia. Adrenalectomized animals drank 1% glucose in saline instead of water, except when treated with cortisone. All animals were fasted 24 hours before the experiment. Glucagon¶ (Eli Lilly and Co., lot No. 208-158B-197: 2.5 or 5 mg/kg) or epinephrine (0.15 mg/kg) and glucose (1 or 2 g/kg in 5% solution) were injected intraperitoneally at the beginning of the experiment. Cortisone (1 or 5 mg/kg) was injected intramuscularly daily for 10 days up to 48 hours before the experiment. Insulin (Ultralente Lilly, 0.05 u/rat) was injected subcutaneously in a single dose at the beginning of the experiment and was followed by a second injection of glucose 5 hours later. After 20 minutes, 2 or 24 hours, the animals were decapitated, samples of liver and of left gastrocnemius muscle were removed as quickly as possible and placed in tared centrifuge tubes containing 30% KOH. Glycogen was isolated and hydrolyzed by the method of Good, Kramer and Somogyi(8), and resulting glucose determined according to Nelson(9). Each experiment consisted of an experimental group of rats treated with glucagon or epinephrine and a control group of rats treated with saline. The statistical significance of the difference between mean experimental and control values was calculated according to Fisher(10).

Results. Results are presented in Table I. In adrenalectomized rats the results were as follows: 1) Fasting liver glycogen was very low and no significant changes were noted 2 hours after injection of glucagon. Twenty-four hours after the injection there was a significant but small rise in liver glycogen. 2) Treatment with cortisone caused a partial restoration of liver glycogen toward normal values. This restoration was more pronounced

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** Three control animals gave abnormally low muscle glycogen values. If these are eliminated, the reduction due to epinephrine becomes statistically significant, as indicated by values in parentheses.

TABLE I. Effect of Glucagon and Epinephrine on Liver and Muscle Glycogen of Adrenalectomized (Adx) and Hypophysectomized (Hyphex) Rats Fasted 24 Hr (mg/100 g of Fresh Tissue).

No. and type of rats	Time after inj., hr	Glucagon, mg/kg	Cortisone, mg/kg	Epinephrine, mg/kg	Insulin, U/rat	Glucose, g/kg	Glycogen: Liver Avg	± S.E.	Liver % change	Muscle Avg	± S.E.	Muscle % change
14 Adx*						2	30	2.2		277	26	
13 †	2	2.5				"	31	3.4	+ 3§	363	31	+31§
10						"	17	2.3		356	29	
10	24	2.5				"	27	3.0	+ 59‖	401	26	+12§
8(5)¶						"	38	3.7		277(358)	40(23)	
8	⅓			.15		"	28	2.8	- 26§	185	38	-30§(-48)‖
8						"	22	3.5		356	27	
8	2			"		"	21	2.8	- 5§	357	29	+ 7§
6			1			"	184	22				
8	24	5	"			"	220	29	+ 25§			
7			5			"	630	77				
7	"	5	"			"	878	28	+ 40‖			
6			"			"	261	26		210	14	
6	⅓		"	.15		"	19	2	- 93‖	196	20	- 7§
6			"			"	252	22		276	36	
6	2		"	"		"	796	62	+215‖	126	81	- 54‖
8 Hyphex						"	40	3				
5	"	5				"	39	2	- 2.5§			
7						"	22	2				
4	24	5				"	25	1.8	+ 13§			
6			5			"	642	115		411	19	
6	2	5	"			"	32	2.5	- 95‖	397	30	- 3§
12‡			"			"	571	73		411	19	
12‡	24	5	"			"	772	98	+ 37§	486	23	+18§
6			"			"	642	115		411	19	
5	⅓		"	.15		"	74	14	- 88‖	185	41	-55‖
6			"			"	642	115		411	19	
6	2		"	"		"	196	72	- 70‖	92	18	-78‖
6					.05	4	21	6.5		271	23	
6	24	5			"	"	49	10	+133‖	290	21	-10§

* Muscle values for 8 rats. † Muscle values for 7 rats. ‡ Muscle values for 6 rats. § $P > 0.05$. ‖ $P \leq 0.05$. ¶ Three control animals gave abnormally low muscle glycogen values. If these are eliminated, the reduction due to epinephrine becomes statistically significant, as indicated by values in parentheses.

in rats receiving cortisone in daily doses of 5 mg/kg than in rats receiving 1 mg/kg. 3) Twenty-four hours after injection of glucagon in adrenalectomized cortisone-treated rats there was a significant increase in liver glycogen above control values. This increase was statistically significant only in rats treated with cortisone in daily doses of 5 mg/kg. 4) Glucagon caused no significant changes in muscle glycogen. 5) Epinephrine caused a temporary decrease in liver and muscle glycogen. This decrease was not statistically significant. 6) In adrenalectomized cortisone-treated animals, epinephrine caused a marked and significant decrease in liver glycogen, followed 2 hours later by a significant decrease in muscle glycogen and by a marked and significant increase in liver glycogen above the initial values.

In hypophysectomized rats, the results were as follows: 1) In animals receiving no cortisone, liver glycogen was very low and did not change significantly 2 or 24 hours after injection of glucagon. 2) In rats treated with cortisone, liver glycogen decreased significantly 2 hours after injection of glucagon and returned to, but not above, initial values 24 hours later. 3) Twenty-four hours after injection of glucagon, liver glycogen of insulin-treated hypophysectomized rats was significantly increased. 4) Muscle glycogen was not changed significantly by glucagon injections. 5) Twenty minutes after injection of epinephrine there was a marked reduction in liver and muscle glycogen. Both were still significantly below initial values 2 hours thereafter.

Discussion. Liver glycogen of fasted, untreated adrenalectomized rats was many times lower than that of fasted normal animals, as reported by other investigators(5). Injection of glucagon or epinephrine did not cause any immediate further decrease, perhaps because an irreducible minimum had already been reached. The delayed increase in liver glycogen averaged 10 mg/100 g of tissue and, although statistically significant, was inadequate to raise the values above those generally found in adrenalectomized rats. Cortisone treatment increased fasting liver glycogen and restored the glycogenolytic effect. In these animals glycogen rebound averaged 248 mg/100 g of tissue. These results suggest that adrenals are a factor in the rebound due to glucagon as they are in that due to epinephrine(11). Recently, Root(12) reported that daily glucagon injections produced an increase in liver glycogen of rats adrenalectomized 48 hours before beginning of treatment. These animals may be comparable to the adrenalectomized animals treated with cortisone up to 48 hours before the experiment and may have sufficient residual adrenal cortical hormones to permit glycogen rebound. Hypophysectomy, like adrenalectomy, reduced liver glycogen and the immediate glycogenolytic effect of glucagon was detectable only in those animals in which the glycogen stores had been replenished by cortisone, although it still remained far below normal values. No secondary liver glycogen increase was noted in hypophysectomized rats whether treated with cortisone or not, while a marked and significant rebound was noted in hypophysectomized rats treated with insulin. These results suggest that the delayed glycostatic effect of glucagon is greatly diminished in the absence of the adrenals or when the adrenal cortical activity is reduced to a minimum by hypophysectomy. It is possible that a pituitary factor, perhaps growth hormone, capable of maintaining a normal insulin function(13) may be necessary for this phenomenon, in agreement with the observation that growth hormone has no glycostatic effect in alloxan diabetic rats(14). The results described in this and in preceding papers of this series show that the immediate liver glycogenolysis induced by glucagon is followed by marked increase in liver glycogen in normal rats, in adrenalectomized rats treated with cortisone and in hypophysectomized rats treated with insulin, but not in alloxan-diabetic rats and in untreated adrenalectomized or hypophysectomized animals. It is believed, therefore, that this rebound is not a direct effect of glucagon, but the result of a secondary secretion of insulin, a hormone known to increase liver glycogen(15,16). Glucagon hyperglycemia causes an almost immediate secretion of insulin(17), while the effect of insulin on liver glycogen is slow(18,

19). In this manner a quick acting hormone like glucagon could cause delayed effects. The glycogenolytic effect of epinephrine in muscle of adrenalectomized rats was not modified significantly by cortisone treatment, in agreement with the belief that epinephrine acts directly on muscle phosphorylase. On the other hand, glucagon, which does not stimulate muscle phosphorylase, does not seem to exert any direct action on muscle glycogen. The occasional increase in muscle glycogen observed in normal animals following glucagon injection(7) may be the result of adrenal cortical stimulation, as shown by the fact that it does not occur in adrenalectomized and in hypophysectomized rats. The only changes in muscle glycogen in these animals were those due to the well known glycogenolytic effect of epinephrine(1,5,6).

Summary and conclusions. The effect of glucagon and of epinephrine on liver and muscle glycogen of adrenalectomized and hypophysectomized rats with or without treatment with cortisone or insulin was studied. 1) Epinephrine caused an immediate decrease in muscle glycogen in all cases and a decrease in liver glycogen in animals treated with cortisone. 2) In untreated adrenalectomized rats, liver glycogen was very low and further decrease due to epinephrine was not significant. No secondary increase in liver glycogen was noted. 3) Two hours after injection of glucagon, no significant changes in liver and muscle glycogen of untreated adrenalectomized or hypophysectomized animals were noted. 4) Twenty-four hours after the injection of glucagon in untreated adrenalectomized rats there was a significant, but very small increase in liver glycogen. Muscle glycogen was unchanged. No significant changes were noted in liver and muscle glycogen of untreated hypophysectomized rats. 5) In cortisone treated adrenalectomized or hypophysectomized rats, glucagon caused a significant decrease in liver glycogen, 2 hours after injection. 6) No secondary increase in liver glycogen was noted in cortisone treated hypophysectomized rats. 7) Glucagon caused a secondary increase in liver glycogen in cortisone treated adrenalectomized rats and in hypophysectomized rats treated with insulin. 8) No significant changes in muscle glycogen of treated or untreated rats were noted following glucagon injection. 9) The results are consistent with the hypothesis that glycogenolysis and hyperglycemia are the primary effects of glucagon and epinephrine and that the delayed increase in liver glycogen and occasionally of muscle glycogen, observed after injection of these hormones, is the result of a secondary secretion of insulin aided by the "permissive" action of the adrenal cortex. 10) The importance of time factors in this "rebound" phenomenon is emphasized.

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