hibitory since the tissue, *in vitro,* survived a temperature of 36-37°C for a prolonged period. The relatively slow rate of growth of the tumor tissue possibly contributes to the difficulty of implantation on the membrane. There was no evidence of alteration in the type of growth of tumor tissue during the course of 20 heterotransplants, such as was noted elsewhere during passage of frog renal carcinoma in newts(3) and during passage of a murine carcinoma in eggs(5).

Summary. The frog renal carcinoma (Lucké) has been propagated on the chorioallantoic membrane of hens' eggs for 20 serial passages. Growth was irregular.

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Glycogen Stores in Glucagon-Treated Rats. II. Effect of Alloxan Diabetes.* (22333)

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Chronic treatment with small doses of glucagon causes no change in liver glycogen(1), while large doses may cause a significant increase(2). Further study of this seemingly paradoxic effect of the "glycogenolytic" hormone demonstrated that, although single intraperitoneal injections of large doses of glucagon into normal rats cause an immediate decrease in liver glycogen, this is followed 24 hours later by a marked increase above normal levels(3). When the animals are sacrificed 24 hours after the last glucagon injection, as is done in chronic experiments, the short-lived glycogenolytic effects are no longer detectable because glycogen has been resynthesized. This resynthesis of glycogen may be due to 3 causes: 1) reversal of the phosphorylase reaction(3); 2) increased secretion of adrenal cortical hormones, in a manner similar to that which follows the injection of epinephrine(4); or 3) increased secretion of insulin, as a result of glucagon hyperglycemia (5). The purpose of this work was to investigate the third hypothesis by studying the effect of glucagon and epinephrine on liver glycogen, muscle glycogen and adrenal ascorbic acid in alloxan-diabetic rats.

Materials and methods. Seventy-two adult Sprague-Dawley male albino rats were used. The animals were kept on a diet of Purina checkers *ad libitum* and fasted for 24 hours before the experiments. Alloxan diabetes was produced according to the method of Sturtevant(6). Since glycogen deposits of alloxanized animals vary with the severity of the diabetes(7-9), only rats with established diabetes of more than 6 weeks' duration and with severe (4+) glycosuria and polyuria were used.‖ Glucagon (Eli Lilly and Co., lot no. 208-158B-197; 2.5 or 5.0 mg/kg),¶ epinephrine (0.15 mg/kg) and glucose (1 g/kg) were injected intraperitoneally. After 2 or 24 hours, the animals were decapitated, samples of liver and of gastrocnemius muscle and both

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¶ Glucagon was a gift of Dr. O. K. Behrens of the Lilly Research Laboratories.

TABLE I. Effect of Glucagon and of Epinephrine on Liver and Muscle Glycogen and on Adrenal Ascorbic Acid of Alloxan-Diabetic Rats. mg/100 g of fresh tissue. Fasting time 24 hr; glucose 1 g/kg.

No. of rats	Time after inj. (hr)	Glucagon (mg/kg)	Epinephrine (mg/kg)	Glycogen — Liver — Avg	Liver — SE_m	Liver — % change	Glycogen — Muscle — Avg	Muscle — SE_m	Muscle — % change	Adrenal ascorbic acid — Avg	SE_m	% change
5	2	—	—	574	± 74		387	± 42		380	± 24	
5		5	—	102	± 11	−82*	316	± 60	−19	306	± 21	−20
4	2	—	—				310	± 47		403	± 32	
6		5	—				559	± 10	+80*	290	± 15	−28*
5	24	—	—	932	± 67		413	± 24		365	± 44	
5		5	—	1045	± 94	+12	319	± 35	−23	321	± 16	−12
5	24	—	—	1348	±159					434	± 21	
5		5	—	1140	±101	−16				411	± 26	− 5
5	24	—	—	639	± 84		307	± 45		326	± 18	
5		5	—	544	± 95	−16	383	± 55	+24	302	± 26	− 7
4	⅓	—	—	1811	±162		212	± 47		270	± 34	
6		—	0.15	1356	± 84	−25	114	± 11	−46	211	± 22	−22
6	⅓	—	—	4180	±154		319	± 25		368	± 15	
6		—	0.15	2165	±190	−49*	205	± 28	−36*	267	± 14	−27*

* $P < 0.05$ or better.

adrenal glands were removed as rapidly as possible. Liver and muscle were placed in tared centrifuge tubes containing 30% KOH. Glycogen was determined according to the method of Good, Kramer and Somogyi(10), supplemented by the method of Nelson(11). The adrenal ascorbic acid was determined according to the method of Roe and Kuether (12). Each experiment consisted of a group of rats treated with glucagon or epinephrine and a control group treated with saline. All animals received glucose 24 hours before death. The statistical significance of the difference between mean experimental and control values was calculated according to Fisher (13).

Results. The results, presented in Table I were as follows: 1) 2 hours after the injection of glucagon there was a marked and significant decrease in liver glycogen accompanied, in 1 experiment, by a marked and significant increase in muscle glycogen; 2) 24 hours after the injection of glucagon the liver and muscle glycogen had returned to control values; 3) in no case did liver glycogen increase above the control values; 4) no significant changes in adrenal ascorbic acid were observed after glucagon injection, except in one experiment where a significant decrease was noted; 5) twenty minutes after the injection of epinephrine there was a decrease in liver and muscle glycogen and adrenal ascorbic acid. These changes were not statistically significant; 6) 2 hours after the injection of epinephrine, the reduction in liver and muscle glycogen and adrenal ascorbic acid became more pronounced and statistically significant; 7) the average weight of the adrenal glands of alloxan diabetic animals was 38.6 mg/100 g of body weight compared to 16.3 mg/100 g in the normal controls ($P < 0.01$).

Discussion. The liver and muscle glycogen content of fasted alloxan-diabetic animals is similar to that of fasted normal rats(3), indicating that well established severe alloxan diabetes does not interfere with glycogen storage. This is in accord with the observation of others(8,9,14-16). The results indicate also that alloxan diabetes does not interfere with the glycogenolytic effect of glucagon and epinephrine, but completely prevents the secondary increase in liver glycogen observed in normal animals 24 hours after the injection of glucagon and 2 hours after the injection of epinephrine. This suggests that insulin is necessary for this phenomenon. The adrenal ascorbic acid which, in the normal animal, decreases markedly following glucagon or epinephrine injections does not appear to be significantly changed in most alloxan diabetic rats. This may be due to the fact that in alloxan diabetes, a state of increased adrenal

activity already exists, as suggested by the greater weight of the adrenal glands. Although hypertrophy in itself does not prove hyperfunction(17), increased adrenal cortical activity in alloxan diabetic rats has been observed also by other workers(8,18). In 1 out of 4 experiments, the injection of glucagon was followed by a significant increase in muscle glycogen, confirming previous observations in normal animals(3,19). This phenomenon appears to be contrary to the observation that glucagon inhibits the synthesis of muscle glycogen produced by insulin(20), and probably is due not to glucagon itself, but to secondary insulin or adrenal cortical secretion(3). Its occurrence in severely diabetic rats makes the first possibility unlikely. On the other hand, the decrease in adrenal ascorbic acid coincident with the increase in muscle glycogen in one experiment suggests that the phenomenon may be the result of adrenal cortical stimulation.

Summary and conclusions. 1) The effects of glucagon and of epinephrine on liver and muscle glycogen and adrenal ascorbic acid of alloxan diabetic rats were studied. 2) Twenty minutes after injection of epinephrine a decrease in liver and muscle glycogen was observed. The decrease was still greater after 2 hours. 3) Two hours after injection of glucagon there was a decrease in liver glycogen and, in one experiment, an increase in muscle glycogen. 4) Twenty-four hours after injection of glucagon, liver glycogen had returned to, but not above, the initial level. 5) The adrenal glands of alloxan diabetic rats are significantly larger than those of normal rats. The injection of glucagon or epinephrine may further stimulate adrenal cortices, as indicated by occasional decrease in their ascorbic acid content. 6) It is concluded that: a) the glycogenolytic effects of glucagon and epinephrine are unimpaired in alloxan diabetes, and b) the delayed increase in liver glycogen, observed in normal rats after injection of epinephrine or glucagon, requires the presence of an intact insulin-secreting system capable of being stimulated by epinephrine or glucagon hyperglycemia.

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