Time Course of Glucocorticoid Dependent Changes in Cardiac Glycogen Concentration* (34036)

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We have previously reported that the concentration of glycogen in the hearts of rats is markedly reduced following chronic adrenalectomy (1). Furthermore, this decrease is prevented by the administration of the synthetic glucocorticoid, dexamethasone. The purposes of the present study were (a) to describe the time course of the decrease in cardiac glycogen concentration after acute adrenalectomy of rats, (b) to determine whether glucocorticoids would increase cardiac glycogen levels of the adrenalectomized rat, and if so (c) to determine the time course of restitution of the cardiac glycogen concentration to control values following administration of glucocorticoid.

Methods and Materials. Male, albino Sprague-Dawley rats (285–350 g) were anesthetized with ether, and bilaterally adrenalectomized via a dorsal lumbar incision. Sham-operated rats were similarly prepared except that the adrenals were not removed. Adrenalectomized rats were given 0.9% NaCl to drink whereas sham-operated rats were given tap water to drink. All rats were fed Purina rat chow, *ad libitum*.

Acute adrenalectomy. Hearts from adrenalectomized and sham-operated rats under pentobarbital anesthesia, [sham-operated (45 mg/kg), adrenalectomized (30 mg/kg), and adrenalectomized + dexamethasone (35 mg/kg)] were collected 6, 12, and 24 hr after operation. The hearts were frozen *in situ* with tongs cooled to the temperature of liquid nitrogen as previously described (1). The initial surgical procedure was performed between 8:00 and 11:00 a.m. Hearts were also collected from intact unoperated anesthetized rats at the same times.

Chronic adrenalectomy. Hearts were similarly collected from intact rats and from rats adrenalectomized 10 days earlier. Hearts of adrenalectomized animals were collected at 1, 2, 4, and 24 hr after a single intramuscular injection of dexamethasone (Decadron, Merck), and from another group of adrenalectomized rats injected with the dexamethasone vehicle (each ml contained creatinine, 8 mg; sodium citrate, 10 mg; sodium bisulfate, 3.2 mg; pH adjusted to 7.3 with NaOH) 1–4 hr prior to heart collection. All sample collections in this series were performed between 8:00 and 11:00 a.m.

Glycogen determination. Glycogen was assayed by the anthrone method from NaOH digests of tissue as previously described (1, 2). All values for glycogen are reported as milligrams of glycogen per gram of tissue (wet wt).

Results. Effect of adrenalectomy. In all groups, the cardiac glycogen concentration was significantly lower in adrenalectomized rats than in sham-operated rats (Fig. 1). For comparison, previously reported (1) values for cardiac glycogen are given for hearts collected at random times 10–14 days after adrenalectomy.

The cardiac glycogen concentrations of sham-operated rats were dependent upon the time of day the hearts were collected. The glycogen concentration was highest in hearts collected in the morning and lowest in hearts collected in the evening. A similar effect persisted in acutely adrenalectomized rats. In rats sacrificed 24 hr after adrenalectomy, the glycogen concentration of the hearts collected in the morning were higher ($p<0.05$) than

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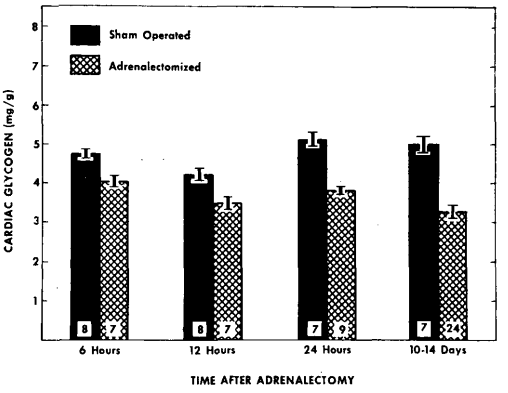

FIG. 1. Time course of cardiac glycogen depletion after adrenalectomy; all surgical procedures were performed in the morning. Samples were collected in the afternoon (6-hr group), evening (12-hr group), and following morning (24-hr group). The groups of adrenalectomized rats labeled "O," received the dexamethasone vehicle. Heights of bars indicate means; brackets indicate standard errors of the means; nos. inside the bars indicate the number of rats used; glycogen concentration given in mg/g of tissue (wet wt).

hearts of rats collected in the evening 12 hr after adrenalectomy.

Although not shown in Fig. 1, the cardiac glycogen concentration of sham-operated rats did not vary significantly from that of intact rats at any of the time periods shown in Fig. 1.

Effect of dexamethasone. The reduced concentration of cardiac glycogen 10 days after adrenalectomy was not significantly increased 1 and 2 hr after a single intramuscular 200-μg dose of dexamethasone. However, it was significantly increased after 4 hr ($p<0.01$), and reached a level which was not significantly less than that of the sham-operated rats, 24 hr after operation (Figs. 1 and 2). Twenty-four hr after a single injection of 200 μg of dexamethasone to adrenalectomized rats, the cardiac glycogen concentration was completely restored to that level seen in intact unoperated rats, whose hearts were collected at the same time of the day (Fig. 2).

Discussion. These results demonstrate rapid changes in the cardiac glycogen concentration of rats following the withdrawal of adrenal steroids by adrenalectomy and following the administration of glucocorticoids in chronically adrenalectomized rats. The time course of the increase in liver glycogen after glucocorticoid administration to adrenalectomized rats is also 3–4 hr (3). The total glycogen transferase (glycogen synthetase) activity of rat heart is reduced by adrenalectomy and increased by dexamethasone administration (1). The rapidity of the changes in cardiac glycogen concentration shown here suggests a direct action of glucocorticoids upon the cardiac metabolism of glycogen. The effects of adrenalectomy and dexamethasone on glycogen transferase activity suggests that this may possibly be via an effect on the synthesis of glycogen transferase or on the interconversion of transferases I and D.

The present results also show a diurnal rhythm in the concentration of cardiac glycogen in the rat. The glycogen is highest in the morning and lowest in the evening. The endogenous secretion of adrenal steroids is known to follow a similar pattern (4). This suggests that one factor responsible for the observed diurnal rhythm of cardiac glycogen

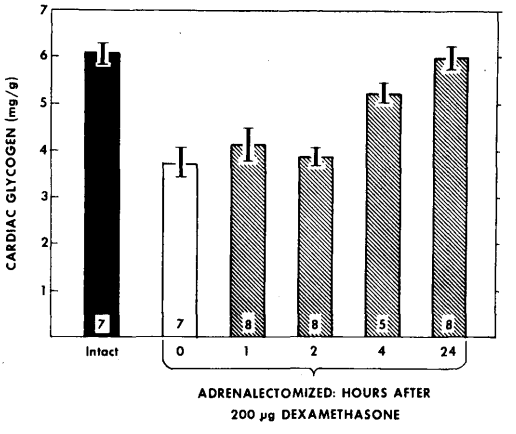

FIG. 2. Time course of repletion of cardiac glycogen in rats adrenalectomized for 10 days after a single intramuscular injection of 200 μg of dexamethasone. The group labeled zero was injected with dexamethasone vehicle 1–4 hr prior to heart collection; all samples were collected in the morning. Heights of bars indicate means; brackets indicate standard errors of the means; nos. inside the bars indicate the number of rats used; glycogen concentrations given in mg/g of tissue (wet weight).

concentration may be the diurnal fluctuations in plasma corticosteroid concentration. The data from rats adrenalectomized for 12 and 24 hr, however, suggest that this rhythm may persist after adrenalectomy. Thus, other factors may partially or wholly account for the diurnal changes in the cardiac glycogen concentration.

The fact that about 85% of an exogenously administered dose of dexamethasone is metabolized within 24 hr also suggests that the effects of glucocorticoids are relatively rapid. Part of the 2–4 hr latent period is due to the absorption time of the steroid which is about 15–30 min (5). In adrenalectomized rats with an impaired circulatory system (6) this process is probably slower than in animals with functioning adrenal glands.

Summary. Cardiac glycogen concentration decreases significantly in rats within 6 hr after adrenalectomy, and gradually decreases further over the next 18 hr. Superimposed upon these decreases are diurnal variations which reach a peak in the morning (8 to 11 a.m.) and decline to a low point in the evening (8 to 11 p.m.). Dexamethasone (200 μg) significantly increases the cardiac glycogen concentration 67% within 2–4 hr after injection and completely restores the glycogen concentrations to normal levels within 24 hr.

1. Daw, J. C., Lefer, A. M., and Berne, R. M., Circulation Res. **22**, 639 (1968).

2. Roe, J. H. and Dailey, R. E., Anal. Biochem. **15**, 245 (1966).

3. Long, C. N. H. and Smith, O. K., *in* "The Human Adrenal Cortex" (A. R. Currie, T. Symington, and J. K. Grant, eds.) p. 268. Williams and Wilkins, Baltimore, Maryland (1962).

4. Zimmerman, E. and Critchlow, V., Proc. Soc. Exptl. Biol. Med. **125**, 658 (1967).

5. Melby, J. C. and St. Cyr, M., Metabolism **10**, 75 (1961).

6. Ballard, K., Lefer, A., and Sayers, G., Am. J. Physiol. **199**, 221 (1960).

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