Discussion. Results of DOC treatment indicate a clear separation between life maintenance and restoration of ovarian-compensatory-hypertrophy: 30 μg is almost completely effective in supporting life, whereas 4 or more times that amount is required to restore the ovarian growth effect. We have recently suggested(2) that the failure of adrenalectomized rats to respond to hemicastration resulted from the abnormal salt and water metabolism that follows adrenal removal. The present study may not be entirely commensurate with this hypothesis since a much lower dose is adequate to maintain life than is necessary to restore the ovarian response. Corticosterone administration also restores the ovarian response, although the data suggest only a partial restoration of the ovarian compensatory hyperthrophy.

Summary. Desoxycorticosterone will restore the ability of adrenalectomized rats to respond to hemicastration with ovarian compensatory hypertrophy; however complete restoration of the response was only obtained at doses 3-4 times those necessary to preserve life. Corticosterone-treated, adrenalectomized rats show a recovery of the ovarian response toward normal, but only at doses exceeding those necessary for life maintenance.

1. Edgren, R. A., Parlow, A. F., Peterson, D. L., Jones, R. C., Endocrinology, 1965, v76, 97.

2. Edgren, R. A., Peterson, D. L., Acta Endocrinol., 1964, v47, 485.

3. Peterson, D. L., Edgren, R. A., Jones, R. C., Endocrinol., 1964, v29, 255.

4. Mainland, D., Murray, I. M., Science, 1952, v116, 591.

Received May 22, 1967. P.S.E.B.M., 1967, v125.

Studies on Cardiac Glycogen in Normal and Thyroidectomized Rats.* (32344)

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Hypothyroidism in rats has been shown to increase the content of glycogen in the heart, but the mechanism responsible for this alteration has not been elucidated(1). From other studies it is known that cardiac glycogen is increased under circumstances of sustained fat mobilization(2,3) and is depleted by catecholamines(4). The present studies were designed to explore the effect of fasting and of catecholamines on the cardiac glycogen of normal and thyroidectomized rats.

Methods and materials. Male Holtzman rats were maintained on tap water and Purina Labena Chow for at least 4 weeks before study. Thyroidectomy was performed on 130 g rats as previously described(5) and hypothyroidism was assessed by failure of gain in weight(6). Any animal weighing more than 200 g 4 to 6 weeks after thyroidectomy was discarded; normal controls weighed between 250 and 400 g after the same length of time.

Experimental procedure. In experiments on fasting rats animals were deprived of food for 18 to 24 hours. The animals were anesthetized with pentobarbital and in some experiments blood was drawn from the abdominal aorta, following which the heart was promptly removed, weighed and dropped in 30% potassium hydroxide in a boiling water bath. Hearts were digested for at least one hour and analyzed for glycogen as previously described(7). Cardiac glycogen by this procedure was 5.8 $\pm$ .3 μg/mg and compared favorably with glycogen values of 5.0 $\pm$ .7 μg/mg determined on hearts frozen immediately after thoracotomy and before weighing. Free fatty acids were determined by the method of Dole(8) on 0.5 ml aliquots of plasma. Isoproterenol was given in a dose of 50 μg/kg subcutaneously at various intervals before death or in various doses by the

* This study was supported in part by Grants 2A-5166, A-612, and AM-09897 from Nat. Inst. Health.

TABLE I. Effect of Thyroidectomy and Fasting on Cardiac Glycogen and FFA Concentrations.

	Normal	Thyroidectomized	P*
	Cardiac glycogen (μg/mg)		
Fed	3.32 ± .17 (17)†	5.66 ± .37 (15)	<.001
Fasting	5.12 ± .22 (16)	6.09 ± .23 (16)	<.01
P‡	<.001	N.S.	
	Plasma FFA (μEq/l)		
Fed	677 ± 66 (6)	632 ± 28 (7)	N.S.
Fasting	1095 ± 47 (6)	936 ± 66 (6)	N.S.
P‡	<.001	<.001	

* Normal *vs* thyroidectomized.
† Mean ± S.E.; the number of observations is indicated in parentheses.
‡ Fed *vs* fasting.

intraperitoneal route 5 minutes before sacrifice.

Results. The effect of fasting on the cardiac glycogen of normal and thyroidectomized rats is shown in Table I. Fasting significantly increased cardiac glycogen in normal but not in hypothyroid rats although both groups showed an increase in plasma free fatty acids (FFA) (Table I). To test further whether an increase in plasma FFA could raise cardiac glycogen, 15 thyroidectomized rats were deprived of food and 7 of them given 1.5 ml of oleic acid by stomach tube at the beginning of an 18-hour fast; five of 10 normal rats also received oleic acid. The plasma FFA were higher in the animals fed oleic acid (Table II), but the cardiac glycogen was unchanged in the thyroidectomized rats and increased in the normal rats.

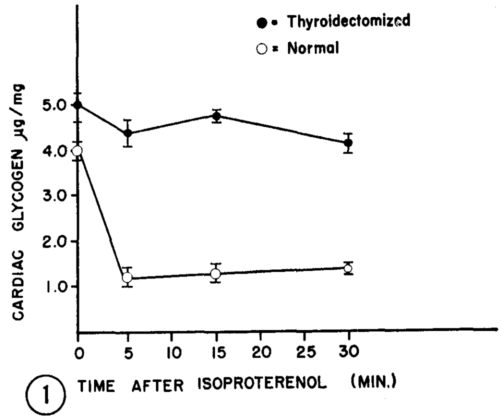
Studies on the changes in cardiac glycogen

TABLE II. Effect of Oleic Acid on Cardiac Glycogen and Plasma Free Fatty Acids of Fasted Normal and Fasted Thyroidectomized Rats.

	No. animals	Cardiac glycogen, μg/mg	Plasma free fatty acids, mEq/l
Normal			
Control	5	5.4 ± .34*	590 ± 69
Oleic acid†	5	6.9 ± .33	810 ±134‡
Thyroidectomized			
Control	8	7.4 ± .30	729 ± 54
Oleic acid†	7	8.2 ± .61	916 ± 54

* Mean ± S.E.M.
† 1.5 ml of oleic acid by stomach tube at beginning of an 18-hr fast.
‡ 3 values.

and plasma FFA after the administration of isoproterenol are shown in Fig. 1 and 2 and Tables III and IV. In fed normal rats 50 μg/kg of isoproterenol given subcutaneously produced a prompt fall in cardiac glycogen (Fig. 1 and Table III), but in the fed hypothyroid animals cardiac glycogen remained unchanged



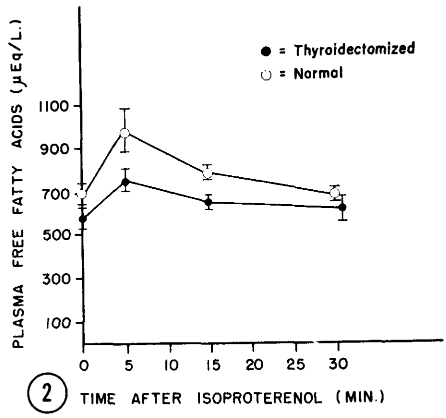


FIG. 1. Effect of isoproterenol on cardiac glycogen of normal and hypothyroid rats. 50 μg/kg of isoproterenol was injected subcutaneously and the animals sacrificed after exsanguination at various times. Each point represents the mean of 8 animals and vertical bar through each point represents the standard error of the mean.

FIG. 2. Effect of isoproternol on plasma free fatty acids of normal and hypothyroid rats. 50 μg/kg of isoproterenol was injected subcutaneously and blood drawn at times indicated. Each point in this Figure represents the same animals depicted in Fig. 1.

for at least 30 minutes after the drug was administered. Plasma FFA were significantly increased in 5 minutes in both normal and thyroidectomized rats (Fig. 2 and Table III)

TABLE III. Effects of Isoproterenol on Cardiac Glycogen and Plasma FFA of Normal and Thyroidectomized, Fasting Rats.

	Cardiac glycogen, μg/mg	P	Plasma FFA, mEq/l	P
Normal				
Control	6.5 ± .11*		913 ±119	
Isoproterenol‡	1.9 ± .39	<.001	1527 ± 21	<.001
Thyroidectomized				
Control	8.4 ± .54		1075 ± 99	
Isoproterenol	8.9 ± .80	N.S.†	1490 ± 91	<.02

* Mean ± S.E.M.; 5 rats/group.
† Not statistically significant, P >.05.
‡ 50 μg/kg administered subcutaneously 10 min previously.

and had returned to control values by 15 minutes. To show that the lack of response in hypothyroid rats was not due to inadequate absorption, isoproterenol was given intraperitoneally in increasing doses to a group of fed normal and thyroidectomized rats 5 minutes before sacrifice (Table IV). In the normal animals there was a 50% reduction in cardiac glycogen with a dose of isoproterenol as low as 5 μg/kg and a progressively greater depletion with each increasing dose. In the thyroidectomized animals there was a 20% reduction in each of the treated groups, but there was no further decrease even when the dose of isoproterenol was raised to 5000 μg/kg, 1000 times the lowest effective dose in normal rats. In fact, the cardiac glycogen of the hypothyroid rats after the highest dose was still significantly above that of the untreated normal rats.

Discussion. Increasing the concentration of plasma FFA by feeding oleic acid(3), or by

TABLE IV. Effect of Increasing Doses of Isoproterenol on Cardiac Glycogen of Normal and Thyroidectomized Rats.

Dose isoproterenol, μg/kg†	Cardiac glycogen, μg/mg Normal	Thyroidectomized
0	3.1 ± .24*	5.5 ± .29
5	1.8 ± .19	—
50	1.3 ± .55	4.3 ± .35
500	.5 ± .06	3.6 ± .46
5000	—	4.1 ± .25

* Mean ± S.E.M.; 5 rats/group.
† Isoproterenol given by intraperitoneal route.

fasting(2), increases cardiac glycogen. In the present experiments on thyroidectomized rats, neither feeding oleic acid nor fasting had any effect on the cardiac glycogen, although both procedures increased the plasma FFA. It has been shown that the concentration of FFA in the plasma is reduced in hypothyroidism (9) and that adipose tissue from thyroidectomized animals has a reduced response to lipolytic hormones(1,10). The elevation of cardiac glycogen in thyroidectomized rats is therefore not attributable to increased circulating levels of FFA.

Catecholamines can decrease(4) and sympathectomy increase(11) the cardiac glycogen. Treatment with thyroxine also decreases(12) while hypothyroidism increases the concentration of glycogen in the heart(1). Depletion of the cardiac glycogen is preceded by an increase in the ratio of active to inactive phosphorylase(13). Catecholamines and thyroxine increase the fraction of phosphorylase present in the active form, while hypothyroidism reduces it(12). The sensitivity of phosphorylase to activation by norepinephrine is increased by pretreatment with thyroxine(12), and as shown in the present experiments, glycogenolysis in response to isoproterenol is reduced by thyroidectomy. These observations, taken as a whole, suggest that the increased concentration of glycogen in the hearts of thyroidectomized rats may result from decreased sensitivity to catecholamines.

These observations apparently are at variance with those of Hornbrook *et al*(12) who reported that intravenous injections of norepinephrine can deplete glycogen and activate phosphorylase in hypothyroid rats. The reasons for this discrepancy are not readily apparent but may reside in the intrinsic difference between isoproterenol and norepinephrine or in the routes by which the drugs were administered. That isoproterenol is absorbed from subcutaneous sites is shown by the fact that a dose of 50 μg/kg given by this route raised FFA within 5 minutes in both normal and hypothyroid rats. However, this dose of isoproterenol given subcutaneously, or doses up to 100 times greater, given by the intraperitoneal route did not deplete cardiac glycogen in hypothyroid rats. Further evidence that isoproterenol is absorbed from the subcutaneous site is provided by the fact that salivary glands of normal and hypothyroid rats hypertrophy to the same extent after treatment with isoproterenol(14).

Our data and those of others(12) implicate the thyroid status as one factor in regulating the level of cardiac glycogen and the sensitivity of phosphorylase to activation by catecholamines. Recent reports from several laboratories have failed to demonstrate any effect of changes in the thyroid status on the chronotropic or inotropic responses of the hearts to catecholamines(15,16). However, Mayer, Cotten and Moran(17) showed that low doses of norepinephrine produced an inotropic response without activating phosphorylase in the heart. Williamson and Jamieson(18) studying the isolated, perfused rat heart similarly found that the inotropic response to norepinephrine could be dissociated from glycogenolysis. Thus, the metabolic and mechanical responses to catecholamines can be dissociated under appropriate conditions and provide precedent for the suggestion that the thyroid status may alter only the sensitivity of the metabolic and not the mechanical responses of the heart to catecholamines.

Summary. The present experiments show that the concentration of cardiac glycogen in thyroidectomized rats is consistently higher than in fasted or fed normal rats and that fasting or feeding oleic acid has no significant effects on the cardiac glycogen of hypothyroid animals. In addition, we have shown that isoproterenol produces a dose-dependent decrease in glycogen content in hearts from normal rats, but that it has little effect on the cardiac glycogen of hearts from thyroidectomized rats.

The authors wish to thank Dr. E. B. Astwood for his continuing interest and to acknowledge the excellent technical assistance of Mrs. Elaine Cline and Mr. Malcolm Morrison.

1. Bray, G. A., Goodman, H. M., Endocrinology, 1965, v76, 323.
2. Adrouny, G., Russell, J. A., ibid., 1956, v59, 241.
3. Bowman, R. H., Am J. Physiol, 1959, v197, 1017.
4. Hornbrook, K. R., Brody, T. M., J. Pharm. Exp. Ther., 1963, v140, 295.
5. Bray, G. A., J. Clin. Invest, 1964, v43, 285.
6. Evans, E. S., Rosenberg, L. L., Evans, A. B., Koneff, A. A., Endocrinology, 1964, v74, 770.
7. Kahan, J. Arch. Biochem., 1953, v47, 408.
8. Dole, V. P., J. Clin. Invest., 1956, v35, 150.
9. Goodman, H. M., Knobil, E., Am. J. Physiol., 1961, v201, 1.
10. Debons, A. F., Schwartz, I. L., J. Lipid Research, 1961, v2, 86.
11. Jellinek, M., Kaye, M. P., Nigh, C. A., Cooper, T., Am J. Physiol., 1964, v206, 971.
12. Hornbrook, K. R., Quinn, P. V., Siegel, J. H., Brody, T. M., Biochem. Pharm., 1965, v14, 925.
13. Haugaard, N., Hess, M. E., Pharmacol. Rev., 1965, v17, 27.
14. Bray, G. A., Proc. Soc. Exp. Biol. & Med., in press.
15. Van der Schoot, J. B., Moran, N. C., J. Pharm. Exp. Ther., 1965, v149, 336.
16. Margolius, H. S., Gaffney, T. E., ibid., 1965, v149, 329.
17. Mayer, S. E., Cotten, M. de V., Moran, N. C., ibid., 1963, v139, 275.
18. Williamson, J. R., Jamieson, D., Nature, 1965, v206, 364.

Received May 12, 1967. P.S.E.B.M., 1967, v125.