than a minor role was provided by the finding that CCl_4 administration does not depress the endotoxin-inactivating ability of the spleen. Thus, the presence of a normal spleen does not constitute adequate protection against even small doses of endotoxin if liver function is severely impaired.

Removal of the spleen did not detectably enhance the susceptibility of normal animals to bacterial endotoxin. More significantly, even when the liver was severely damaged by CCl_4, so that the animals were more than 100 times as sensitive as normal animals to endotoxin, splenectomy did not result in a further increase in susceptibility.

The experiments reported herein fail to provide evidence that the spleen participates in any important way in the response of either normal guinea pigs or those with severe liver damage to the injection of bacterial endotoxin.

Summary. Homogenates of guinea pig liver, spleen, kidney and (to a lesser degree) heart are capable of detoxifying bacterial endotoxin *in vitro*. Since only the liver and spleen appear to remove significant amounts of endotoxin from the circulation, these two organs would seem to be the ones in which the inactivation process might be of some physiological significance *in vivo*. Previous studies suggested that the liver may have an important function in the inactivation of endotoxin which gains access to the body. The experiments reported herein are consistent with this hypothesis, but provide no evidence that the spleen plays any significant role in the response of either normal guinea pigs or those with liver damage to administration of endotoxin.

I am indebted to S. B. Formal for many valuable suggestions, and to J. G. Watson, S. Austin and A. L. Fisher, Jr., for excellent technical assistance.

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Received September 2, 1964. P.S.E.B.M., 1965, v118.

Effect of Altered Thyroid Status upon Epinephrine Induced Lipolysis *in vitro*.* (29803)

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Because of similarities in their peripheral effects, an interrelationship between the thyroid hormones and catecholamines has long been suspected. It has been postulated that some of the actions of thyroid hormones may result from increased tissue sensitivity to the effects of epinephrine(1-3). The serum concentration of free fatty acids (FFA) is elevated in hyperthyroidism(4). Since triiodothyronine has no lipolytic effect *in vitro*(5) and since epinephrine is a very potent fat mobilizer both *in vitro*(6) and *in vivo*(7,8), it seemed appropriate to study the effect of

* This study was supported by grants from USPHS.

TABLE I. Release of FFA (μEq/g/Hr) from Tissues of Control, Thyroidectomized and Thyroxine-Treated Rats. No. of animals in parentheses.

Group	Basal	Epinephrine .005 μg/ml	Epinephrine 10 μg/ml
Control	(46) 1.549 ± .127*	(46) 2.709 ± .242	(18) 9.076 ± .649
Thyroidectomy	(31) .916 ± .114	(31) 1.611 ± .165	(13) 7.851 ± .445
p *vs* control	<.001	<.001	>.10
Thyroxine	(21) 1.959 ± .147	(21) 3.714 ± .328	(10) 9.309 ± .587
p *vs* control	<.05	<.02	>.70

* Standard error of mean.

alteration of thyroid status upon the fat mobilizing action of epinephrine *in vitro*.

Material and methods. Male rats of the Charles River Strain were used. Rats were divided into "thyroxine-treated," "thyroidectomized," and "control" groups. Thyroxine-treated rats were given a single daily intraperitoneal injection of 0.5 ml of a solution of l-thyroxine† in normal saline containing 200 μg per ml, for 10 to 14 days prior to use, the last injection being on the day of experiment. Thyroidectomized rats had undergone surgical thyroidectomy under ether anesthesia 21-28 days prior to use. At the time of each experiment, completeness of thyroidectomy was checked by careful dissection and examination of the neck. Control animals were untreated. All animals were maintained on Purina Laboratory Chow and water *ad lib* until 24 hours prior to experiment, at which time they were denied food but allowed water *ad lib*.

Rats were lightly anesthetized with pentobarbital, both epididymal fat pads removed and 2 or 3 pieces, weighing 50 to 100 mg, were cut from the thin portion of each pad. These pieces were weighed on a torsion balance and each placed into one of 4 small flasks containing one ml of Krebs-Ringer Phosphate buffer (pH 7.35-7.45) containing 5% defatted human albumin‡ from which titrable FFA had been extracted as described by Hollenberg(9). Epinephrine Injection U.S.P.§ containing one mg epinephrine hydrochloride per ml was diluted 1:1000 with normal saline and 0.005 ml of the dilute solution was added to 2 of the flasks, so that the final epinephrine concentration in the medium was 0.005 μg per ml. In some experiments, 0.01 ml of undiluted Epinephrine Injection U.S.P. was added to 2 additional flasks, the final epinephrine concentration being 10 μg per ml. The unstoppered flasks were then shaken gently for 3 hours at 37°C in a Dubnoff Metabolic Incubator with air as the gas phase. At the end of the incubation period, the adipose tissue was removed from the flask and the flask contents analyzed for FFA by the method of Dole(8). On some days, the flask contents were immediately frozen following removal of the tissue and the analysis performed the next day.

Release of FFA was expressed as microequivalents per gram of tissue per hour of incubation. The mean increase in FFA release induced by epinephrine (mean Δ ep) in a group of N rats was calculated as $\frac{\sum \Delta \text{ ep}}{N}$ where Δ ep is the difference between FFA release rate of epinephrine-treated tissue and that of untreated tissues (basal) of the same rat. Mean percent increase was calculated as $\frac{\sum \frac{\Delta \text{ ep}}{\text{basal release rate}} \times 100}{N}$.

Results. Epididymal fat pads taken from thyroxine-treated rats were considerably smaller than those of control or thyroidectomized rats and were strikingly hyperemic. The mean release of FFA from tissues incubated under basal conditions and with epinephrine in control, thyroidectomized and thyroxine-treated rats is shown in Table I. Tissues of thyroidectomized animals released significantly less FFA under basal conditions

† Sigma Chemical Co., St. Louis, Mo.

‡ Fraction V (from human plasma), E. R. Squibb & Sons, New York, generously supplied by American National Red Cross.

§ Adrenaline® Chloride Solution, Parke, Davis & Co., Detroit, Mich.

TABLE II. Increase in FFA Release and Percent Increase Produced by Epinephrine .005 μg/ml.*

Group	Increase (mean Δ ep)	% Increase
Control	1.160 ± .194	92.3 ± 14.1
Thyroidectomy	.695 ± .121	143.1 ± 39.3
p *vs* control	<.05	>.20
Thyroxine	1.755 ± .227	113.9 ± 43.5
p *vs* control	>.05	>.60

* All values are mean ± standard error of mean.

than those of controls (0.916 *vs* 1.549). Basal release of thyroxine-treated animals was greater than that of controls (1.959 *vs* 1.549); this difference was of borderline statistical significance ($0.1>P>.05$). The difference in release between thyroxine-treated and thyroidectomized animals was highly significant ($P<.001$).

Release of FFA from tissue exposed to the lower concentration of epinephrine was significantly greater than the basal release in all groups (controls $P<.001$, thyroidectomy $P<.001$, thyroxine $P = .01$). Release of FFA was significantly increased by thyroxine pre-treatment and significantly decreased by thyroidectomy as compared with controls. At this concentration of epinephrine, the difference in release between thyroxine-treated and thyroidectomized animals was statistically highly significant ($P<.001$).

Table II shows results for basal release and release with the smaller dose of epinephrine expressed as net increase and percent increase for each experiment. The net increase in thyroxine-treated animals was greater than that of controls (1.755 *vs* 1.160). This difference was of dubious statistical significance ($0.1>P>.05$). Net increase in thyroidectomized animals was significantly smaller than that of controls (0.695 *vs* 1.160). When thyroidectomized animals were compared with thyroxine-treated animals, the difference was highly significant ($P<.001$). However, if the enhanced lipolysis produced by epinephrine were expressed not as net increase but as percent increase, neither group differed significantly from controls, or from each other ($P>.50$).

Although at the high concentration of epinephrine release of FFA from adipose tissue of thyroidectomized animals (7.851) was somewhat smaller than that of controls (9.076) and the corresponding value in thyroxine-treated animals (9.369) somewhat greater, these differences were not statistically significant (Table I). The difference between thyroidectomized and thyroxine-treated rats was also not statistically significant ($.10>P>.05$).

Discussion. Although it is widely believed on the basis of hemodynamic studies in hyperthyroidism(10) that there exists a "dynamic interrelationship" between the thyroid hormones and the catecholamines, it has recently been pointed out(11) that the increase in hemodynamic effects of epinephrine in hyperthyroidism could result from decreased myocardial binding and inactivation of epinephrine rather than from altered physiologic responsiveness of the heart to catecholamines. Investigation of the thyroid-epinephrine relationship on fat mobilization *in vivo* has yielded conflicting results. Infusion of epinephrine in patients with thyroid disease by Harlan *et al*(12) produced a greater increase in serum FFA in hyperthyroid than in hypothyroid subjects. More recently, similar experiments(13) have failed to demonstrate this difference. Goodman and Knobil(14) reported that hypophysectomized monkeys failed to respond to an injection of epinephrine with an increase in serum FFA. Pretreatment with thyrotropin or triiodothyronine but not with corticotropin or adrenal steroids restored the response. Similar experiments(15) using hypophysectomized dogs, however, revealed restoration of response to epinephrine after pre-treatment with corticotropin or adrenal steroids.

Adipose tissue of hypophysectomized rats showed no lipolytic response *in vitro* to epinephrine at a concentration of 0.05 μg per ml(16). Administration of thyrotropin induced only a small increase in responsiveness. Triiodothyronine administration completely restored responsiveness. Both cortisol and growth hormone, however, also completely restored responsiveness. Thyroid hormone, thus, does not appear to be essential for the lipolytic response to epinephrine by

adipose tissue of hypophysectomized dogs and rats.

The present studies confirm the potent lipolytic action of small concentrations of epinephrine *in vitro,* and are consistent with the finding of several investigators(17-19) that hypothyroidism decreases *in vitro* release of FFA from adipose tissue. In contrast to others, however, we observed a significant lipolytic response to epinephrine by adipose tissue of hypothyroid rats. Debons and Schwartz(17) and Deykin and Vaughan (19) reported complete abolition of the lipolytic action of epinephrine in propylthiouracil (PTU) treated rats. The possibility of an extra-thyroidal effect of PTU upon lipolysis should be considered, however, since PTU has been shown to have peripheral actions (20). Although Felt *et al*(18) report no significant lipolytic effect of epinephrine on tissues of thyroidectomized rats, their data show a mean value for FFA in incubation medium containing epinephrine which, though not statistically significant, is more than twice that in epinephrine-free medium.

The fact that previous studies have been performed using non-fasted animals may be of importance since fasting has been shown to increase greatly the release of FFA *in vitro*(6) and to raise serum FFA *in vivo*(8).

Although the results of the present study reveal that adipose tissue of thyroxine-treated rats released more FFA in the presence of epinephrine (0.005 μg/ml) than did tissues of thyroidectomized rats, the release expressed in terms of percent increase did not differ in any group. Comparison of hyperthyroid and euthyroid rats in the studies of Debons and Schwartz(17) and Deykin and Vaughan(19) reveals that there was no significant difference in the percent increase of FFA released with epinephrine although the absolute release of FFA was clearly higher in the hyperthyroid animals. The experiments of Felt *et al*(18) in which thyroidectomized rats were used reveal a mean basal release of FFA from tissues of euthyroid rats of 0.189 meq per liter and a release of 0.395 meq per liter after epinephrine; an increase of 109%. For hypothyroid rats, the comparable figures are 0.081 meq per liter basal and 0.191 meq per liter after epinephrine; an increase of 136%. In a more recent study Felt *et al*(21) have shown that phentolamine blocked the *in vitro* lipolytic response to epinephrine by adipose tissue of both euthyroid and hyperthyroid rats. Even with phentolamine added, however, tissues of hyperthyroid rats still released more FFA than did euthyroid tissues, suggesting that factors other than endogenous catecholamine may be involved in the effect of hyperthyroidism upon lipolysis.

In the present work, the FFA release from adipose tissue of hypothyroid and euthyroid rats was essentially the same when exposed to a concentration of epinephrine (10 μg/ml) large enough so that differences in tissue sensitivity might presumably be abolished. It would thus appear that the maximum lipolytic response is independent of the status of thyroid function.

Thus, while the present study leaves in doubt the question of whether thyroid hormone *in vivo* actually changes tissue sensitivity to the fat mobilizing action of epinephrine, the data suggest that the difference between hypothyroid and hyperthyroid rats in the absolute quantity of FFA released from adipose tissue in response to *in vitro* epinephrine may be a function of the basal lipolytic activity of these tissues, since the relative changes are the same. Comparison of the lipolytic effect of epinephrine with that of other fat mobilizing hormones, *e.g.,* corticotropin, upon tissues of animals with altered thyroid function might be of value in clarifying this point.

Summary. The effect of pre-treatment with thyroxine and of thyroidectomy upon the *in vitro* lipolytic response of adipose tissue to epinephrine has been studied. Release of FFA from adipose tissue of thyroxine-treated animals was significantly greater than that of tissues of thyroidectomized animals, and the absolute increase produced by epinephrine was also larger. Adipose tissue of thyroidectomized rats exhibited a significant lipolytic response to epinephrine. The fractional increase induced by a dilute concentration of epinephrine was independent of thyroid status. The lipolytic response of tis-

sues of hypothyroid, hyperthyroid and euthyroid rats to a high concentration of epinephrine was essentially the same. It is suggested that the magnitude of the lipolytic response to physiologic doses of epinephrine may be related to the basal lipolytic activity of the adipose tissue.

The technical assistance of Miss Patricia Gordon and Mrs. Louise Melendy is gratefully acknowledged.

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Received September 2, 1964. P.S.E.B.M., 1965, v118.

Enzyme Activities in the Developing Muscle and Liver of Normal and Genetically Dystrophic Chick Embryos. (29804)

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Asmundson and Julian(1) reported muscular dystrophy as a genetic anomaly in a strain of New Hampshire chicks. They attributed this condition to a recessive gene which also affects the body conformation. The age at which the characteristic symptoms of avian muscular dystrophy could be detected varied from 4 to 12 weeks. Cornelius *et al*(2), in a study using the same strain of chicks, reported that 4 weeks to 18 months old dystrophic birds exhibited higher activities of serum aldolase and glutamic-oxaloacetic transaminase (GOT) in serum and tissues when compared to normal birds. Zalkin and Tappel(3) reported that oxidation of succinate is impaired in tissue from vit. E-deficient rabbits. Allen *et al*(4) found that activity of cytochrome *c* reductase was elevated in muscles from vit. E-deficient rabbits while cytochrome *c* oxidase was decreased during the first week of the deficiency and then approached normal level. The purpose of this investigation was to determine the activities of the succinic dehydrogenase, GOT and cytochrome *c* oxidase in embryos and day-old chicks from the genetically dystrophic and normal strains of New Hampshire chicks in an effort to elucidate the effect of the dystrophic gene on the activity of these enzymes.

Procedure. Eggs were collected daily from hens maintained on a practical type diet. The muscular dystrophy strain of birds was from a stock originally obtained from Asmundson and Julian in 1958 and maintained at this station. A normal strain of New