The influence of thyroxine and other hormones on hepatic TPN-cytochrome reductase activity

The rate of hepatic fatty acid synthesis is markedly depressed in the diabetic rat and is elevated in the hypophysectomized animal[1]. This information, together with our recent observation that fatty acid synthesis requires TPNH as an electron donor[2], has prompted us to study the influence of hormonal factors upon the relative activities of enzymes catalyzing the oxidation and reduction of TPN.

During these studies it has been found that TPN-cytochrome *c* reductase activity in the livers of hypophysectomized rats is invariably much lower than the remarkably constant activities found in the livers of normal rats (Table I). The activity of this enzyme is similarly lowered in the livers of thyroidectomized animals (Table I). This diminished activity can regularly be restored to normal or even elevated values by the administration of thyroxine to thyroidectomized (Table I) or hypophysectomized (Fig. 1) animals. A large part of this response occurs within the first 24 hours following the administration of thyroxine, but smaller additional increments in activity result from further daily treatments with the hormone. In addition, liver homogenates prepared from thyroxine-treated normal rats consistently display elevated TPN-cytochrome *c* reductase activity. In this case the response appears more slowly, and the activity continues to rise steadily during more than a week of daily thyroxine treatments (Fig. 2).

TABLE I

THE EFFECTS OF HYPOPHYSECTOMY, THYROIDECTOMY AND THYROXINE ADMINISTRATION ON TPN-CYTOCHROME *c* REDUCTASE ACTIVITY IN RAT LIVER HOMOGENATES*

Condition of rat	*Number of rats*	*TPN-cytochrome c reductase specific activity***	*Specific activity % of normal control*
Normal	27	13.0 (± 2.4)§	100
Hypophysectomized	11	5.6 (+ 1.1)	43
Thyroidectomized	3	4.8 (± 0.5)	37
Thyroidectomized-thyroxin-treated***	3	9.3 (± 2.5)	71.5

* Standard assay conditions were as follows: A portion of rat liver was homogenized in 2.5 volumes 0.25 *M* sucrose, and the large particles were removed by centrifugation at 10,000 × *g* for 20 minutes. The supernatant fraction was assayed for TPN-cytochrome *c* reductase by a slight modification of the method of HOGEBOOM AND SCHNEIDER[3]. Conditions were so arranged that activity was proportional to enzyme concentration.

** Specific activity $= \frac{\mu\text{moles of cytochrome } c \text{ reduced}}{\text{g protein} \times \text{minute}}$

*** 1 mg DL-thyroxine administered intraperitoneally daily for 3–4 days.

§ The figures in parentheses are the standard deviations.

These changes in TPN-cytochrome *c* reductase activity appear to be related rather specis fically to the thyroid state of the animals since no significant changes in the activity of thir enzyme were detected in the livers of adrenalectomized, castrated, or alloxan-diabetic rats, no- was the low enzymic activity in hypophysectomized rats altered by treatment with growth hormone, cortisone, or ACTH.

The activity of DPN-cytochrome *c* reductase[4] was also measured in many of these experiments. However, the activity of this enzyme was affected comparatively little by the various experimental conditions. In addition, no effects of hypophysectomy were found on the activities of glucose-6-phosphate dehydrogenase[5] or transhydrogenase[6]. It may thus be concluded that the changes observed in TPN-cytochrome *c* reductase activity do not occur as the result of a general variation in enzymic activities.

A series of preliminary measurements have been made of TPN diaphorase activity in the livers of normal, hypophysectoimzed, thyroidectomized, and thyroxine-treated thyroidectomized rats (Table II). No statistically significant differences in this enzymic activity were detected among these groups of animals. It has been reported recently that purified DPN-cytochrome *c* reductase has high DPN-diaphorase activity[7], and it has been postulated[4] that the same flavoprotein moiety is responsible for both cytochrome reductase and diaphorase activities. Pyridine nucleotide diaphorases having no cytochrome reductase activity represent, according to this

view[7], cytochrome reductases in which metal has been lost or in which the mode of attachment of the prosthetic groups has been altered. If this hypothesis is correct, the results presented here indicate that it is not the concentration of TPN-cytochrome reductase apoenzyme, but rather the activity of the enzyme toward cytochrome *c* as an electron acceptor which is dependent upon the availability of thyroxine.

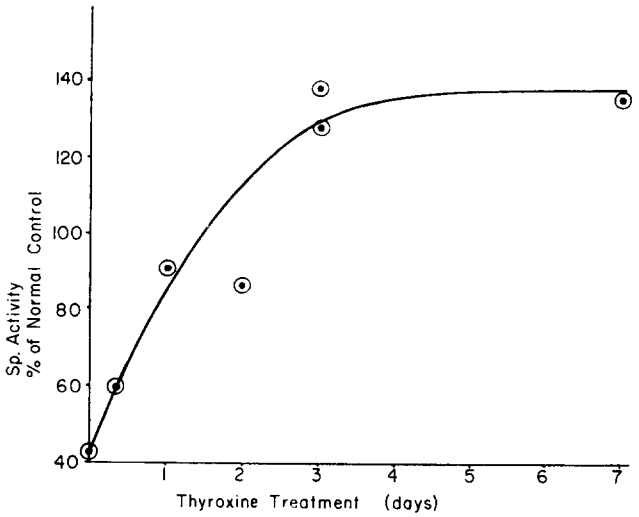

Fig. 1. The effect of *in vivo* thyroxin administration on hepatic TPN-cytochrome *c* reductase activity in the hypophysectomized rat. 1 mg DL-thyroxin administered daily by intraperitoneal injection to each rat. Condition of assay as in footnote to Table I.

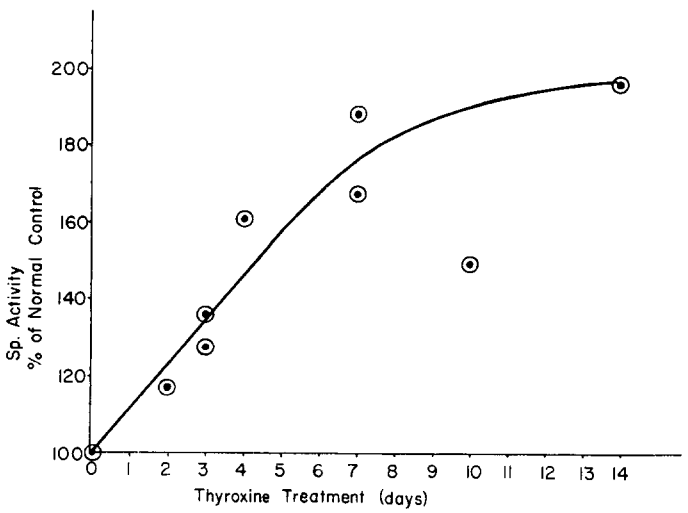

Fig. 2. The effect of thyroxin administration on hepatic TPN-cytochrome *c* reductase activity in the normal rat. 1 mg DL-thyroxin administered daily by intraperitoneal injection to each rat. Conditions of assay as in footnote to Table I.

It is not revealed by the present experiments whether the observed effect of thyroxine on TPN-cytochrome reductase activity represents a primary site of physiological action of the hormone. However, of the numerous enzymes which have been studied in tissues of hyper- and hypothyroid animals[8], none, to our knowledge, has been found to show such marked depression of activity in the hypothyroid state, or to respond so rapidly to thyroxine administration. In ad-

TABLE II

HORMONAL EFFECTS ON TPN-DIAPHORASE ACTIVITY*

Condition of rats	*Number of rats*	*TPN diaphorase Specific activity***
Normal	10	37.0 (± 10.0)
Thyroidectomized	3	34.3 (± 7.0)
Hypophysectomized	4	31.9 (± 6.3)
Thyroidectomized-thyroxin treated***	4	33.5 (± 1.8)

* Conditions of assay were essentially as described in footnote to Table I, except that $3.6 \cdot 10^{-5} M$ 2,6-dichlorophenol-indophenol was substituted for cytochrome *c* as an electron acceptor.

** Specific activity = $\frac{\mu\text{moles 2,6-dichlorophenol-indophenol reduced}}{\text{g protein} \times \text{minute}}$.

*** 1 mg DL-thyroxin injected intraperitoneally daily for 1–4 days.

dition, in no previous case has evidence been obtained to indicate that variation in enzymic activity was not due to variation in the concentration of the protein moiety of the enzyme being studied.

In summary, the activity of hepatic TPN-cytochrome *c* reductase appears to be under thyroid control, and it is suggested that control of the activity of this enzyme may represent a primary site of action of the thyroid hormone.

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Effect of hypophysectomy on dehydrogenase activity of rat tissues

High levels of activity of glucose-6-phosphate (G6P) dehydrogenase have been reported for the adrenal cortex and lactating mammary glands[1,2]. Measurements of the activities have also been made in a variety of mammalian tissues. It was suggested that the direct oxidative pathway might play a significant role in adrenal metabolism since the cortex showed high activity[2].

Selected tissues from control and hypophysectomized (postoperative 7 days) rats* were assayed for G6P dehydrogenase and 6-phosphogluconate (6PG) dehydrogenase activities. The two-substrate method of GLOCK AND MCLEAN[4] was used. Each cell contained 0.24 m*M* tris (hydroxy methyl) amino methane buffer pH 7.45, 0.4 μM triphosphopyridine nucleotide and suitable aliquots of the supernatant from tissue homogenates to a total volume of 3.0 ml. The

* Control and hypophysectomized rats were purchased from Hormone Assay Laboratories, Chicago, Ill.