

Effect of Hyperthyroidism on Distribution of Adenosine Phosphates and Glycogen in Liver.*† (24928)

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The experiments described below were undertaken with two objectives. The first derives from the postulate that the mechanism of action of the thyroid hormone consists of a dissociation of phosphorylation from oxidation, in a manner analogous to the effect of dinitrophenol. Such a dissociation was found by Maley and Lardy(1) in lowering the P:O ratio of isolated liver mitochondria from thyrotoxic rats. The *in vivo* equivalent might be expected to manifest itself as a shift in the balance between ATP and the products of dephosphorylation, ADP and AMP, under steady state conditions in the intact animal. The second objective was as a test of the hypothesis of Bloom *et al.*(2) that the fraction of liver glycogen which can be extracted by cold TCA§ is more active metabolically than the fraction which is not so extractable, which can be isolated by treatment with hot KOH solution.

Methods. Experiments were carried out on guinea pigs because of their sensitivity to thyroxin. Males weighing at least 500 g were maintained on Purina guinea pig chow and water *ad lib*. Oxygen consumption under conditions approximating basal was determined by modification of the method of Watts and Gourley(3), using a large vacuum type desiccator as the animal chamber. When a number of consistent readings were obtained over several days, the experimental animals were given daily injections of L-thyroxin subcutaneously, in dose of 100 µg/kg. The thyroxin was

dissolved in minimum volume of 0.1 N NaOH, and diluted with 0.9% solution of NaCl. Two weeks of this treatment usually increased oxygen consumption by at least 50%. A few animals were more refractory and required a daily dose of 150 µg/kg. One normal and one thyrotoxic animal were worked up simultaneously. Under pentobarbital anesthesia, supplemented by ether when necessary, the liver was excised and dropped immediately into a large volume of freezing mixture of dry ice and ether. Subsequent operations were carried out in cold room at 1-2°C. The frozen liver was broken into small pieces in a tissue crusher chilled with dry ice. A weighed quantity was transferred to a Waring blender, and treated with approximately 5 volumes of 10% solution of TCA. The blender was run for about 2 minutes, to obtain complete precipitation of proteins. The suspension was centrifuged, the supernatant filtered, and the residue extracted twice with 5% TCA solution. The combined filtrate and washings were extracted with ether to remove the TCA, the solution made ammoniacal, and brought up to definite volume. An aliquot of this was taken for precipitation of glycogen by ethanol. The bulk of the solution was then passed over a column of Dowex-1 resin to adsorb the P compounds present. The column was washed with water, then with a liter of 0.01 M NH₄Cl. The sequence of eluents used was: 0.002 N HCl, which removed AMP; 0.01 N HCl, which removed first some nucleotide matter containing bases other than adenine, and then ADP; 0.01 N HCl plus 0.04 M KCl, which eluted additional nucleotide compounds with other bases than adenine; and finally with 0.02 N HCl plus 0.6 M KCl, to elute ATP. Elution was at a rate of approximately 50 ml/hour, and 2-hour fractions were collected by automatic fraction collector. Progress of the elution was followed by measurements at 260 and 280 mµ in a Beckman

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§ Abbreviations used are: TCA for trichloroacetic acid, and ATP, ADP, and AMP, for adenosine tri-, di-, and mono-phosphates, respectively.

TABLE I. Effect of Hyperthyroidism Produced by Thyroxin on Adenosine Phosphates and Glycogen of Liver in Guinea Pigs.

	AMP	ADP	ATP	TCA-extractable glycogen, mg/g	Non-extractable glycogen, mg/g
	$\mu\text{M/g}$				
Normal (7 exp.)	$.945 \pm .25$	$1.49 \pm .17$	$.99 \pm .09$	31.4 ± 1.7	$3.6 \pm .6$
Hyperthyroid (7 exp.)	$.77 \pm .19$	$1.82 \pm .10$	$.86 \pm .11$	$1.9 \pm .3$	$3.4 \pm .6$

Values as mean and stand. error of mean.

Model DU spectrophotometer. The residue from the TCA extraction was digested with 30% KOH solution and the glycogen isolated by precipitation with ethanol. The material was dissolved in water, proteinaceous material precipitated by addition of TCA, and the glycogen re-precipitated by ethanol. Glycogen determinations were made by the anthrone method(4) using purified glycogen isolated from rabbit liver as a standard.

Results obtained are given in Table I. It is evident that ATP comprises less than one-third of total adenosine phosphates present in liver in water-soluble forms, and that ADP is the form present in highest concentration. This distribution makes it possible to ascertain whether there is any shift in the balance in the thyrotoxic state. Neither with respect to total adenosine phosphates nor to distribution between ATP, ADP and AMP, was any significant difference found between normal and thyrotoxic animals. These data, therefore, lend no support to the theory that thyroid hormone produces a dissociation of phosphorylation from oxidation. However, there is still the possibility that such a shift in the balance between ATP and the dephosphorylation products might be obtained acutely, as a result of a single injection of a sufficiently

large dose of tri-iodothyronine.

The decrease in glycogen content of the liver, which is well known as an effect of the hyperthyroid state, is clearly seen to be limited to that fraction which is extractable by TCA. This finding strengthens the hypothesis of Bloom *et al.*(2) that TCA-extractable glycogen is more active metabolically than the fraction which cannot be extracted from the liver by this means.

Summary. Chronic hyperthyroidism induced in guinea pigs by daily injection of thyroxin has no effect on concentrations of ATP, ADP, and AMP in the liver. This finding gives no support to the postulate that thyroxine produces its metabolic effects by dissociating phosphorylation from oxidation. Reduction in glycogen content of liver that results from chronic hyperthyroidism is entirely in the fraction extractable by TCA.

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Effect of Reserpine on Oxytocin and Lactogen Discharge in Lactating Rats.* (24929)

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It is generally accepted that oxytocin as well as lactogen are reflexly released from the hypophysis in lactating animals and that both hormones are important factors in determin-

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