Effect of Thyroxine Administration on Enzymes Associated with Glucose Metabolism in the Liver.* (31956)

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Glucose-6-phosphatase (G-6-Pase) and fructose-1,6-diphosphatase (FDPase) have a prominent role in the production of glucose from lactate and other glucose precursors. The activities of these enzymes are increased significantly when protein or fructose, both of which must go through FDPase and G-6-Pase, are fed as high percentage of the diet(1). Diabetes(2,3) and treatment with hydrocortisone (4) also increase the activities of both of these enzymes. All these conditions appear to induce the synthesis of enzymes involved in gluconeogenesis. Recent reports indicate that conditions causing increased gluconeogenesis also decrease liver pyruvate kinase (PK) activity. This is consistent with the findings that PK activity is decreased by starvation(5), diabetes(6), and hydrocortisone treatment (Freedland *et al,* unpublished work). The decrease in PK activity would permit more phosphoenolpyruvate to proceed to glucose, as *in vivo* the reaction catalyzed by PK is essentially unidirectional toward pyruvate formation.

Since thyroxine causes marked increase in G-6-Pase(7) activity, it was of interest to study if this increase is also related to gluconeogenesis. It was felt that studies on FDPase and PK in the liver after thyroidectomy and thyroxine treatment might answer this question.

Methods. Male Sprague-Dawley rats weighing between 100 to 150 g were used throughout and offered food (65% glucose, 25% casein, 5% corn oil, 4% salt and 1% vitamins) and water *ad libitum* in individual screened bottom metal cages. L-thyroxine was dissolved in 0.01 N NaOH and administered intraperitoneally for 5 days at a dose of 1 mg/rat/day to both normal and thyroidectomized animals.

The animals were stunned by a sharp blow on the head, decapitated, exsanguinated, the livers rapidly and completely removed, weighed, chilled, and a portion was homogenized in 19 volumes of 0.1 M potassium citrate (pH 6.5) and used for determination of G-6-Pase(8) by the release of inorganic phosphate at 37°. A second portion was homogenized in 9 volumes of 0.14 M KCl, centrifuged at 30,000 × *g* for 30 minutes at 0-4°, the supernatant fluid used for the assay of FDPase(9) and PK(10) after appropriate dilution. The two assays were followed at 340 mμ, measuring TPNH formation and DPNH utilization respectively, using a Gilford Multisample Recording Spectrophotometer at 25°. Another accurately weighed portion of the liver (100-200 mg) was used for the determination of glycogen (11). Protein(12) was determined both on the whole homogenate and KCl supernatant fluid. The reaction rate was linear with enzyme concentration for rates greater than twice the most rapid measured.

Results and discussion. The results shown in Table I indicate that thyroidectomy caused decreases in liver G-6-Pase, FDPase, and PK activities. The decrease was most marked in G-6-Pase where the activity was reduced to one-third of the normal. FDPase and PK activities were lowered to one-half normal. Thyroxine treatment increased G-6-Pase activity in both the normal and thyroidectomized animals as expected, the relative response was greater in thyroidectomized animals since the base value of the enzyme activity was much lower in these animals. FDPase activity remained unchanged on thyroxine treatment of thyroidectomized animals and even decreased in normal animals injected with thyroxine. This decrease may indicate that under this condition FDPase is not needed to as great an extent as other enzymes. Another report(13) indicates no change in FDPase activity in the liver of rats fed thyroid powder. It is important to point out here that FDPase may become relatively

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TABLE I. Effect of Thyroidectomy and Thyroxine Treatment on Rat Liver Constituents.

	Normal		Thyroidectomized	
	Control	Thyroxine treated	Control	Thyroxine treated
g liver/100 g body wt	4.94± .44†	4.01± .06	3.42± .23	3.78± .14
Soluble protein (mg/100 g body wt)	593 ±17	634 ± 15	399 ±28	588 ± 40
Total protein (mg/100 g body wt)	1353 ±34	1785 ±106	778 ±90	1410 ±161
Glycogen (mg/100 g body wt)	165 ±23	Not measurable	140 ± 5	Not measurable
Enzyme activity*				
Glucose-6-phosphatase	70.6 ± 3	147 ± 4.9	23.3 ± 2.2	100 ± 8.8
Fructose-1,6-diphosphatase	22.6 ± 1	13.7 ± .5	11.7 ± 1	11.9 ± .3
Pyruvate kinase	138 ± 9.4	101 ± 7.2	64.7 ± 6.2	78.4 ± 6.9

* Corrected for endogenous. Expressed in μmoles/min/100 g body wt.
† Mean ± S.E.

more limiting in respect to gluconeogenesis in hyperthyroidism due to an increase in AMP concentration resulting from increased ATPase (14) activity. It has been shown that AMP is a potent inhibitor of FDPase(9) and, in the case of *in vivo* preparations, increasing concentrations of AMP caused decreasing rates of gluconeogenesis(15), which was attributed to inhibition of the FDPase. Thus, the decrease in total FDPase activity accompanied by an increase in AMP concentration would not tend to accelerate gluconeogenesis. The PK activity of thyroidectomized animals injected with thyroxine showed no significant change although G-6-Pase activity increased about 4-fold in these animals.

The increases observed in liver-G-6-Pase activity may be related to the increased synthesis of microsomal protein(16) and possibly an increase in the amount of total microsomes (17) by thyroxine. If one is to extrapolate from many conditions (*i.e.*, high protein or high fructose diet, starvation, diabetes, and hydrocortisone treatment) causing an increase in FDPase activity and decrease in PK activity (with the exception of high fructose diet), it would appear that thyroxine does not cause an increase in gluconeogenesis. This would further be consistent with the observation that thyroxine treatment failed to increase glutamic-pyruvic transaminase(18) activity which increases in many gluconeogenic conditions(19). Many gluconeogenic conditions have been reported to decrease or at least fail to increase 4 enzymes: glucose-6-phosphate dehydrogenase(20,21), 6-phosphogluconate dehydrogenase(20,21), malic enzyme(22), and citric cleavage enzyme(23). All these enzymes(24) have been shown to increase after thyroxine treatment. Thus, it appears on the basis of the activities of these 3 enzymes in the liver that thyroxine probably does not increase gluconeogenesis.

Summary. The activities of 3 liver enzymes: glucose-6-phosphatase, fructose-1,6-diphosphatase, and pyruvate kinase were studied in thyroidectomized and hyperthyroid rats. Thyroidectomy considerably decreased all 3 activities. Thyroxine treatment resulted in an increase of glucose-6-phosphatase activity but caused decrease in fructose-1,6-diphosphatase activity of normal animals and no change in pyruvate kinase activity of thyroidectomized animals. This contradictory picture has been discussed as thyroxine causing no increase in gluconeogenesis. The specific increases in glucose-6-phosphatase activity may be explained by the increased synthesis of microsomal protein on thyroxine treatment.

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Long-Term Synthesis of Antibody *in vitro*.* (31957)

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Antibody synthesis at the cellular level is a relatively rapid process. Yet the full expression of the immune response in terms of participation of different cell types, accumulation and degradation of circulating antibodies, diversity of immunoglobulins and evocation of immunologic memory requires extended observations. All of these aspects can be readily followed in the intact host but heretofore they have not been scrutinized in organ culture. Some authors terminated their observations after 2 or 3 days(1-3). In the work of others the organ culture ceased to function after a relatively short period of time(4-6). Among the various tissues studied, the lymph node has proved to be the most useful organ for demonstrating *in vitro* induced secondary responses, whereas under similar conditions the spleen has been shown to produce antibodies less frequently, and to our knowledge there are no reports of the ability of thymic tissue to form antibodies *in vitro*. The present report describes a method which has permitted long-term antibody production in culture of fragments of lymph nodes and spleen and, in addition, has demonstrated immunologic activity on the part of thymic explants.

Materials and methods. Organ culture. The medium employed consisted of solution 199 (7) supplemented by 1 μg of hydrocortisone succinate. Penicillin (100 units/ml), streptomycin (50 μg/ml) and Fungizone (2 μg/ml) were added. In some experiments this medium was further supplemented with normal rabbit serum in a ratio of 25 parts of serum to 75 parts of medium. The organ cultures were prepared from tissues of rabbits previously immunized with bovine serum albumin (BSA). In most experiments an effort was made to obtain the tissue from animals whose antibodies had declined significantly from the initial high levels. Spleen, popliteal lymph nodes and thymus were diced into fragments measuring no more than 1-2 mm in the longest dimension. These were explanted onto discs

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