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THE REDUCTION OF TRIPHENYLTETRAZOLIUM BY HYPERTROPHIED TISSUES*

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Abstract—Hypertrophy of the myocardium, adrenal gland and thyroid gland was induced in adult male and female albino rats. Tetrazolium reductase levels of the hypertrophied tissues were determined by means of the triphenyltetrazolium technique. Control and experimental organ weights were measured to quantitate increases in tissue mass. No significant change occurred in the endogenous levels of dehydrogenase in cardiac or adrenal gland tissue, when work hypertrophy was induced in those tissues. Increased gland weight or increased gland work in the case of the thyroid gland was found to be associated with increases in tetrazolium reductase levels which were significantly elevated above control levels. In cardiac tissue slices from bilaterally thryroidectomized animals significant reductions in endogenous dehydrogenase levels were observed. The implications of these observations are discussed.

TETRAZOLIUM salts have been employed in recent years as indicators of changes in tissue metabolism under a variety of experimental conditions.[1–3] Although the specific metabolic implications of the reduction of tetrazolium by tissues are partially speculative, the reaction serves as a useful method for the determination of metabolic alterations in tissue slices.[4] The current status of the tetrazolium technique has been well reviewed by Pearse.[5] Triphenyltetrazolium chloride is desirable for use in quantitative studies because of its low toxicity for tissues, the monochromatic character of the formazen resulting from the dye reduction, and a high rate of diffusion into tissues.

This investigation was designed to determine if hypertrophy or hyperplasia of various tissues could be related to metabolic changes within the tissues, as indicated by quantitative alterations in the reduction of triphenyltetrazolium chloride by tissues.

METHODS

Hypertrophy was induced in thyroid, adrenal and cardiac tissues of male and female adult albino rats of the Denver strain by the following methods.

(a) *Thyroid.* Thiouracil was administered in the drinking water as a 0·1 per cent solution for 10 days. A second series of animals was subjected to surgical hemithyroidectomy of the right lobe.

(b) *Adrenal.* A group of animals was subjected to unilateral adrenalectomy, the right adrenal gland being removed.

(c) *Heart.* Animals were injected subcutaneously for 14 days with synthetic l-thyroxin (as synthroid Na) in saline in a dose of 75 μg/rat per day. l-Tri-iodothyronine

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was administered subcutaneously for 14 days to a second group of rats in a dose of 50–75 μg/rat per day. A third series of animals was subjected to bilateral thyroidectomy.

Endogenous dehydrogenase activity of the tissues was measured by the quantitative tetrazolium reduction technique,[6] as modified for use with tissue slices.[7] Triphenyltetrazolium chloride was employed as the indicator in a modified Krebs solution,[4] at a pH of 7·4. This pH was maintained in all determinations because of the possibility of non-specific reduction of tetrazolium at higher pH values, as shown by Seligman.[8] Aerobic incubation of the tissues was carried out for 2 hr at 37 °C with shaking at 60 cycles/min. The amount of dye reduced by the tissue after extraction with acetone, was quantitated by colorimetry at 470 mμ. All values thus obtained were converted to μg of dye reduced per mg of air-dried tissue. Control tissues were inserted in each group of experimental determinations.

All animals were killed by a sharp blow on the head and the appropriate tissue was rapidly removed, sliced and incubated. The experimental periods were as follows: thiouracil administration, 10 days; unilateral thyroidectomy, 7 days; l-thyroxine and l-tri-iodothyronine administration, 14 days; bilateral thyroidectomy, 7 days; unilateral adrenalectomy, 7 days. Heart weights represent the total wet weight of the right and left ventricles and the intraventricular septum. Adrenal and thyroid glands were air-dried after acetone extraction and weighed.

TABLE 1. ORGAN WEIGHTS AND TISSUE DEHYDROGENASE ENZYME ACTIVITY

Organs and tissues	No. animals	Mean weight of organs in mg. *	s.e.	No. of animals	μg of dye reduced/mg dry tissue mean	s.e.
Thyroid						
Control	16	1·57	± 0·08	16	15·3	±0·63
Unilateral thyroidectomy	14	1·70†	± 0·05	14	19·4	±0·74‡
Thiouracil 0·1%	19	5·00	± 0·30‡	19	35·4	±0·94‡
Adrenal						
Control	35	2·33	± 0·09	34	25·0	±0·63
Unilateral adrenalectomy	10	3·93	± 0·25‡	10	24·1	±0·64
Control	59	322·90	± 3·43	18	21·1	±0·55
l-Thyroxine	32	417·00	± 6·80‡	12	21·8	±0·94
l-Tri-iodothyronine	9	478·00	±19·80‡	9	20·0	±0·72
Bilateral thyroidectomy	24	296·00	± 6·30	20	16·5	±0·56‡

* Thyroid and adrenal weights, air-dried; heart weights, wet tissue/100 g body weight.

† Weight of remaining lobe × 2.

‡ Difference is statistically significant, $P < 0·0001$, when compared to control values.

RESULTS AND DISCUSSION

The results of this study are tabulated in Table 1. Although significant increases in both heart and adrenal gland weights were observed, no change occurred in the endogenous levels of the dehydrogenase of the hypertrophied tissues. The enzyme levels observed in the control adrenal glands are in close agreement with those

reported by other workers.[9] These data suggest that the increases in tissue mass which were demonstrated were not accompanied by an increase in the functional metabolic processes related to reductase activity within the cells of adrenal gland or cardiac tissue.

In thyroid glands rendered hyperplastic and hypertrophic by thiouracil administration a significant increase in reductase levels was demonstrated in association with the increase in tissue mass. The lobes of the thyroid gland remaining after hemi-thyroidectomy showed an elevated endogenous dehydrogenase level in the absence of increased tissue weight. Although there was no perceptible increase in the reductase level in cardiac tissue from animals exposed to relatively large amounts of l-thyroxine or l-tri-iodothyronine, the relative lack of endogenous thyroxine in the bilaterally thyroidectomized animals was associated with a reduced tissue level of dehydrogenase in the myocardium.

The question of primary interest raised by the results of this study concerns the lack of response of the reductase level to hypertrophy of the cardiac muscle and the adrenal gland tissue, as contrasted to the large elevation of tetrazolium reduction observed in the enlarged thyroid and in thyroid tissue after hemi-thyroidectomy. It is possible that the metabolic response to increased tissue mass in the heart and the adrenal gland involved enzyme systems which are not DPN- or TPN-dependent reductases. Cascarano and Zweifach[10] have shown that tetrazolium reduction in tissues slices is related to substrate formation by DPN- and TPN-dependent dehydrogenases. It would follow, then, that metabolic changes occurring in hypertrophied or hyperplastic cells and mediated by enzymes other than such dehydrogenases, would not be reflected in changes in the levels of tetrazolium reductase. This fact has been demonstrated in the case of the adrenal glands by histochemical methods.[11] Increases in endogenous dehydrogenase were recorded in both enlarged thyroid glands and in non-enlarged thyroid gland lobes remaining 7 days following hemi-thyroidectomy. Therefore, hypertrophy alone is not a primary casual factor in the elevation of reductase levels in thyroid tissue. Under the conditions which prevailed in these experiments it is likely that increased functional activity in thyroid cells, as opposed to similar activity in cardiac or adrenal cells, is the initiating factor for the observed effect on reductase activity which is specific for thyroid tissue. It is apparent that under these circumstances stimulation of thyroid tissue by thyrotropin may be the operative mechanism in the elevation of certain endogenous dehydrogenases. It has been shown that chronic administration of l-thyroxine with a concomitant reduction of thyrotropic stimulation results in decreased reductase levels in thyroid tissues.[12] It is possible that stimulation of thyroid gland cells by thyrotropin causes an increase in oxidative potential within the cells which is reflected by equivalent increases in DPN- or TPN-dependent dehydrogenases.

The significant decrease in reductase levels which occurred in cardiac tissue of thyroidectomized animals indicates simply that physiological amounts of thyroid hormone are required for maintenance of normal reductase levels in the myocardium. Relatively large amounts of circulating amounts of thyroid hormones did not increase dehydrogenase levels in cardiac tissue. An explanation for this finding may lie in the reports of Barker[13] and Laursen[14] which show that endogenous dehydrogenase activity, as measured by tetrazolium reduction, represents only from 3 to 5 per cent of the total metabolic activity of tissues expressed as oxygen consumption.

The data herein presented support the conclusion that alterations in cellular reductase levels in thyroid tissue are associated with increases in specific cellular function. Hypertrophy *per se* does not initiate changes in the activity of dehydrogenases dependent upon DPN or TPN in cardiac or adrenal gland tissue. The relationship between thyrotropic stimulation of the thyroid gland and endogenous tetrazolium reductase activity in thyroid cells represents an interesting problem for future study.

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