Metabolism of l-Thyroxine and l-3:5:3′-Triiodothyronine by Homogenates of Rat Skeletal Muscle.* (23222)

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It has been shown recently(1) that tissue slices and homogenates of rat brain can deiodinate and possibly deaminate l-thyroxine and l-3:5:3′-triiodothyronine. Comparative studies using rat liver and kidney proved technically difficult because incubation of thyroxine and triiodothyronine with either slices or homogenates of these 2 tissues produced between 4 and 7 different iodinated metabolites. Most of these products were not formed by brain preparations, in which the iodinated metabolites were mainly inorganic iodide and what appeared to be the deaminated analogues of thyroxine and triiodothyronine. Subsequently it was found that the metabolites produced from homogenates of muscle resembled, to some extent, those produced by brain homogenates. These observations are described in the present report.

Methods. About 2-3 g of skeletal muscle were removed from each hind leg of unanaesthetized, decapitated male or female rats (75-150 g). Muscle was rapidly chilled in ice, trimmed and cut into fragments of approximately 100 mg. The tissue was then homogenized in a Waring blendor run at 65-75 V for 3 minutes.‡ For incubation studies a concentration of 1 g of muscle per 15 ml of suspension medium was used. Radioactive (I^{131}) l-thyroxine§ and l-3:5:3′-triiodothyronine§ were used for incubation with homogenates. Incubation time ranged from 15 minutes to 6 hours. Substrate levels between 10^{-3}M and 2 x 10^{-9}M were obtained by addition of non-radioactive thyroxine and triiodothyronine when necessary. After incubation each sample was chromatographed directly on paper to determine the amount of I^{131} remaining as unchanged substrate, as well as the amount of each of the metabolites. Comparative studies were simultaneously done on brain homogenates from the same animals. Analytical procedures, the composition of the suspension medium, conditions of incubation and control experiments were the same as those previously reported for rat cerebral tissue(1).

Results. Since anaerobic conditions failed to show any significant difference, only results of incubation under aerobic conditions are reported. The rates of disappearance of thyroxine and triiodothyronine at different substrate levels are shown in Fig. 1. The data here have been expressed as percentage of the total homogenate radioiodine remaining after incubation as either thyroxine or triiodothyronine. Simultaneous studies with brain homogenates were similar to those reported previously(1) and are not repeated here.

The disappearance of radioactive thyroxine and triiodothyronine from muscle homogenates was most rapid during the first 2 hours of incubation. At roughly the same substrate levels rates of disappearance were substantially faster with muscle homogenates than those obtained with homogenates of brain per g of tissue. For example, brain suspension was almost inactive on thyroxine and triiodothyronine at a concentration of about 10^{-6} M, while similar muscle suspension (1 g tissue/15 ml medium) metabolized approximately 60% of thyroxine and 35% of the triiodothyronine within 3 hours of incubation. This difference might indicate a higher concentration, in muscle, of the enzyme(s) involved in the metabolism of the 2 hormones. In both muscle and brain homogenates thyroxine was degraded more rapidly than tri-

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‡ Most of the connective tissue which collects around the blades of the blendor was removed after one min. Homogenization was then continued for an additional 2 min.

§ Obtained from Abbott Laboratories, Oak Ridge, Tenn.

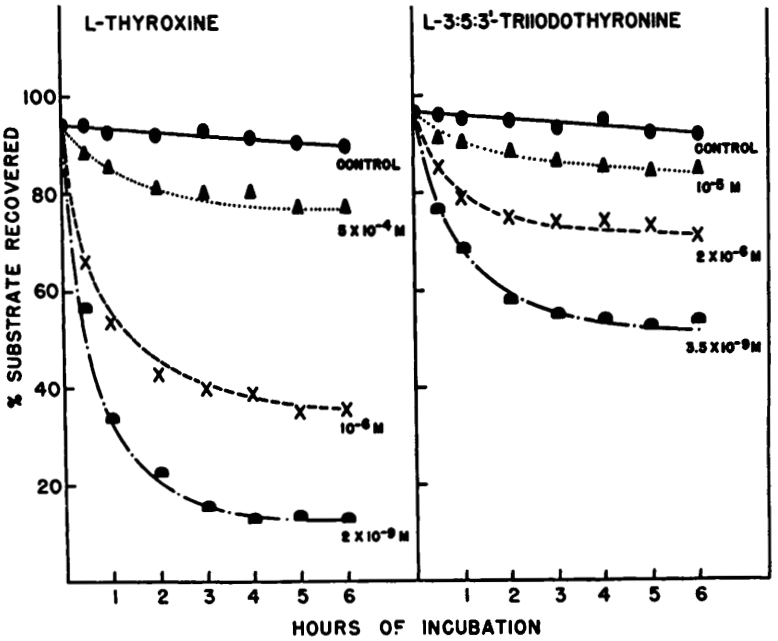

FIG. 1. Rates of degradation of I^{131}-labelled l-thyroxine and l-3:5:3′-triiodothyronine incubated with rat muscle homogenate at different substrate concentrations. Values given represent percentage of total I^{131} in homogenates found as unchanged substrate at different time intervals. c = control incubation with boiled tissue.

iodothyronine. An explanation for this difference is not apparent.

Chromatographic analysis of the iodinated products obtained after incubation of labelled thyroxine and triiodothyronine with muscle and brain homogenates is shown in Table I.

The major I^{131}-labelled metabolite in all cases‖ was inorganic iodide. In the case of muscle homogenates, inorganic iodide appeared to be the main labelled metabolite from thyroxine or triiodothyronine since, in all studies, at least 90% of the I^{131} of the product formed was found in this fraction. As much as 10-15% of the total I^{131} was found as their acetic and/or propionic acid analogues (it was not possible to distinguish chromatographically between the acetic and propionic acid analogues of the 2 hormones) within 1-2 hours of incubation with brain homogenates. Using muscle homogenates, however, it was not possible to obtain sufficient radioactivity to elute and re-chromatograph any of the non-iodide fractions of the iodinated metabolites. 3:5:3′-Triiodothyroacetic acid, identified in muscle of thyroidectomized rats injected with L-T_3 by Roche and co-workers(2) was not found to be present as a metabolite of incubation in this work.

Both muscle and brain homogenates were inactivated by heating at 100°C for 20 minutes. Treatment of brain preparations with 10^{-3} M $HgCl_2$ blocked all deiodination but did not interfere with conversion of thyroxine and triiodothyronine to other non-deiodinated products. In muscle preparation 10^{-3} M $HgCl_2$ blocked all deiodination but, as may be seen in Table I, since the non-deiodinated products represented such a small fraction of the total I^{131} it was difficult to be certain of any effect on conversion of thyroxine and triiodothyronine to other products.

The process of homogenization of the tissue is not responsible for deiodination or lack of deamination of l-thyroxine and l-3:5:3′-triiodothyronine since no significant qualitative difference was obtained by incubation with skeletal muscle fragments.

‖ In considering the qualitative analysis of radioiodinated metabolites, it should be emphasized that the iodine atoms in molecules of thyroxine and triiodothyronine used for this work were not uniformly labelled.

TABLE I. Breakdown of I^{131} Labelled l-Thyroxine (T_4) and l-3:5:3′-Triiodothyronine (T_3) by Rat Brain and Muscle Homogenates (1 g Tissue/15 ml Medium).

Tissue	Hr incubation	l-Thyroxine, 2×10^{-9}M: % total I^{131} distributed as* T_4	I^-	Or.	Others†	l-3:5:3′-Triiodothyronine, 3.5×10^{-9}M: % total I^{131} distributed as* T_3	I^-	Or.	Others†
Muscle	.5	57.1	40.4	1.3	+	76.6	22.7	+	1.4
	1	34.5	63.5		2.0	69.0	29.0	2.0	
	3	17.3	79.1	3.6		56.6	32.4		1.0
	6	12.6	84.2	2.5	+	53.0	35.5	1.5	
Boiled muscle	6	86.1	12.0	+	+	92.4	6.8	+	
Brain	1	68.7	15.9	+	14.8	80.1	9.2	+	10.3
	3	60.4	27.0	3.8	8.8	69.3	23.4	2.7	4.6
	6	51.0	33.2	3.8	2.0	64.8	34.2	1.0	
Boiled brain	6	89.8	10.2			90.6	8.3	+	

* + = Less than 1%; Or. = Fraction of I^{131} immobile in chromatography.
† In case of the brain this group includes acetic acid and/or propionic acid analogues of T_4 and T_3.

Summary. From the present study it can be concluded that brain and muscle have certain similarities as well as differences in the metabolism of thyroxine and triiodothyronine. The deiodinating enzyme is present in both the tissues but the deaminating activity found in brain preparations is virtually absent in skeletal muscle. The nature of the enzymes as well as other factors involved in the metabolism of these hormones require further clarification and are being studied.

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Inhibitory Effects of Cortisone Acetate and Hydrocortisone on Growth of Fibroblasts.* (23223)

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Diverse evidence has been presented by several investigators concerning the direct action of cortisone acetate and 17-hydroxycorticosterone on growth of fibroblasts *in vitro*. The technics have differed as to methods of culture and application of the test agents(1-7).

The purpose of the present work was to study the effect of cortisone and hydrocortisone on a single cell system. The results indicate that addition of these steroids to cultures of actively proliferating fibroblastic cells inhibits their growth and induces development of abnormal cytologic changes.

Materials and methods. The steroids, cortisone acetate and hydrocortisone (free alcohol) were supplied through the courtesy of Dr. Frederick Heath, Merck & Co. Cortisone was diluted in aqueous vehicle to contain 5 mg ml and further diluted to desired strength with Earle's balanced salt solution (8) prior to combining with the culture media. Hydrocortisone was prepared according to the method of Grossfeld and Ragan(3). The original vehicle was removed from this

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