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Thyroid hormones control lysosomal enzyme activities in liver and skeletal muscle*

(intracellular proteases/protein turnover/thyroxine/triiodothyronine/hyperthyroidism and hypothyroidism)

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ABSTRACT **Because protein degradation in liver and skeletal muscle is increased by thyroid hormones and decreased by thyroidectomy, we investigated the influence of thyroid hormones on the level of lysosomal enzymes. Hypophysectomized rats received daily injections of L-thyroxine or L-triiodothyronine. After 3 days of this regimen, homogenates of liver and skeletal muscle showed a 2- to 3-fold increase in the activities of cathepsin D, cathepsin B, and other lysosomal enzymes including leucine aminopeptidase, acid phosphatase, β-galactosidase, *N*-acetylglucosaminidase, and α-mannosidase. In liver, this effect reflected increased enzyme activity in the two subcellular fractions that normally contain lysosomes. Titration of cathepsin D with pepstatin indicated that the increase in this activity resulted from an increase in the number of enzyme molecules. These effects occurred with both pharmacologic (thyrotoxic) and physiologic (growth-promoting) doses of thyroid hormones. Liver and skeletal muscle from thyroidectomized rats had approximately 50% of the normal levels of lysosomal enzyme activities. Under these various conditions, heart and kidney, tissues in which protein degradation does not appear to be influenced by thyroid hormones, showed no significant changes in lysosomal hydrolases. Thus, thyroid hormones regulate proteolytic and other lysosomal enzyme activities in those tissues in which these hormones influence protein degradation. Many characteristic features of hyperthyroidism and hypothyroidism may result from changes in levels of lysosomal enzymes.**

In mammalian cells, several hormones are known to regulate the average rates of protein degradation (1, 2). For example, in liver, insulin decreases overall proteolysis (3, 4) whereas glucagon stimulates this process (5, 6), and these effects are important in regulating protein content of this organ (2). Recent studies (7, 8) in this laboratory demonstrated that thyroid hormones also have important effects on average rates of protein degradation in addition to other known effects in promoting protein synthesis. Thyroidectomy or hypophysectomy, either of which greatly diminishes thyroid hormone production, decreased protein degradation in skeletal muscles of rats. Physiologic doses of thyroxine (T4) or triiodothyronine (T3) induced muscle growth and increased both protein synthesis and protein degradation. Higher doses of these hormones increased protein degradation further but did not change protein synthesis rates beyond those observed with growth-promoting doses. Thus, the severe muscle wasting observed in hyperthyroidism appears to result from increased protein degradation. In addition, thyroid hormones were found to increase protein degradation in liver but not in heart or kidney (7). The present studies were undertaken to clarify the mechanism by which thyroid hormones influence protein catabolism.

Mammalian tissues contain both lysosomal and nonlysosomal proteases (2). Although certain proteins (e.g., proteins with abnormal conformations) appear to be degraded by a nonlysosomal mechanism (9, 10), there is appreciable evidence that lysosomes also play an important role in intracellular protein degradation (2). Glucagon, for example, increases protein degradation, probably by promoting autophagocytosis, a process that involves the fusion of primary lysosomes with membrane-bounded cellular material (11, 12). The resulting lysosomes (autophagosomes), which seem to contain substrates for degradation, are denser and sediment more rapidly than do primary lysosomes (13). Thus, glucagon causes a redistribution of lysosomal hydrolases to a denser subcellular fraction but does not affect the total amounts of lysosomal enzymes. A lack of insulin or amino acids leads to increased proteolysis, probably by similar mechanisms (14).

We have investigated whether the effects of T4 and T3 on protein degradation are associated with changes in the overall levels of lysosomal proteases or in their subcellular distribution. The content of various lysosomal hydrolases was investigated in liver and skeletal muscle as well as in heart and kidney, in which T3 and T4 do not affect protein degradation.

MATERIALS AND METHODS

Animals and Hormone Treatments. Normal, hypophysectomized, and thyroidectomized CD male rats were obtained from Charles River Breeding Laboratory and maintained on Purina Rat Chow and tap water. The animals weighed 60–70 g at the time of surgery and were not used until at least 8 weeks later, by which time the hypophysectomized rats had grown to 80–100 g and the thyroidectomized rats, to 130–140 g. T4 or T3 (Sigma Chemical Co.) was dissolved in 50 mM NaOH containing 0.8% NaCl and injected subcutaneously. Control animals received alkaline saline.

Preparation of Tissue Homogenates. Animals were killed by cervical dislocation. The liver was gently perfused with ice-cold 0.15 M KCl through the portal vein. The liver was then rinsed with ice-cold 0.25 M sucrose, weighed, and minced. All subsequent procedures were carried out at 0–4°. A 20% (wt/vol) homogenate was prepared in 0.25 M sucrose (containing 1 mM EDTA adjusted to pH 7.0) with a Dounce-type homogenizer. The homogenates of several livers were combined, filtered through four layers of gauze, and subjected to the following fractionation scheme.

The crude liver homogenate was centrifuged at 600 × *g* for 10 min. A portion of the supernatant (the postnuclear homogenate) was saved and the remainder was centrifuged at 3300 × *g* for 10 min. The pellet (the heavy mitochondrial fraction) was saved and the supernatant was centrifuged at 16,300 × *g* for 20 min. The resulting pellet (the light mitochondrial frac-

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Abbreviations: T4, L-thyroxine; T3, L-triiodothyronine.

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Table 1. Effect of T3 administration on lysosomal enzyme activities in liver homogenates from hypophysectomized rats

Enzyme	Units/g liver		T3, %	Units/mg protein × 10^3		T3, %
	Control	T3	control	Control	T3	control
Cathepsin D	12.8	27.1	212	50	145	290
Cathepsin B	6.8	17.9	263	27	97	359
Leucine amino-peptidase	0.60	1.17	194	2.48	5.04	203
Acid phosphatase	3.60	6.47	179	14.1	29.6	210
β-Galactosidase	0.070	0.128	183	0.273	0.696	255
N-Acetylglucos-aminidase	1.80	3.42	190	7.39	16.9	229
α-Mannosidase	3.42	6.72	196	2.32	5.60	241

Four hypophysectomized rats (100 g) were treated for 6 days with T3 (200 μg/day). Four others received alkaline saline and served as controls. The animals were killed 24 hr after the final injection. Similar results were obtained in five additional experiments. Cathepsin B was assayed with hemoglobin as substrate.

Table 2. Titration of liver cathepsin D activity from control and T3-treated rats with pepstatin

	Enzyme activity		Conc. of pepstatin causing 50% inhibition	
	Units × 10^{-4}/ml	% control	mM	% control
Untreated control	7	100	1.43	100
T3-treated				
2 μg/100 g/day	14	200	3.03	212
200 μg/100 g/day	20	296	4.47	313

Hypophysectomized rats were treated with either dosage of T3 or alkaline saline for 6 days. Postnuclear homogenates were prepared from livers of each group and assayed for cathepsin D activity. For each group, increasing concentrations of pepstatin were added to a constant amount of enzyme preparation.

tion) and the supernatant were saved. The pellets were resuspended in 50 mM acetic acid/sodium acetate buffer, pH 5.0. All isolated fractions were homogenized with a motor-driven glass Duall homogenizer and subjected to one freeze-thaw cycle. Preliminary experiments showed that this method was as effective as Triton X-100 treatment in releasing latent lysosomal enzyme activities.

Heart, kidney, and mixed leg muscles (soleus, extensor digitorum longus, and gastrocnemius) were also removed, weighed, and homogenized (10%, wt/vol) in 0.15 M KCl using the Duall homogenizer. The resulting homogenates were passed through four layers of gauze and subjected to two freeze-thaw cycles.

Enzyme Assays. Cathepsin D (EC 3.4.23.5) was estimated by a modification of the protease assay described by Kowit *et al.* (15). Hemoglobin (Sigma bovine type II) was used as a substrate and the reaction was carried out at pH4.0 in a sodium acetate/acetic acid buffer (0.10 M). Production of fluorescamine-reactive material was linear for 2–3 hr and proportional to the amount of extract. In cardiac and skeletal muscle, which have much less protease activity than does liver or kidney, autolysis was used to estimate proteolytic activity. These tissues were homogenized as described above and dialyzed for 12 hr against 0.15 M KCl. In a typical assay, 4.5 ml of the dialyzed homogenate was added to 0.5 ml of 1.0 M sodium acetate/acetic acid buffer, pH 4.0. Measurements were carried out as described (15). Autolytic activity was linear for several hours.

Cathepsin B (EC 3.4.22.1) was estimated in two ways. (*i*) The hydrolysis of hemoglobin was assayed as described above but in the presence of 0.5 μM pepstatin, an inhibitor of cathepsin D. Addition of sulfhydryl activating agents (16) was not necessary in this assay because of the high protein concentrations. (*ii*) The hydrolysis of α-*N*-benzoylarginine-β-naphthylamide was used with a modification of the assay of Doebber and Miller (17). A unit of activity is defined as 1 μmol of β-naphthylamine formed per min.

Leucine aminopeptidase [α-aminoacyl-peptide hydrolase (cyterol), EC 3.4.11.1] was assayed with leucyl-β-naphthylamide as described by McDonald *et al.* (18), and β-naphthylamine was measured as described above. Activities of other lysosomal enzymes including acid phosphatase (EC 3.1.3.2), β-galactosidase (EC 3.2.1.23), *N*-acetylglucosaminidase (EC 3.2.1.50), and α-mannosidase (EC 3.2.1.24) were measured as described by Barrett (16). A unit of activity is defined as 1 μmol of product formed per min. Protein was measured on acid-precipitated samples by the biuret method with crystalline bovine serum albumin as a standard (19).

RESULTS

To determine the effect of thyroid hormones on liver content of lysosomal enzymes, hypophysectomized rats were injected with either T3 (200 μg/100 g body weight) or alkaline saline for 6 days. The activities of various proteases and other lysosomal enzymes were increased 2- to 3-fold in the homogenates of livers of treated animals (Table 1). Similar effects were obtained in analogous experiments when the same concentration of T4 was administered (data not shown). These increases cannot be attributed to changes in nonprotein liver mass or to a selective decrease of nonlysosomal enzyme proteins because these effects were evident whether the results were expressed as activity/per g of liver or as activity per mg of protein. Additional experiments indicated a similar increase in lysosomal proteases of hepatocytes isolated from T3-treated rats (unpublished data).

An increase in enzyme activity can result from an increase in the number of enzyme molecules or from a change in their activity. Experiments were therefore carried out to test whether the number of molecules of cathepsin D increases upon treatment with T3. Pepstatin, a potent inhibitor of cathepsin D (20), binds in a 1:1 molar ratio to the active site of this enzyme (21). This agent was used to titrate cathepsin D activity in liver homogenates from control and T3-treated animals (Table 2). In homogenates from T3-treated animals, which had 3 times more enzymatic activity than did controls, approximately 3 times more pepstatin was required to inhibit 50% of the cathepsin D activity. In additional experiments in which thyroid hormone treatment caused a 2-fold increase in activity, approximately 2 times more pepstatin was necessary for 50% inhibition. These data indicate that T3 increases cathepsin D activity by increasing the number of active enzyme molecules.

Most physiologic effects of thyroid hormones are not evident for several days after their administration to hypothyroid animals (22). Fig. 1 shows that, 24 hr after the first injection of T3, little or no change was evident in lysosomal enzyme activities. However, by 72 hr, the lysosomal enzyme activities reached maximal levels.

Upon differential centrifugation of tissue homogenates, lysosomes sediment at similar rates as mitochondria. Table 3 shows that lysosomal enzyme activities increased in both the heavy mitochondrial and light mitochondrial cell fractions.

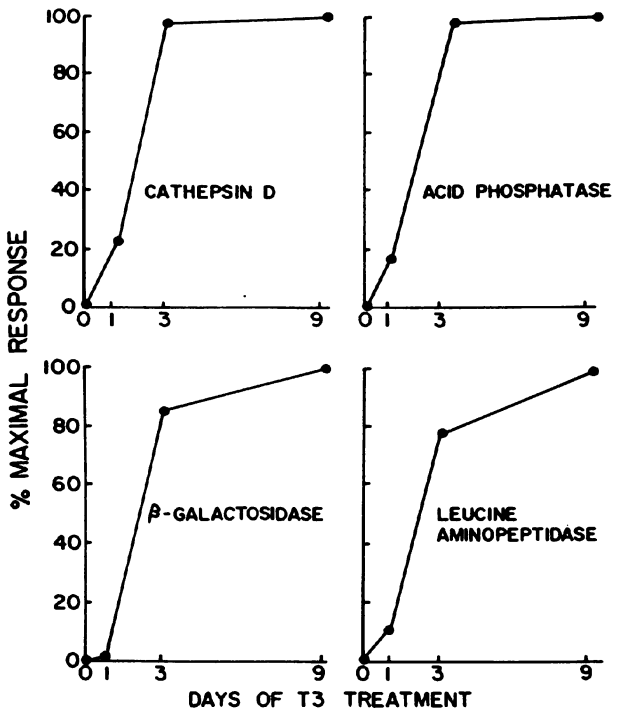

FIG. 1. Hypophysectomized rats (100 g) were divided into groups of four. The animals received daily injections of T3 (200 μg per day) for 0, 1, 3, or 9 days prior to sacrifice. Animals not receiving T3 injections received alkaline saline injections, and all were sacrificed on the same day. Lysosomal enzymes were assayed in the postnuclear homogenate, and the activities from the animals treated with T3 for 9 days were taken as "the maximal response" (100%).

Because normally these two subcellular fractions accounted for 70–90% of the total activity of a lysosomal enzyme, thyroid hormones increased the total cellular content of these enzymes but did not change their relative subcellular distribution.

After 6–9 days of treatment with these large doses of thyroid hormones, the rats appeared hyperthyroid; they were obviously more active and lost approximately 10% of their initial body and liver weight. Similar increases in lysosomal enzyme activities also occurred when physiologic doses of T3 (2 μg/100 g body wt per day) were injected into hypophysectomized rats (Table 4). This dose caused a small but significant increase in body weight and should be equivalent to the amount normally produced by rats of this size (23).

To investigate further whether thyroid hormones normally influence the levels of lysosomal enzymes, livers from normal and thyroidectomized rats were compared. The proteolytic and other lysosomal enzyme activities decreased approximately 50% in thyroidectomized animals. These changes resulted from alterations in the heavy mitochondrial and light mitochondrial cell fractions (Table 5), as was observed in experiments on T3 treatment (Table 3). Thus, thyroid hormones play a physiologic role in the regulation of overall lysosomal enzyme content in liver.

Lysosomal enzyme activities were also measured in skeletal muscle, kidney, and heart in analogous experiments. In skeletal muscle, as in liver, lysosomal enzyme activities increased after administration of either physiologic or thyrotoxic doses of T3 to hypophysectomized rats (Table 6). However, these same treatments did not significantly affect lysosomal enzyme activities in either kidney or heart (Table 7). On the other hand, in skeletal muscles from thyroidectomized rats these enzyme levels were reduced to approximately 50% of normal levels. Thyroidectomy had no effect on these activities in kidney but reduced them slightly (20%–30%) in heart.

Table 3. Effect of T3 administration to hypophysectomized rats on lysosomal enzyme activities in liver cell fractions

Enzyme	Cell fraction	Units/mg protein × 10^3 Control	T3	T3, % control
Cathepsin D	HM	66	478	724
	LM	131	402	307
	S	16	49	306
Cathepsin B	HM	2.8	14.3	507
	LM	5.7	14.4	249
	S	—	—	—
Leucine aminopeptidase	HM	3.21	13.3	414
	LM	5.41	12.3	227
	S	1.04	1.52	146
Acid phosphatase	HM	12.7	59.0	465
	LM	24.5	65.3	267
	S	11.8	19.4	164
β-Galactosidase	HM	1.9	5.1	268
	LM	2.0	5.2	260
	S	—	—	—
N-Acetylglucos-aminidase	HM	25.1	44.7	178
	LM	36.0	70.3	195
	S	3.6	6.8	188
α-Mannosidase	HM	7.2	16.0	222
	LM	12.0	27.5	224
	S	1.0	1.3	130

Animals were treated as described in Table 1. Heavy mitochondrial (HM), light mitochondrial (LM), and supernatant (S) cell fractions were prepared as described in *Materials and Methods*. Similar results were obtained in four additional experiments and when results were expressed as units per g of liver. For the enzymes studied, fraction HM contained 25%–35% and fraction LM contained 40%–60% of the total activities in the postnuclear homogenate. Cathepsin B was assayed with the synthetic substrate.

DISCUSSION

These studies have demonstrated changes in the activities of the lysosomal proteases cathepsin D and cathepsin B under conditions such that thyroid hormones alter protein degradation (7, 8). The increase in cathepsin D activity appeared to result from an increased number of enzyme molecules (Table 4). Because there is increasing evidence that lysosomal proteases participate in intracellular protein degradation, it is attractive to suggest that T3 and T4 regulate protein degradation by altering levels of these enzymes, although direct evidence for this conclusion is lacking.

These hormones thus appear to regulate protein degradation by a different mechanism than that of glucagon or insulin. Glucagon seems to increase protein degradation in liver by inducing autophagocytosis (11, 12). This process involves the subcellular redistribution of lysosomal enzymes from "substrate-poor" primary lysosomes (isolated primarily in the light mitochondrial cell fraction) to "substrate-rich" autophagic vacuoles (isolated primarily in the heavy mitochondrial cell fraction). Insulin, which inhibits overall protein degradation, can prevent such alterations in lysosomal enzyme distribution (14). By contrast, thyroid hormones or thyroidectomy altered the total activity of these enzymes in liver without affecting their distribution in different cell fractions (Table 3).

The increased lysosomal enzyme activity in the heavy mitochondrial fraction may indicate that autophagocytosis is indeed promoted by thyroid hormones. Studies with glucagon

Table 4. Comparison of effects of anabolic and catabolic doses of T3 on lysosomal enzyme activities in rat liver cell fractions

Enzyme	Cell fraction	Units/mg protein × 10^3 Untreated control	Anabolic dose	Catabolic dose
Cathepsin D	PNH	104	236	306
	HM	266	803	1210
	LM	333	1420	1250
Cathepsin B	PNH	1.8	3.2	4.4
	HM	4.6	6.5	8.7
	LM	6.9	11.7	13.5
Acid phosphatase	PNH	8.6	16.0	20.5
	HM	12.1	19.9	22.0
	LM	19.5	41.3	42.3
N-Acetylglucosaminidase	PNH	10.6	23.2	26.5
	HM	25.2	43.0	48.6
	LM	38.0	66.7	72.3

Hypophysectomized rats (100 g) were divided into groups of five. The control group received daily injections of alkaline saline for 6 days. Two other groups received daily injections of 2 μg of T3 per day (anabolic dose) or 200 μg of T3 per day (catabolic dose) for 6 days. The anabolic dose caused the animals to increase their body weight by 5%. The catabolic dose caused the animals to lose 10% of initial body weight. For each enzyme, the total activity recovered in heavy mitochondrial (HM) and light mitochondrial (LM) fractions together accounted for 70%–90% of the total activity in the postnuclear homogenate (PNH). Similar results were obtained when the data were expressed as units per g of liver. Cathepsin B was assayed with synthetic substrate.

and insulin suggest that the rate of protein degradation correlates closely with protease activity in autophagosomes (11–13). By increasing protease levels in primary lysosomes, which are the source of hydrolases used for autophagocytosis, T3 and T4 may also ensure that autophagosome information can be increased for prolonged periods (in contrast to the short-term effects of glucagon).

Table 5. Effect of thyroidectomy on lysosomal enzyme activities in liver cell fractions

Enzyme	Cell fraction	Units/mg protein × 10^3 Control	TDX	TDX, % control
Cathepsin D	PNH	200	80	40
	HM	550	154	28
	LM	1270	490	39
Cathepsin B	PNH	2.3	1.5	65
	HM	5.1	2.5	49
	LM	14.8	9.3	62
Acid phosphatase	PNH	16.2	11.1	68
	HM	23.3	11.3	69
	LM	57.7	29.8	52
N-Acetylglucosaminidase	PNH	30.7	18.3	60
	HM	54.3	25.3	46
	LM	128	68.7	53

Liver cell fractions (see Table 4) were prepared from control and thyroidectomized (TDX) rats of the same weights (130–140 g). Similar effects were observed in four additional experiments and for the enzymes leucine aminopeptidase, β-galactosidase, and α-mannosidase (data not shown). Also, similar effects of thyroidectomy were obtained when the data were expressed as units per g of liver. The total activities recovered in fractions HM and LM together accounted for 60%–90% of the total activity in the PNH. Cathepsin B was assayed with synthetic substrate.

Table 6. Effect of thyroid hormones on lysosomal enzyme activities in skeletal muscle

Enzyme	T3 Administration* Hypophysectomized control	Anabolic dose	Catabolic dose	Thydoidectomy† Normal	TDX
Cathepsin D	2.47	3.36	3.60	3.44	1.06
Cathepsin B	0.98	1.77	1.97	1.49	0.42
Acid phosphatase	2.63	3.17	4.38	1.8	1.2
N-Acetylglucosaminidase	0.49	0.73	0.95	2.9	1.1

Data shown as units/mg of protein × 10^3. Similar results were observed in four additional experiments and when data were expressed as units per g of muscle.
* Hypophysectomized rats were treated as described in Table 4.
† Normal and thyroidectomized (TDX) rats of the same weight (140–150 g) were used.

The time course of the effects of thyroid hormones on protein degradation (7) and lysosomal enzyme activities (Fig. 1) are also significantly different that that of glucagon and insulin. Glucagon and insulin exert their effects within 1 hr whereas the effects of thyroid hormones were not evident for 2–3 days. A lag of several days may reflect the time required for transcription of new mRNA species, synthesis of lysosomal enzymes, or formation of new lysosomes. Also, lysosomal enzymes have relatively long half-lives (24) and thus changes in their levels should be relatively slow. A similar lag precedes various other effects of thyroid hormones including the increased activity of several mitochondrial enzymes in liver [e.g., α-glycerophosphate dehydrogenase, citrate synthetase, and cytochrome *c* (25)].

Changes in lysosomal enzyme activities were observed in liver and skeletal muscle in response to thyroid hormones which also affect protein degradation in these tissues (7). By contrast, thyroid hormones did not significantly affect protein degradation (7) or lysosomal enzyme activities in the heart or kidney (Table 7).

The nonproteolytic lysosomal enzymes, including acid

Table 7. Lack of effect of T4 administration or thyroidectomy on lysosomal enzyme activities in heart and kidney

Tissue	Enzyme	T4 administration* Control	T4	Thyroidectomy† Normal	TDX
Kidney	Cathepsin D	11.0	10.6	5.60	7.20
	Cathepsin B	NT	NT	5.72	6.61
	Acid phosphatase	15.3	12.3	17.4	18.9
	Leucine aminopeptidase	16.8	16.3	NT	NT
	N-Acetylglucosaminidase	29.2	30.1	10.2	10.5
	α-Mannosidase	NT	NT	11.6	11.3
Heart	Cathepsin D	1.55	1.60	0.70	0.49
	Acid phosphatase	1.90	2.06	2.75	1.80
	N-Acetylglucosaminidase	2.37	2.29	3.53	2.77

Data shown as units/mg of protein × 10^3. Similar results were seen in two additional experiments and when expressed as units per g of tissue. NT, not tested.
* Animals were treated as described in Table 1 but with T4 instead of T3. Controls were hypophysectomized.
† Normal and thyroidectomized (TDX) rats of the same weight (140–150 g) were used.

phosphatase, β-galactosidase, *N*-acetylglucosaminidase, and α-mannosidase, increased and decreased in parallel with the proteases in liver and skeletal muscle. Parallel changes in the content of all these enzymes may result from alterations in either the number of lysosomes per cell or the number of enzymes per lysosome. Coordinate regulation of a wide variety of lysosomal enzymes may ensure that the carbohydrate and lipid portions of glycoproteins and lipoproteins are degraded together with the polypeptides. Furthermore, because T3 and T4 also control levels of lysosomal RNase, DNase, and acid lipases (unpublished observations), the turnover of other nonprotein cellular constituents may also be regulated by thyroid hormones. Concomitant increases in the activities of various lysosomal enzymes have been reported previously under other conditions. For example, addition of increasing concentrations of serum to cultured mononuclear phagocytes increased the levels of cathepsin D, acid phosphatase, and β-glucuronidase (26). In addition, several lysosomal enzymes appear to increase in muscles of dystrophic animals (27, 28) (although invasion by foreign cells may contribute to these effects).

It seems unlikely that the effects described here with thyroid hormones can be accounted for by invasion of liver or muscle by cells rich in lysosomes. In related studies we found that isolated hepatocytes from T3-treated rats contained 2- to 3-fold greater lysosomal hydrolase activities than did hepatocytes from hypothyroid rats. In addition, morphologic studies have not demonstrated invasion of muscle by leukocytes during hyperthyroidism (29). Weissman and Siegal (30) reported increased levels of β-glucuronidase in serum of hyperthyroid patients. It is very unlikely that the present findings can be accounted for by contamination of tissues with serum lysosomal enzymes. The liver, for example, was initially perfused to remove blood prior to homogenization, and heart and kidney, which are more likely to be contaminated with blood, did not show the effects seen in skeletal muscle or liver. The origin of serum β-glucuronidase is unknown but may result from leakage from liver or other tissues. Thus, the present results may explain the earlier observations (30).

In addition to effects on growth and protein degradation, many other features of thyroid disease may result from altered lysosomal enzyme activities. For example, hypothyroidism is characterized by the accumulation of extracellular hyaluronic acid (31), perhaps as a consequence of decreased levels of the lysosomal enzyme hyaluronidase. Similarly, the elevated concentrations of plasma cholesterol in hypothyroid patients (32) may result from decreased levels of cholesterol esterase, the rate-limiting lysosomal enzyme for the breakdown of cholesterol esters (33–35). Thyroid hormone treatments return plasma levels of cholesterol to normal (22), perhaps by increasing levels of this enzyme. Indeed, preliminary results indicate that thyroid hormones influence the levels of cholesterol esterase and hyaluronidase in the same fashion as they affect other lysosomal enzymes.

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1. Goldberg, A. L., Howell, E. M., Li, J. B., Martel, S. B. & Prouty, W. F. (1974) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 1112–1120.
2. Goldberg, A. L. & St. John, A. C. (1976) *Annu. Rev. Biochem.* **45**, 747–803.
3. Miller, L. L. (1965) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **24**, 737–744.
4. Mortimore, G. E. & Mondon, C. E. (1970) *J. Biol. Chem.* **245**, 2375–2383.
5. Miller, L. L. (1960) *Nature* **185**, 248.
6. Woodside, K. H., Ward, W. F. & Mortimore, G. E. (1970) *J. Biol. Chem.* **249**, 5458–5463.
7. Griffin, G. E. & Goldberg, A. L. (1978) *J. Biol. Chem.*, in press.
8. Goldberg, A. L., Griffin, G. E. & Dice, J. F., Jr. (1977) in *Pathogenesis of Human Muscular Dystrophies* (Excerpta Medica, Amsterdam), pp. 376–385.
9. Etlinger, J. D. & Goldberg, A. L. (1977) *Proc. Natl. Acad. Sci USA* **74**, 54–58.
10. Knowles, S. E. & Ballard, F. J. (1976) *Biochem. J.* **156**, 609–617.
11. Deter, R. L. (1971) *J. Cell Biol.* **48**, 473–489.
12. Deter, R. L., Baudhuin, P. & deDuve, C. (1967) *J. Cell Biol.* **35**, C11–C16.
13. Mortimore, G. E. & Neeley, A. N. (1975) in *Intracellular Protein Turnover*, eds. Schimke, R. T. & Katunuma, N. (Academic Press, New York), pp. 265–279.
14. Schworer, C. M. & Mortimore, G. E. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 338.
15. Kowit, J. D., Choy, W.-N., Champe, S. P. & Goldberg, A. L. (1976) *J. Bacteriol.* **128**, 776–784.
16. Barrett, A. J. (1972) in *Lysosomes: A Laboratory Handbook*, ed. Dingle, J. T. (North Holland Publishing Co., Amsterdam), pp. 110–122.
17. Doebber, T. W. & Miller, L. L. (1976) *Anal. Biochem.* **70**, 39–44.
18. McDonald, J. K., Zeitman, B. B. & Ellis, S. (1970) *Nature* **225**, 1048–1049.
19. Gornall, A. G., Bardawill, C. S. & David, M. M. (1949) *J. Biol. Chem.* **177**, 751–766.
20. Umezawa, H., Aoyagi, T., Morishima, H., Hamada, M., Takeuchi, T. & Umezawa, H. (1970) *J. Antibiot.* **23**, 259–262.
21. Woessner, J. F., Jr. (1972) *Biochem. Biophys. Res. Commun.* **47**, 965–970.
22. Greer, M. A. & Solomon, D. M., eds. (1974) *Handbook of Physiology* (American Physiological Society, Washington, DC), Sect. 7, Vol III, pp. 391–411; 469–478.
23. Denckla, W. D. (1974) *J. Clin. Invest.* **53**, 572–581.
24. Arias, I. M., Doyle, D. & Schimke, R. T. (1969) *J. Biol. Chem.* **244**, 3303–3305.
25. Winder, W. W., Baldwin, K. M., Terjung, R. L. & Holloszy, J. O. (1975) *Am. J. Physiol.* **228**, 1341–1345.
26. Cohn, Z. A. & Benson, B. (1965) *J. Exp. Med.* **121**, 835–848.
27. Tappel, A. L., Zalkin, H., Caldwell, K. A., Desai, I. D. & Shibko, S. (1962) *Arch. Biochem. Biophys.* **96**, 340–346.
28. Iodice, A. A., Chin, J., Perker, S. & Weinstock, I. M. (1972) *Arch. Biochem. Biophys.* **152**, 166–174.
29. Adam, R. D. (1975) *Diseases of Muscle* (Harper & Row, New York), pp. 475–482.
30. Weissman, G. & Siegal, R. L. (1970) *Proc. Soc. Exp. Biol. Med.* **134**, 812–813
31. Robbins, S. L. (1974) in *Pathological Basis of Disease* (W.B. Saunders Co., Philadelphia, PA), pp. 1013–1024.
32. Peters, J. P. & Mann, E. B. (1943) *J. Clin. Invest.* **22**, 715–720.
33. Goldstein, J. L., Dana, S. E., Faust, J. R., Beaudet, A. L. & Brown, M. L. (1975) *J. Biol. Chem.* **250**, 8487–8495.
34. Beaudet, A. L., Lipson, M. H., Ferry, G. D. & Nicols, B. L., Jr. (1974) *J. Lab. Clin. Med.* **84**, 54–61.
35. Burke, J. A. Schubert, W. K. (1972) *Science* **176**, 309–310.