

CONTROL OF MITOCHONDRIAL TURNOVER UNDER THE INFLUENCE OF THYROID HORMONE

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ABSTRACT

The effect of thyroid hormone on the turnover of mitochondrial DNA and protein was studied in rat heart and liver. Changes in turnover were observed in both thyroidectomized and normal rats following administration of thyroid hormone. In heart and liver the turnover of mitochondrial DNA and protein was slower in thyroidectomized rats than in normal rats. The turnover of mitochondrial DNA and protein was affected similarly following the administration of thyroid hormone, suggesting that mechanisms which control turnover of mitochondrial constituents may be predicated upon a major part of the mitochondrion. In heart a decreased rate of degradation contributes to the increase in total mitochondrial protein. Mitochondrial DNA, labeled before administration of thyroid hormone, turns over, after the start of thyroid hormone administration, at a different rate from that in newly synthesized DNA. The different turnover rates suggest that in liver the pre-existing population of mitochondria is being replaced by another population synthesized under new physiological conditions.

INTRODUCTION

Many previous reports (Fletcher and Sanadi, 1961; Cuzner et al., 1966; Marver et al., 1966; Bailey et al., 1967; Beattie et al., 1967; Swick et al., 1968; Beattie, 1969; de Bernard et al., 1969; Druyan et al., 1969; Gross et al., 1969) deal with the turnover of mitochondrial constituents in mammalian tissues in the normal physiological state. Since thyroid hormone has marked effects on mitochondrial function (Tata, 1964; Hoch, 1968), it is of interest to determine whether the turnover rate differs in the hypothyroid, normal, and hyperthyroid states, and since mitochondrial mass is a function of both the rate of synthesis and the rate of degradation, one may also ask how changes in these two rates contribute to any changes in mitochondrial mass following the administration of thyroid hormone.

An experiment in which the rate of turnover of

mitochondrial constituents is changed may also contribute information on whether or not there is a major subunit of the mitochondrion which turns over as a single entity, since if such a subunit exists, the turnover rates of its constituents would be affected in the same manner.

In the experiments reported here the effect of thyroid hormone administration on mitochondrial DNA and protein turnover was studied with the following aims: to determine the turnover rates in different thyroid states; to determine whether changes in synthesis or degradation or both result in changes in the mass of mitochondrial protein; to determine whether the turnover rates of mitochondrial DNA and protein are affected in the same manner and to the same extent; and to determine whether mitochondria synthesized before thyroid hormone administration turn over after

thyroid hormone administration at the same rate as newly synthesized mitochondria.

METHODS

Two experiments of similar design were performed. In experiment I, the hypothyroid state and its transition to the euthyroid or slightly hyperthyroid state were studied. In experiment II, the normal or euthyroid state and its transition to the strongly hyperthyroid state were studied.

Experiment I was performed on female Sprague-Dawley rats which had been surgically thyroidectomized (Hormone Assay Laboratories, Chicago, Ill.) at 10 wk of age. The rats were maintained on Purina rat diet *ad libitum*, and water supplemented with a small amount of CaCl_2 for 10 wk. Their weights were checked at weekly intervals, and any animal whose weight increased was discarded. In rats thyroidectomized at this age the total absence of thyroid function can be diagnosed by the cessation of growth. After several months some increase in weight does occur, but this is probably due to the accumulation of myxedema. At the start of experiment I the rats weighed between 173 and 197 g each; they were divided into eight matched groups of three rats each. A group was killed each week for 7 wk, with an additional group at $2\frac{1}{2}$ wk (groups 0, 1, 2, $2\frac{1}{2}$, 3, 4, 5, and 6). Groups 0, 1, and 2 received no thyroid hormone. Groups $2\frac{1}{2}$ -6 received 20 μg each of *l*-thyroxine (Sigma Chemical Company, St. Louis, Mo.) on alternate days by intraperitoneal injection, starting with the day on which group 2 animals were killed and continuing to the end of the experiment. Experiment II was performed in a similar manner on normal adult female Sprague-Dawley rats weighing between 225 and 247 g each. Eight groups of three rats each were killed according to the time schedule described in experiment I. Thyroid hormone was administered similarly, but at a dose of 300 μg per rat on alternate days.

Preparation of Tissue Fractions from Liver

The rats were killed by decapitation and the livers were removed, cut into small pieces with scissors, and chilled in 0.3 M sucrose, 0.01 M Tris-HCl, pH 7.4, 0.1 mM EDTA (S.T.E. buffer).¹ All subsequent steps were performed at 0-4°C. The livers were pooled, blotted dry, weighed, and homogenized as described previously (Gross et al., 1969). An aliquot of the homogenate was kept for protein, DNA, and enzyme determinations, and the remainder was centrifuged at 500 g for 10 min in the SS-34 rotor of the RC2-B

¹S.T.E. buffer used in this paper for sucrose-Tris-EDTA; S.T.M. buffer used in this paper for sucrose-Tris-Mg.

Sorvall centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.). Mitochondria were purified from the supernatant by one further centrifugation at 500 g for 10 min, centrifugation of the supernatant at 12,000 g for 12 min, incubation of the resuspended pellet with pancreatic DNase and MgCl_2 , three further centrifugations at 9000 g for 10 min, centrifugation through a sucrose gradient (0.8-2.0 M) in the 25.2 rotor of the Spinco L2-65 ultracentrifuge (Spinco Division, Beckman Instruments, Inc., Palo Alto, Calif.), and reprecipitation of the mitochondria in the Sorvall centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.). All subcellular fractions were finally resuspended in S.T.E. buffer and frozen.

Preparation of Tissue Fractions from Heart

After removal of the livers, the hearts were removed and the coronary arteries were flushed with 5 ml of cold 0.3 M sucrose, 0.05 M Tris-HCl, pH 7.4, and 0.0003 M MgCl_2 (S.T.M. buffer).¹ The atria were trimmed away and the ventricles were weighed and homogenized as previously described (Gross et al., 1969). A small fraction of the ground muscle was removed, weighed, and extensively homogenized separately for determinations of total protein, total DNA, and enzyme. Mitochondria were isolated as described for liver, but omitting the DNase step. All fractions were finally suspended in S.T.M. buffer and frozen.

Chemical Determinations

Protein determinations were made in duplicate on all homogenate and mitochondrial samples by the method of Lowry et al. (1951). DNA determinations were made in duplicate on all homogenate samples by Burton's modification of the diphenylamine method (1956).

Respiratory and Enzymatic Determinations

Oxygen consumption was determined in a chamber, constructed in this laboratory, which is similar to that described by MacLagan and Sheahan (1950). Because of considerable variations among values determined successively on the same rats, changes in oxygen consumption following thyroid hormone administration were determined in a separate series of experiments. Six thyroidectomized rats and six normal rats were studied approximately three times per week over a 4 wk period. Thyroid hormone was administered as described above, and the oxygen consumption of the rats was determined over the next 8 wk. Each determination was made on two rats simultaneously over a 15-20 min period, after a 10 min period for equilibration.

Succinate cytochrome *c* reductase activity was

measured on all homogenate and mitochondrial samples at 37°C in a 3 ml system by the method of Rabinowitz and de Bernard (1957).

Succinate cytochrome *c* reductase determinations on any one tissue were all made during the same session on an aliquot that had been extensively dispersed in an all-glass homogenizer and stored separately for this purpose. Duplicate determinations were made on the refrozen, rethawed aliquot. This method was employed because freezing and thawing results in an increase in activity of the enzyme as compared with the fresh preparation, and subsequent freeze-thaw cycles result in only a slight reduction in activity. Since prolonged storage in the frozen state produced no detectable change in activity, greater consistency could be obtained by analyzing all samples at the same time.

Cytochrome *c* oxidase determinations were made on most homogenate and mitochondrial samples from experiment I by the method of Cooperstein and Lazarow (1951), as modified by de Duve et al. (1955). These determinations were performed on unfrozen tissue only. The results for heart tissue were fairly consistent, but the data for liver tissue showed considerable variation, even after stimulation of cytochrome *c* oxidase activity by the addition of Lubrol (ICI America Inc., Stamford, Conn.) (Schnaitman and Greenawalt, 1968).

Isotopic Labeling

Animals were labeled in an identical fashion in experiments I and II. Proteins were labeled with the nonreusable isotope guanidine-¹⁴C-arginine (Swick et al., 1968) (New England Nuclear Corp., Boston, Mass., 5 mCi/mole). Each animal in groups 0, 1, and 2 received 10 μCi arginine-¹⁴C by intraperitoneal injection 2 days before the animals in group 0 were killed. (In experiment II, animals in group 2½ were also labeled at this time; in experiment I, this group was not labeled.) Animals in groups 3, 4, 5, and 6 received an injection of arginine-¹⁴C 1 day before the animals in group 3 were killed, i.e., 6 days after the first injection of *l*-thyroxine.

DNA was labeled with methyl-³H-thymidine (Schwarz Bio Research Inc., Orangeburg, N.Y., 6 Ci/mole) and with methyl-¹⁴C-thymidine (Schwarz Bio Research, 45 mCi/mole). All animals received 465 μCi of ³H-thymidine by intraperitoneal injection 2 days before the animals in group 0 were killed. Each animal in groups 3, 4, 5, and 6 received 9 μCi of thymidine-¹⁴C by intraperitoneal injection 1 day before the animals in group 3 were killed (in addition to the initial injection of thymidine-³H).

Purification of Liver Mitochondrial DNA

DNA was purified from liver mitochondria as described previously (Gross et al., 1969; method 2);

this method entails deproteinization with phenol, incubation with ribonuclease, chromatography on a column of methylated albumin kieselguhr, denaturation and renaturation, and preparative CsCl-gradient centrifugation. It has been shown (Gross et al., 1969) that when mitochondrial DNA is purified by this method, fractions of the final CsCl gradients are obtained which contain pure mitochondrial DNA as characterized by analytical ultracentrifugation. 20 μg of highly purified SP-01 (*Bacillus subtilis* phage) DNA was added to each mitochondrial preparation at the start of the purification procedure. The main purpose of this addition was to attempt a rough approximation of the amount of mitochondrial DNA in each mitochondrial preparation from the recovery of SP-01 DNA in the final CsCl gradient; it also served as a density marker, and may have improved yields of mitochondrial DNA by acting as a carrier. Purification of DNA from heart mitochondria was not attempted, since the yield of mitochondria from three rat hearts is too low to permit reliable determinations of the specific activity of the DNA.

Radioactivity Determinations

Specific activity of proteins was determined in duplicate on all mitochondrial preparations. Approximately 5–10 mg of protein was precipitated at 0–4°C by the addition of 25% TCA (trichloroacetic acid) to a final concentration of 5%. The precipitate was collected by low-speed centrifugation, washed once with 5% TCA, and again collected by low-speed centrifugation. It was then resuspended in 3 ml of 5% TCA and incubated at 90°C for 30 min to hydrolyze DNA, cooled, and collected by centrifugation again. The pellet was dissolved by the addition of 3 ml of 0.5 N NaOH, and incubated at 45°C for 1 hr, after which it was centrifuged at 9000 *g* for 10 min and the small pellet was discarded. The supernatant was neutralized with 1.5 ml of 1 N HCl, and brought to 5% with respect to TCA concentration by the addition of 25% TCA in the cold. The reprecipitated proteins were collected once more by low-speed centrifugation. Lipids were extracted by washing with 5 ml of ethanol-ether (3:1) twice, and once with 5 ml of acetone at room temperature. The final pellet was dried overnight in the centrifuge tubes and dissolved the next day in 1.2 ml concentrated formic acid. Of this solution, 1.0 ml was plated onto planchets, and the formic acid was evaporated by using heat. Radioactivity was determined in a Beckman low-beta 11 counter, (Electronic Instruments Division, Beckman Instruments, Inc., Richmond, Calif.) a total of 2000 counts being collected for each sample. 20 and 50 μliter duplicate aliquots were taken and evaporated for protein determinations.

The specific activity of purified mitochondrial DNA was determined by the collection of the appro-

priate fractions from each gradient on Millipore filters (Millipore Corp., Bedford, Mass.), as described previously (Gross et al., 1969). Radioactivity was determined in a Packard Tri-Carb scintillation spectrometer, and corrections were made for quenching and "spillage" of each isotope by internal standardization.

RESULTS

As described under Methods, the number identifying each group of three animals corresponds to the age in weeks of the animals after the initiation of the experiment. In each experiment, thyroid hormone was administered in the doses described above to all remaining animals at the time of death of those in group 2. Experiment I shows the transition from the hypothyroid to the euthyroid, or, more properly, to the slightly hyperthyroid, state, and experiment II the transition from the normal or euthyroid state to the strongly hyperthyroid state.

Oxygen Consumption

In the thyroidectomized rats, oxygen consumption increased from $2.50 \pm 0.21 \times 10^{-2}$ ml/min per g before administration of thyroid hormone to $3.01 \pm 0.24 \times 10^{-2}$ ml/min per g after 3 wk of treatment. In the normal rats, oxygen consumption increased from $2.96 \pm 0.28 \times 10^{-2}$ ml/min per g to $3.53 \pm 0.20 \times 10^{-2}$ ml/min per g in the corresponding period after treatment with large doses of thyroid hormone. The increase in oxygen consumption occurred in the first 3 wk of administration of thyroid hormone. These values for oxygen consumption are somewhat higher, and the changes are relatively smaller, than those reported by others (Tata et al., 1963; Gustafsson et al., 1965; Roodyn et al., 1965). However, they serve to establish that the dose of thyroid hormone was approximately a replacement dose in experiment I, and was sufficient to produce hyperthyroidism in experiment II.

Liver Mitochondrial DNA Turnover²

The results of experiment I are shown in Fig. 1. The initial decline in specific activity in the hypo-

² It should be emphasized that the figures given for half-lives of mitochondrial DNA and protein in the following sections should be taken literally only where the specific activity declines exponentially and there is no evidence of a change in the total quantity of the constituent. When these conditions

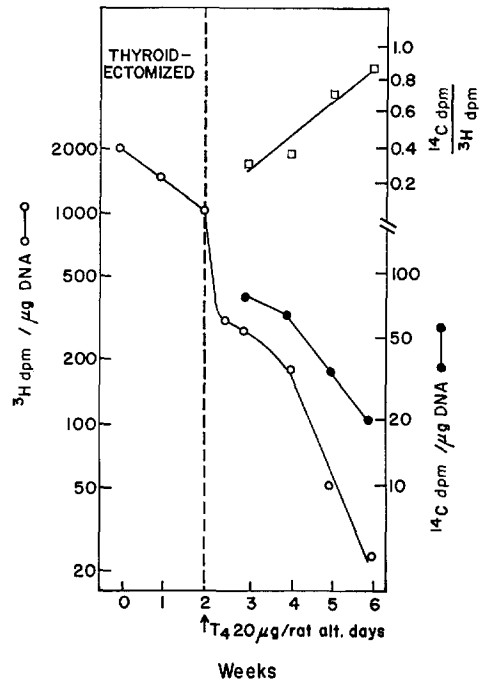


FIGURE 1 Turnover of liver mitochondrial DNA in thyroidectomized rats. All animals received 465 μ Ci methyl-³H-thymidine 2 days before the animals in group 2 were killed; animals in groups 3, 4, 5, and 6 received 9 μ Ci methyl-¹⁴C-thymidine 1 day before the animals in group 3 were killed. Administration of *l*-thyroxine (T_4) was started at the time and in the dose indicated. The specific activities of ³H-labeled DNA (O—O) and ¹⁴C-labeled DNA (●—●) are indicated on logarithmic scales. The ratio of ¹⁴C activity to ³H activity of the DNA of groups 3, 4, 5, and 6 (□—□) is indicated on a linear scale. A free-hand curve has been drawn through each set of points.

thyroid animals (groups 0, 1, and 2) is exponential, and corresponds to a half-life of about 14.0 days. Following the start of treatment with thyroid hormone, there is a very rapid decrease in ³H specific activity (group 2½), which appears to level off to some extent (groups 3 and 4), and then to decline rapidly and exponentially (groups 5 and 6). The half-life determined from ³H DNA specific activity over the last 2 wk is 4.9 days. Thymidine-¹⁴C was administered 6 days after the first injection of thyroid hormone and 1 day before the animals in group 3 were killed. During the last 2

are not satisfied, a "half-life" is given only to simplify description of the changes seen in the specific activity curves.

wk the ^{14}C DNA specific activity declines exponentially with a half-life of 10.0 days. This is more than twice the half-life of the ^3H -labeled DNA in the same preparation. The ratio of ^{14}C specific activity to ^3H specific activity, also shown in Fig. 1, increases threefold between weeks 3 and 6. The difference between the slopes of the two DNA labels is significant at the 1% level (using the t test) over the period from 4–6 weeks.

In Experiment II (Fig. 2), the initial decline in ^3H DNA specific activity in normal animals is exponential and corresponds to a half-life of 9.1 days; this is similar to the half-life of 9.4 days we reported previously (Gross et al., 1969). There is little change in the ^3H specific activity immediately after the start of thyroid hormone administra-

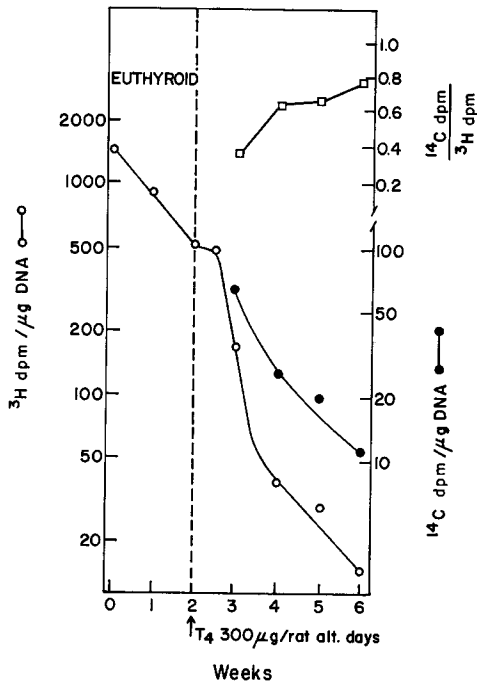


FIGURE 2 Turnover of liver mitochondrial DNA in normal rats. All animals received 465 μCi methyl- ^3H -thymidine 2 days before the animals in group 2 were killed; animals in groups 3, 4, 5, and 6 received 9 μCi methyl- ^{14}C -thymidine 1 day before the animals in group 3 were killed. Administration of l -thyroxine (T_4) was started at the time and in the dose indicated. The specific activities of ^3H -labeled DNA (\circ — \circ) and ^{14}C -labeled DNA (\bullet — \bullet) are indicated on logarithmic scales. The ratio of ^{14}C activity to ^3H activity of the DNA of groups 3, 4, 5, and 6 (\square — \square) is indicated on a linear scale. A free-hand curve has been drawn through each set of points.

tion (group 2 $\frac{1}{2}$), but there is a subsequent rapid decline in specific activity. The half-life of ^3H -labeled mitochondrial DNA during the last 2 wk is 8.4 days. Over the same period, the ^{14}C -labeled DNA has a half-life of 12.0 days. The ratio of ^{14}C to ^3H specific activity increases by a factor of nearly two during the last 3 wk of the experiment.

In order to rule out the possibility that the difference in turnover of ^3H - and ^{14}C -labeled DNA observed in both experiments is due to differential reutilization of the two isotopes, in spite of the fact that they are both labeled in the 5-methyl group, a separate series of experiments was performed. Groups of thyroidectomized, normal, and hyperthyroid animals were simultaneously given the two isotopes used in experiments I and II. Liver mitochondrial DNA was purified from animals killed 2 and 8 days later, and the ratio of ^3H to ^{14}C DNA specific activity was determined. This ratio was constant for animals in each thyroid state. This shows that the apparent half-life of mitochondrial DNA is the same, whichever isotope is used to determine the results.

In contrast to the results obtained for mitochondrial DNA, the ratio of ^{14}C to ^3H specific activity of nuclear DNA was constant in each experiment. This rules out the possibility that the changing ratio of ^{14}C to ^3H specific activity of mitochondrial DNA is due to random variations in uptake and incorporation of the two isotopes.

Quantitation of Liver Mitochondrial DNA

The approximate amounts of mitochondrial DNA in each mitochondrial preparation were estimated from the recovery of SP-01 DNA added in a known amount to each sample at the start of the purification procedure. In the final preparative CsCl gradient, the SP-01 DNA was easily identified as a band heavier than the mitochondrial DNA band and separate from it, and also separate from a small band of denatured nuclear DNA which consistently contaminated all preparations. (The buoyant densities in CsCl of native SP-01, denatured rat nuclear, and renatured rat mitochondrial DNA are, respectively, 1.742, 1.717, and 1.706 g/ml [Gross et al., 1969]. It is assumed that denatured SP-01 DNA renatures rapidly and returns to within 0.003 g/ml of its native buoyant density, as does mitochondrial DNA.) The amount of DNA in each band was calculated from the optical density at 260 μm of

the appropriate fractions of the gradient. From the recovery of SP-01 DNA, an estimate can be made of the amount of mitochondrial DNA in the original mitochondrial sample. It is assumed, however, that recovery of the two DNA's is equal, or that any difference in recovery is consistent for all preparations. The ratio of mitochondrial DNA to mitochondrial protein in each preparation of

mitochondria is given in Table I. There is some variability in these results, as would be expected, but no obvious trend in the ratio of mitochondrial DNA to mitochondrial protein can be seen. A 50% change in the mitochondrial DNA/protein ratio would probably not be detected with certainty; but an increase of 300%, such as has been reported to follow administration of thyroid hormone

TABLE I
DNA Content of Liver Mitochondria in Different Thyroid States

Group	0	1	2	2½	3	4	5	6
	<i>µg DNA per mg mitochondrial protein</i>							
Experiment I	0.095	0.110	0.168	0.090	0.169	0.133	0.213	0.140
Experiment II	0.098	0.130	0.064	0.091	0.070	0.110	0.149	0.100

TABLE II
Total Mitochondrial Protein Calculated from Succinate Cytochrome c Reductase Activities in Whole Tissue and Mitochondria

In Experiment I, groups 0, 1, and 2 were thyroidectomized untreated rats; groups 4, 5, and 6 were thyroidectomized rats, each treated with 20 µg *l*-thyroxine on alternate days. In experiment II, groups 0, 1, and 2 were normal rats; groups 4, 5, and 6 were normal rats each treated with 300 µg *l*-thyroxine on alternate days.

	Experiment I				Experiment II			
	Mean of groups 0, 1, 2	Mean of groups 4, 5, 6	Change	<i>P</i> *	Mean of groups 0, 1, 2	Mean of groups 4, 5, 6	Change	<i>P</i> *
			%				%	
<i>Liver</i>								
Total enzyme activity, µmoles/min/liver	138	191	+38	<0.01	190	261	+37	<0.02
Mitochondrial specific activity, µmoles/min/mg protein	0.350	0.538	+54	<0.01	0.414	0.597	+44	<0.02
Total mitochondrial protein, g/liver	0.39	0.36	-10	N.S.	0.46	0.45	-2	N.S.
<i>Heart</i>								
Total enzyme activity, µmoles/min/heart	5.43	16.4	+202	<0.01	17.6	26.0	+48	<0.05
Mitochondrial specific activity, µmoles/min/mg protein	1.00	1.07	+7	N.S.	0.95	0.98	+3	N.S.
Total mitochondrial protein, mg/heart	5.43	15.3	+182	<0.01	18.9	26.5	+41	<0.05

* Probability values refer to the significance of the difference (using the *t* test) between groups 0, 1, and 2 combined and groups 4, 5, and 6 combined.

(Haldar et al., 1966), could probably be detected. DNA/protein values reported here are all lower by a factor of two to four than those reported in a recent review (Nass, 1969). This may reflect the different methods of determination.

Quantitation of Liver and Heart Mitochondrial Protein

The total liver and heart mitochondrial protein of each group of rats was calculated from the recovery of succinate cytochrome *c* reductase and cytochrome *c* oxidase. As these enzymes are localized in the mitochondrial inner membrane (Sottocasa et al., 1967; Schnaitman and Greenawalt, 1968), the ratio of the total activity in the organ to the activity per unit of protein in the purified mitochondria gives an indication of the total mitochondrial protein of the organ. The mitochondrial protein mass calculated from the succinate cytochrome *c* reductase results is shown in Table II. The total activity of this enzyme in both liver and heart increases following the administration of thyroid hormone; only in the heart, however, was an increase in the total mitochondrial protein demonstrated.

Liver Mitochondrial Protein Turnover

Fig. 3 shows the results of specific activity determinations on mitochondrial proteins in experiment I, in which the transition from hypothy-

roidism to euthyroidism is studied. In untreated thyroidectomized animals the total mitochondrial proteins turn over with a half-life of 9.1 days. After 2 wk of treatment with thyroid hormone in replacement doses, the half-life is 6.3 days. Data for the corresponding periods in experiment II (Fig. 4) indicate a half-life of 4.0 days for mitochondrial proteins in normal rats. 4 days after the start of thyroid hormone administration (group 2½) the slope decreases considerably; 3 days later the decline in specific activity is exponential and corresponds to a half-life of 5.3 days.

Heart Mitochondrial Protein Turnover

The results of experiments I and II are shown in Figs. 5 and 6, respectively. In untreated thyroidectomized rats (Fig. 5), the half-life of mitochondrial proteins is very long. After thyroid hormone administration the apparent turnover remains very slow. If a correction is made for the increase in total mitochondrial protein during this part of the experiment (by multiplying the specific activity by the total mitochondrial protein at each time point), there is very little or no loss of labeled mitochondrial protein. There is evidence that in heart, as in liver, guanidine-¹⁴C-arginine is not reutilized (Aschenbrenner et al., 1970). In untreated normal rats (Fig. 6), heart mitochondrial protein has a half-life of 5.2 days; this is similar to the half-life of 6.1 days also reported from this laboratory (Aschenbrenner et al., 1970). The de-

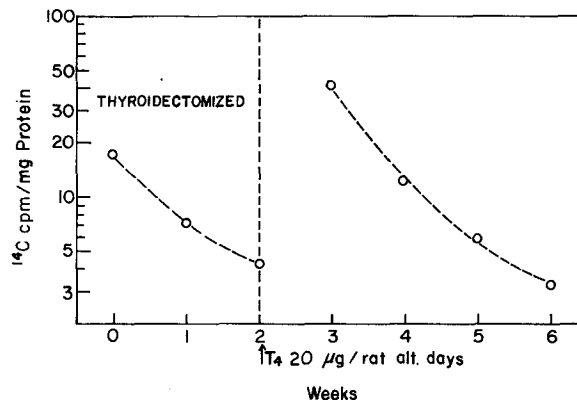


FIGURE 3 Turnover of liver mitochondrial proteins in thyroidectomized rats. Animals in groups 0, 1, and 2 received 10 μ Ci guanidine-¹⁴C-arginine 2 days before the animals in group 0 were killed; animals in groups 3, 4, 5, and 6 received 10 μ Ci guanidine-¹⁴C-arginine 1 day before the animals in group 3 were killed. Administration of *l*-thyroxine (T_4) was started at the time and in the dose indicated. The specific activity (O----O) is indicated on a logarithmic scale on the ordinate. A free-hand curve has been drawn through each set of points.

cline in specific activity ceases in the first 4 days of thyroid hormone administration. The specific activity of the mitochondrial proteins labeled 6 days after the start of thyroid hormone administration appears to decline slowly at first and more rapidly later. Again, correction for the increase in total heart mitochondrial protein suggests that there is very little degradation of mitochondrial

protein for at least 2 wk following administration of the second isotope.

DISCUSSION

The results may be summarized as follows: mitochondrial DNA and protein turn over at a slower

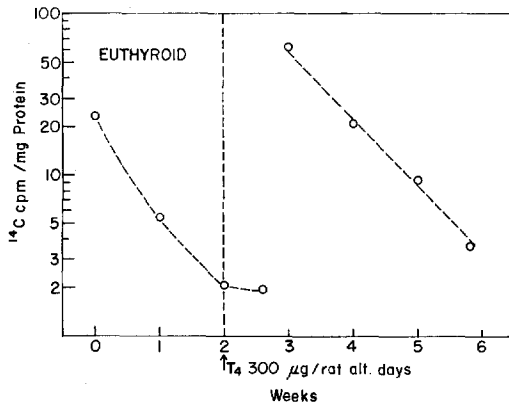


FIGURE 4 Turnover of liver mitochondrial proteins in normal rats. Animals in groups 0, 1, 2, and $2\frac{1}{2}$ received $10\ \mu\text{Ci}$ guanidine- ^{14}C -arginine 2 days before the animals in group 0 were killed; animals in groups 3, 4, 5, and 6 received $10\ \mu\text{Ci}$ guanidine- ^{14}C -arginine 1 day before the animals in group 3 were killed. Administration of *l*-thyroxine (T_4) was started at the time and in the dose indicated. The specific activity ($\text{O}---\text{O}$) is indicated on a logarithmic scale on the ordinate. A free-hand curve has been drawn through each set of points.

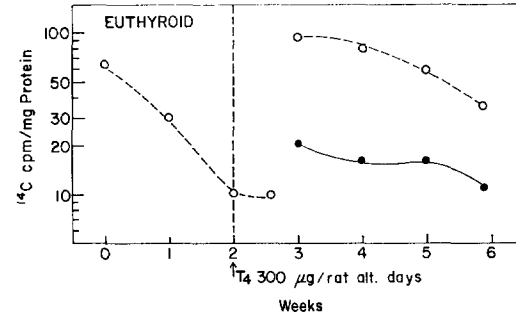


FIGURE 6 Turnover of heart mitochondrial proteins in normal rats. Animals in groups 0, 1, 2, and $2\frac{1}{2}$ received $10\ \mu\text{Ci}$ guanidine- ^{14}C -arginine 2 days before the animals in group 0 were killed; animals in groups 3, 4, 5, and 6 received $10\ \mu\text{Ci}$ guanidine- ^{14}C -arginine 1 day before the animals in group 3 were killed. Administration of *l*-thyroxine (T_4) was started at the time and in the dose indicated. The specific activity ($\text{O}---\text{O}$) is indicated on a logarithmic scale on the ordinate. The activity corrected for increase in mass of heart mitochondrial protein ($\bullet---\bullet$) was calculated by multiplying the specific activity by the total mitochondrial protein for each of groups 3, 4, 5, and 6. Corrected protein specific activities are expressed in arbitrary units. A free-hand curve has been drawn through each set of points.

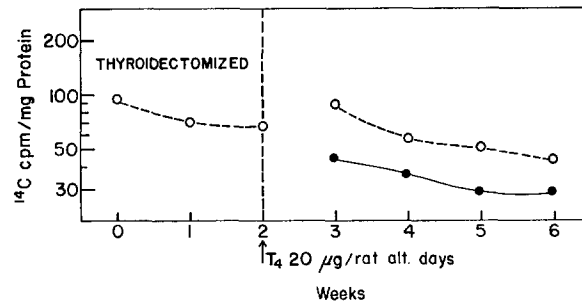


FIGURE 5 Turnover of heart mitochondrial proteins in thyroidectomized rats. Animals in groups 0, 1, and 2 received $10\ \mu\text{Ci}$ guanidine- ^{14}C -arginine 2 days before the animals in group 0 were killed; animals in groups 3, 4, 5, and 6 received $10\ \mu\text{Ci}$ guanidine- ^{14}C -arginine 1 day before the animals in group 3 were killed. Administration of *l*-thyroxine (T_4) was started at the time and in the dose indicated. The specific activity ($\text{O}---\text{O}$) is indicated on a logarithmic scale on the ordinate. The activity corrected for increase in mass of heart mitochondrial protein ($\bullet---\bullet$) was calculated by multiplying the specific activity by the total mitochondrial protein for each of groups 3, 4, 5, and 6. Corrected protein specific activities are expressed in arbitrary units. A free-hand curve has been drawn through each set of points.

rate in hypothyroid rats than in normal rats; mitochondrial DNA and protein turnovers are altered in the same direction and to the same extent following thyroid hormone administration; in heart, a decreased rate of degradation of mitochondrial protein contributes to the increase in the total mitochondrial protein. The observation that we consider to be most interesting is that liver mitochondrial DNA labeled before administration of thyroid hormone turns over at a different rate than DNA that is newly synthesized after administration of thyroid hormone.

Effect of Thyroid State on Mitochondrial Turnover

Liver mitochondrial DNA of untreated thyroidectomized animals turns over more slowly than that of untreated normal animals (groups 0, 1, and 2; Figs. 1 and 2). The half-lives are approximately 14 days and 9 days, respectively. Comparison of the protein turnover rates in the same mitochondria (Figs. 3 and 4) shows that mitochondrial protein also turns over more slowly in thyroidectomized animals. The difference in the half-life of mitochondrial DNA and protein can probably be explained on the basis of reutilization of the thymidine isotope. The guanidine group of arginine has a very small probability of being reutilized (Swick, 1958); relatively short half-lives are a feature of experiments that employ arginine labeled in the guanidine group (Swick et al., 1968), and problems related to the size and turnover of the precursor pool are avoided.

In the case of heart, also, mitochondrial protein turns over more slowly in thyroidectomized animals than in normal animals (groups 0, 1, and 2; Figs. 5 and 6).

The results do not permit us to draw any conclusions about mitochondrial turnover in the hyperthyroid state because a steady state has probably not been reached in the first few weeks of thyroid hormone administration.

Comparison of the Effect of Thyroid Hormone on Protein and DNA Turnovers in Liver Mitochondria

It was noted above that both DNA and protein turn over more slowly in the hypothyroid state than in the normal state. There is additional evidence that mitochondrial DNA and protein are similarly affected by a change in thyroid state.

In experiment I (Fig. 1), the turnover of newly synthesized DNA is more rapid after administration of thyroid hormone (^{14}C -labeled DNA, groups 4, 5, and 6) than before it (^3H -labeled DNA, groups 0, 1, and 2). Fig. 3 shows that newly synthesized protein also turns over more rapidly after thyroid hormone administration (groups 4, 5, and 6) than before it (groups 0, 1, and 2). The similar effect of thyroid hormone on DNA and protein turnover can also be seen in experiment II (Figs. 2 and 4). It is notable that the change in turnover rate of DNA and protein is of the same relative magnitude.

The proteins labeled by a single injection of arginine- ^{14}C necessarily include a very large number of mitochondrial constituents from outer and inner membranes and matrix. It has been shown (Marver et al., 1966; Beattie, 1969; de Bernard et al., 1969; Druyan et al., 1969) that significant differences exist between the turnover rates of these major components. However, the similarity of the effect of thyroid hormone on the turnover of mitochondrial DNA and uncharacterized protein suggests that mechanisms which control mitochondrial turnover may be predicated upon the whole mitochondrion or upon some large subunit consisting of the DNA and much of the protein, rather than upon individual components. Detailed turnover data for several purified mitochondrial constituents would be needed to confirm this hypothesis.

Effect of Changes in Synthesis and Degradation Rates on Mitochondrial Mass

The total mitochondrial protein of heart increases following administration of thyroid hormone (Table II). The increase could occur by an increase in the rate of synthesis or by a decrease in the rate of degradation of mitochondrial proteins. The turnover curves (Figs. 5 and 6) suggest that degradation has been reduced. This is most clearly shown in experiment II, (Fig. 6) in which it is shown that turnover is relatively rapid before thyroid hormone administration. The turnover rate is greatly reduced in the second part of the experiment, but increases towards the end (groups 5 and 6). Correction of the specific activity changes for the increase in total mitochondrial protein shows that very little of the newly synthesized protein is degraded until week 5, by which time the mitochondrial mass has stabilized at a new level. One must be cautious in concluding that

degradation of all mitochondrial proteins has slowed to the extent suggested by the changes in specific activity of groups 3, 4, and 5. It was shown for liver mitochondria that newly synthesized DNA turns over more slowly than previously synthesized DNA. This could apply to protein in the heart mitochondria. It is noteworthy that the specific activity changes in heart mitochondrial protein which suggest decreased degradation are not apparent in liver mitochondrial protein (Figs. 3 and 4), where no net increase in mass was detected.

Differential Turnover of ³H- and ¹⁴C-Labeled DNA in Liver Mitochondria

There is a difference in the turnover rates of ³H- and ¹⁴C-labeled DNA following administration of thyroid hormone which cannot be explained on the basis of differential reutilization of the two thymidine isotopes. Mitochondrial DNA synthesized after the administration of thyroid hormone is evidently more stable than pre-existing DNA. A difference in the replication rate of the new and the old populations of mitochondrial DNA would not by itself account for the difference in turnover. Two other features suggest that the difference in turnover is due to selective degradation of pre-existing mitochondrial DNA. In experiment I, a replacement dose of thyroid hormone was administered to thyroidectomized animals. The half-life of newly synthesized (¹⁴C labeled) DNA in the treated animals during the 4–6 wk period is 10.0 days, about the same as in normal rats. The half-life of the pre-existing DNA (³H labeled) is much shorter, suggesting that the pre-existing DNA is selectively degraded.

There is a steep decline in the specific activity of ³H-labeled DNA following the start of thyroid hormone administration. This is most obvious in experiment I (Fig. 1, group 2½), and cannot be explained solely on the basis of dilution of labeled DNA by newly synthesized DNA. The total mitochondrial DNA would have to increase by a factor of almost three to account for the threefold decrease in ³H-labeled DNA specific activity. Our estimate of the total mitochondrial DNA (Table I) indicates that no net increase in total mitochondrial DNA occurs. We may conclude, therefore, that ³H-labeled DNA is being replaced by newly synthesized unlabeled DNA in the first week of thyroid hormone administration. It is

noteworthy that at the same stage of thyroid hormone administration the liver mitochondria become more active with respect to succinate cytochrome *c* reductase (Table II). If a new population of mitochondrial DNA is replacing the pre-existing population, the phenomenon most likely reflects the behavior of the whole mitochondrion rather than that of the DNA alone. However, we cannot exclude the possibility that mitochondrial DNA is being replaced within relatively stable mitochondria. Nor can we exclude the possibility that heterogeneity of the liver cell population or nonuniform response to thyroid hormone may result in the apparent replacement of one population of mitochondrial DNA with another.

The increase in the turnover rate of mitochondrial DNA in the first week of treatment with thyroid hormone is similar to the phenomenon reported by Tata (1967) in thyroid-induced amphibian metamorphosis. An increase in the turnover of previously labeled cytoplasmic RNA occurs in the liver 2–4 days after the administration of thyroid hormone, and precedes any increase in RNA mass. This raises the very interesting possibility that the replacement of one population of organelles with another perhaps more physiologically appropriate population may be a widespread response to a changing physiological environment.

If our interpretation is correct that one population of mitochondria is being replaced by a more active population, the method of this replacement is at present obscure. There is considerable evidence that mitochondria arise by growth and division of pre-existing mitochondria (Roodyn and Wilkie, 1968). Luck's experiments (1965) show that *Neurospora* mitochondria evolve from one type to another as a single population, indicating that the "progeny" of a mitochondrial division are identical. A more complex situation may be present in mammalian cells. One may speculate that, at some stage in a changing physiological environment, mitochondrial division results in two nonidentical progeny, one corresponding to the pre-existing mitochondrion, the other corresponding to the newly synthesized type. The pre-existing moiety is then eliminated by selective degradation. Inherent in this scheme is the idea that qualitative changes in the mitochondria, such as have been reported by Tata et al. (1963), Roodyn et al. (1965), and Kadenbach (1966), and are seen in the present data (Table

II), are built into new mitochondria rather than brought about by the modification of pre-existing mitochondria.

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