daughter produced within the body from Cs^{137} is differentially metabolized so that, in the living animal, various tissues may have a deficiency or excess of Ba^{137m} as compared with the equilibrium mixture. It can be calculated from the data presented thus far that the gamma levels in the liver, bone, blood, and plasma of living animals in these experiments were 0.8, 3.3, 3.9 and 14 times the values as determined by usual tissue analysis.

In another study, other tissues were examined by the same procedure to see whether there was an excess or deficiency of Ba^{137m}. It was observed that the gamma activity in blood and bone decreased with time as noted before. No deviations from the equilibrium proportions were seen for bone marrow, testes, and brain; there was a suggestion of an excess Ba^{137m} in spleen and a deficiency of Ba^{137m} in kidney. The significance of these results is obscured because of the difficulty of detecting slight deviations from equilibrium against the high levels of Cs^{137}-Ba^{137m} in these tissues.

The source of excess Ba^{137m} in the plasma is most likely the soft tissues. Although the red blood cells probably contribute to the plasma excess, they can only be a minor source since the blood as a whole shows a considerable excess of the Ba^{137m}. The excess of Ba^{137m} in bone is probably the result of two mechanisms: a reflection of the increased plasma levels and a selective accumulation of barium by the bone. Evidently any depletion of the plasma barium by selective accumulation in bone is far outweighed by the entry of barium into the plasma from the tissues.

The fact that there is an excess or deficiency of Ba^{137m} in the living animal and that it can be measured raises some interesting considerations: (i) Since the gamma activities in tissues of the living animal are different from those expected from usual radioassay procedures, the radiation dosage may be different from that calculated from available Cs^{137} levels. (ii) Barium-137m is produced within cells and the opportunity is presented for studying the mechanism by means of which this element is extruded from the cell. (iii) Steady-state levels of Ba^{137m} in clinical cases after Cs^{137} administration may indicate both the state of the circulatory system and tissue metabolism; these factors, among others, should influence the level of excess Ba^{137m} in the blood. (iv) The excess Ba^{137m} in the skeleton offers the possibility of studying the processes of rapid incorporation of alkaline earths into bone independent of the slower process of growth. For example, from a comparison of the excess Ba^{137m} to calcium ratios in the plasma and bone, it was calculated that the exchangeable calcium in the femur diaphyses was 4 to 5 percent; this is in general agreement with other values in the literature as determined by other methods (*4*).

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Effects of Thyroxin on Amino Acid Incorporation into Protein

Abstract. The effect of thyroxin on the in vitro incorporation of DL-leucine-1-C^{14} into the protein of rat liver homogenates has been investigated. Both thyroxin pretreatment in vivo and thyroxin in vitro at a concentration of $1 \times 10^{-5}M$ were found to increase the rate of amino acid incorporation. The increased activity following the thyroxin pretreatment in vivo was found to be localized in the mitochondrial fraction. It is suggested that the acceleration of metabolic rate characteristic of thyroxin action may be secondary to the stimulation of energy-requiring reactions such as protein synthesis.

Recent concepts of the mechanism of action of thyroxin have emphasized its uncoupling effect on oxidative phosphorylation (*1, 2*). This effect, however, is observed only with relatively high concentrations of thyroxin, occurs equally well with both the D- and L- forms (*1*), and, except for changes in oxidative metabolism, explains few of the physiological effects of the thyroid hormone. Many clinical features of thyroid disease suggest a major, if not primary, role of the thyroid hormone in protein metabolism. In immature animals it is involved in growth; in adults it causes pronounced changes in nitrogen metabolism. Furthermore, in the adult brain and testis—organs in which the quantities of protein and lipid turned over per unit time are apparently negligible compared with turnover of carbohydrate, as evidenced by a respiratory quotient of approximately 1 (*3*)—the characteristic acceleration of metabolic rate that is observed in almost all other tissues is absent in hyperthyroidism (*4*).

Previous observations (*5*) have indicated that thyroxin pretreatment in vivo stimulates amino acid uptake into the protein of rat liver slices. To investigate further the apparent relationship between thyroid function and protein synthesis, studies were undertaken to determine the effects of in vivo and in vitro thyroxin administration on the in vitro incorporation of DL-leucine-1-C^{14} into the proteins of rat liver homogenates. Livers from 90- to 150-g fasting, male Sprague-Dawley rats were homogenized by means of glass homogenizers in 5 ml of $0.25M$ sucrose solution per gram of tissue. Homogenization was performed at 0° to 2°C, and tissue fractions were maintained at that temperature through all subsequent operations until final incubation. Intact cells, nuclei, and cell debris were removed by centrifugation at 700*g* for 10 minutes. The supernatant fluid was spun at 54,000*g* for 60 minutes in a Spinco model L ultracentrifuge. The

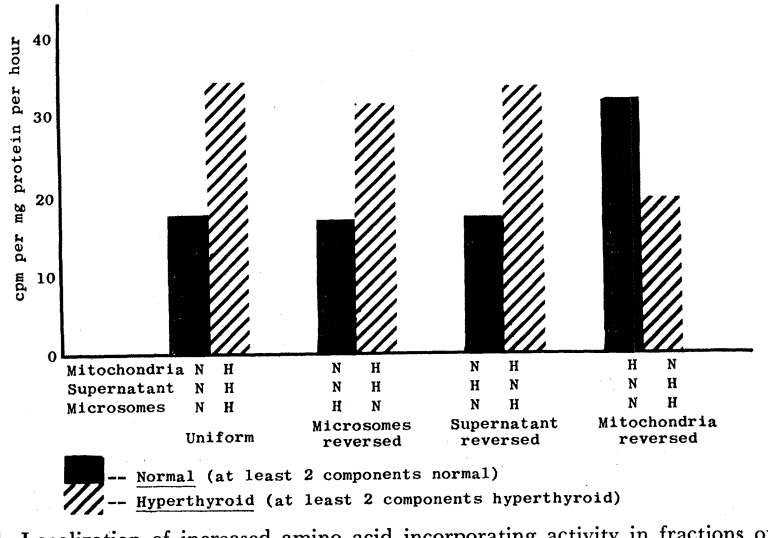

Fig. 1. Localization of increased amino acid incorporating activity in fractions of liver homogenates from rats pretreated with thyroxin. Results are representative of seven such experiments.

sediment, containing both mitochondrial and microsomal fractions, was suspended in appropriate amounts of 0.25*M* sucrose and supernatant fluid to yield a suspension containing particulate fractions and supernatant fluid equivalent to approximately 200 mg and 30 mg of liver, respectively, per 0.45 ml, the quantity of homogenate added to each of the experimental flasks.

In eight experiments rats were paired for age and weight; one received almost daily intraperitoneal injections of 100 μg of sodium thyroxin in 1 ml of 0.01*N* NaOH; the other received equivalent amounts of the NaOH solution alone. After at least six doses in 7 days, homogenates were prepared simultaneously from both animals as described above, and DL-leucine-1-C^{14} incorporation activity in both was measured in parallel flasks in a single combined experiment. Flask contents and incubation procedure are described in the title of Table 1. The reaction was terminated with 12-percent trichloroacetic acid, and the precipitated protein was purified and plated on filter paper by a modification of the method of Siekevitz (*6*). Sample weights were determined from difference in planchet weights before and after plating. Radioactivity was measured with a thin-window Geiger-Mueller counter; total counts collected were sufficient to yield a 3-percent coefficient of variation. Counting rates were corrected for background, self-absorption, and zero time controls. The results are summarized in Table 1. Although protein nitrogen concentrations, as determined by the micro-Kjeldahl technique, were identical in both groups (2.17 mg per flask), leucine incorporation was substantially greater in the homogenates from thyroxin-treated rats.

Table 1. Effects of thyroxin on DL-leucine-1-C^{14} incorporation into protein of rat-liver homogenates. To each flask (25-ml Erlenmeyer) were added 5 μmole of adenosine-5′-monophosphate, 20 μmole of potassium phosphate (*p*H 7.4), 5 μmole of $MgCl_2$, 50 μmole of potassium α-ketoglutarate, 0.8 μmole of DL-leucine-1-C^{14} (specific activity, 5.33 μc/μmole), and 0.45 ml of the appropriate homogenate prepared in 0.25*M* sucrose, as described in the text. In in vitro studies, 0.022 μmole of sodium thyroxin contained in 0.1 ml of 0.01*N* NaOH was added to the experimental flasks; all other flasks received equivalent amounts of the NaOH solution alone. The reaction mixture was brought to a final volume of 1.7 ml with 0.25*M* sucrose. Incubation in air was carried out with shaking in a water bath at 37°C for 1 hour. Zero time controls were included in all experiments.

Item	Activity (count/min mg of protein per hr)	
	Mean	Standard error
Thyroxin pretreatment in vivo (8 rat pairs)		
Normal rat	29.0	± 1.9
Hyperthyroid rat	42.3	± 3.0
Difference	13.3*	± 3.2
Effect (%)	+46	
Treatment with 1.3 × 10^{-5}M thyroxin in vitro (7 experiments)		
Control	26.9	± 1.8
Thyroxin-treated	31.9	± 2.3
Difference	5.0*	± 1.4
Effect (%)	+19	

* Denotes statistical significance; $p < .02$ (determined by method of paired comparison).

In order to localize the source of the increased activity, mitochondria and microsomes were prepared separately from livers of both types of animals by centrifugation at 15,000*g* for 15 to 20 minutes and 105,000*g* for 60 minutes, respectively. All possible combinations of mitochondria, microsomes, and supernatant derived from both homogenates were incubated as described above. Representative results of an experiment of this type are illustrated graphically in Fig. 1. It is clear that most, if not all, of the increased activity is localized in the mitochondrial (15,000*g*) fraction.

In Table 1 are also summarized the results of seven experiments in which the effects of $1.3 \times 10^{-5}M$ thyroxin added in vitro to normal rat liver homogenates were studied under the conditions specified. Although less pronounced, the effects were just as consistent as those observed with thyroxin administration in vivo, a stimulation occurring in every one of the experiments. Similar effects have been observed in several experiments with slightly altered conditions. The effect is erratic with more highly concentrated homogenates and is completely eliminated by doubling the Mg^{++} concentration. Preliminary observations indicate that increasing graded effects occur with thyroxin concentrations between 1×10^{-7} and $1 \times 10^{-4}M$. At $1 \times 10^{-3}M$, the effects of the uncoupling of oxidative phosphorylation supersede, and a marked inhibition of amino acid incorporation occurs, indicating a qualitatively different phenomenon.

To test the possibility that the thyroxin effects may be preservative rather than stimulatory, a few short-term incubations have been performed. The effects of thyroxin pretreatment in vivo are clearly not preservative; they are as great during the linear period of amino acid incorporation as at 60 minutes. The effects of thyroxin in vitro are less clear; they are distinctly present during the linear period but become more pronounced with longer incubation.

The results of these studies (*7*) suggest that uncoupling of oxidative phosphorylation is not a physiological action of thyroxin. They support, rather, the hypothesis that thyroxin stimulates energy-requiring processes, such as protein synthesis, and that its characteristic acceleration of oxygen consumption is secondary to the increased demand.

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Serial Errors in Human Learning: a Test of the McCrary-Hunter Hypothesis

Abstract. An experiment was conducted on 120 human subjects to test the hypothesis that the probability distribution of serial errors is an invariant property of rote memorization. Contrary to the hypothesis, the relative difficulty function was significantly affected by ability to learn. There was a systematic tendency ($p < .05$) for fast learners to commit proportionately more errors in the middle of the sequence.

The present communication reports an experimental test (*1*) of a hypothesis concerning certain learning phenomena advanced by McCrary and Hunter (*2*). Upon reexamining many classical serial position curves, in which distribution of practice had caused unequal reductions in errors at the central and extreme positions of a list, McCrary and Hunter conceived the idea of employing *relative* rather than *absolute* measures of difficulty. To this end, they replotted some earlier data by expressing the mean errors committed at each serial position "as a percentage of the total mean errors" (*2*). The results were as follows: (i) all curves had the typical skewed, bowed form which reveals subjects' greater difficulty with intermediate items than with initial or final items, and (ii) all curves had basically the same form, showing no consistent differences in trend under variation of work conditions.

From these findings, McCrary and