Several adult mice of both sexes from each colony were sacrificed and cobamide coenzymes were isolated from the pooled livers according to TOOHEY and BARKER[12]. Essentially the method consists of the preparation of an acetone powder followed by the extraction of cobamide coenzymes into hot 80% ethanol. The ethanol was removed and after extraction of phospholipids with ether, the solutions were passed through an anion exchange column, the cobamide coenzymes were concentrated with phenol, passed over a cation exchange resin at neutral pH and finally adsorbed and eluted from a 5 × 18 mm column of Dowex-50-Na^+H^+. The absorption spectrum of the purified coenzyme is shown in the Figure along with the spectrum of authentic coenzyme B_{12}. The amount of cobamide coenzyme in the various fractions was determined by the glutamate isomerase assay[16] (Table).

Determination of cobamide coenzyme in mouse liver. This assay was made after passing the coenzyme-containing solution through Dowex-50 at pH 7.0

Condition of mice	Control	Germ-free
Weight of pooled liver tissue in g	10	26
Cobamide coenzyme activity[a], mμmoles/kg	190	239

[a] The values are expressed in terms of the activity relative to that of crystalline coenzyme B_{12} in the glutamate isomerase assay system.

The results indicate that the livers of germ-free mice contain significant quantities of cobamide coenzyme and the values obtained compare very well with those obtained with mice harboring a flora. They also approximated values reported for human and lamb liver, which were 220 and 180 mμmoles, respectively, per kg of fresh liver at the same purification step. It was not possible to identify the specific base of the coenzyme obtained from the germ-free mice due to the limited quantity of tissue available.

The available information on the metabolic activities in tissues from germ-free animals has not indicated any profound abnormalities. Thus, one might have concluded that B_{12} coenzymes would be found in germ-free mouse liver if mammalian methylmalonyl-coenzyme A isomerase catalyzes an obligatory, or even a quantitatively significant, reaction for normal metabolic function.

Zusammenfassung. Die enzymatische Synthese von Cobamid-Coenzymen ist aus Extrakten, die von Mikroorganismen gewonnen wurden, gut bekannt, nicht aber von Säugetiergeweben. Unsere Befunde mit Cobamid-Coenzymen in der Leber von keimfreien Mäusen scheint anzuzeigen, dass die Verwandlung von Vitamin B_{12} in Coenzymform von Enzymen der Säugetiergewebe katalysiert werden kann.

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Thyroxine Administration and the Uptake of Amino Acids by Liver Cell Nucleolus, Nucleus and Cytoplasm, and by Other Cell Types[1]

Several works show the important role of the thyroid hormone on growth in general (GEMMIL[2]; GROSS and PITT-RIVERS[3]; SCOW[4]) and protein synthesis in liver (SOKOLOFF and KAUFMAN[5]; STEIN and GROSS[6]). Others observed that the thyroid hormone induces an enlargement of the nucleoli in liver cells (STENRAM[7]), although the dry matter concentration of this organelle did not change (STENRAM[8]). The present work arose from an attempt to determine whether these various effects of thyroxine are related. The uptake of labelled amino acid was examined by radioautography in the various parts of the cells in liver and other organs.

Eight litter-mates, suckling rats, were used. Four of these rats received single daily intraperitoneal injections of 20 μg of *d-l*-thyroxine per 100 g of body weight, during 6 days. The other four animals served as controls. To label the newly formed proteins, 2 controls and 2 thyroxine treated rats each received one subcutaneous injection of 5 μc/g of body weight of *d-l*-tryptophane-H^3 (specific activity 658 mc/m*M*). The other 2 thyroxine injected rats and corresponding controls each received 5 μc/g of body weight of *d-l*-phenylalanine-H^3 (specific activity 126 mc/m*M*). Both amino acids were labelled in general and prepared by the Radiochemical Centre, Amersham, England. All rats were sacrificed 12 h after the amino acid injection. Organs were fixed in Bouin's fluid, embedded in paraffin, sectioned at 6 μ, and radioautographed with AR10 stripping film (Messrs. Kodak Ltd., London). Exposure time was from 22 to 105 days. Some radioautograms were stained, after processing, with basic fuchsin (BERGERON[9]) while others were left unstained.

The amount of radioactivity was estimated with the help of an ocular grid and grains were counted over 36 μ^2 of cytoplasm in each of 20 cells. In liver cells, the nuclear and nucleolar radioactivity was also assessed. All grains over each of 20 nuclei and nucleoli were counted, the respective areas estimated from the diameters, and the number of grains was also expressed per 36 μ^2 of each structure.

Figure 1 shows that, when injected into intact suckling rats, thyroxine accelerates the uptake of tryptophane-H^3 by liver cell nucleus and cytoplasm. The uptake by cytoplasm of other cell types, however, is decreased (Figure 2). Both amino acids used (tryptophane-H^3 and phenylalanine-H^3) gave similar results, which make us suppose

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that an effect of the hormone on the general protein metabolism was detected, rather than an action upon a specific protein, as was described in other conditions (PAIK and COHEN[10]).

It is known that thyroid hormone stimulates the protein synthesis in liver (SOKOLOFF and KAUFMAN[11]; STEIN and GROSS[6]). This was confirmed by the grain counts presented in this paper, which show in addition that the nuclear incorporation of amino acids by liver cells is also accelerated.

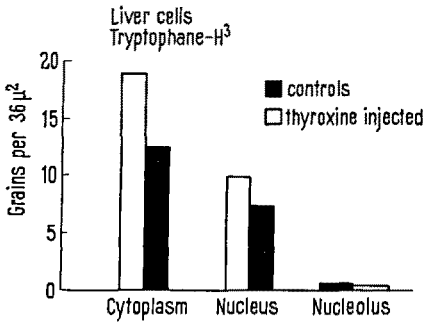

Fig. 1. Shows the effects of thyroxine on the uptake of H^3-labelled tryptophane by liver cell components. The results are represented as silver grains per 36 μ^2 of each structure. The number of grains represented by each bar is the average for 40 cells from 2 rats. The differences in the uptake of either amino acid, between the thyroxine-injected rats and the controls are significant ($P < 0.01$).

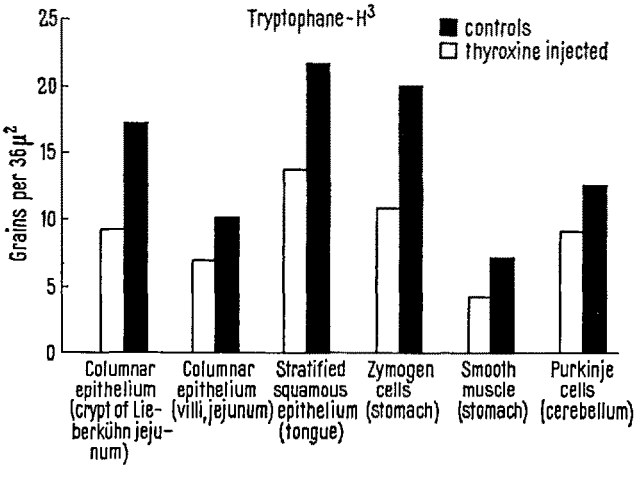

Fig. 2. In several other cell types the effect of thyroxine on amino acid uptake is the reverse of that found in liver. In this figure each bar is the average for 40 cells from 2 rats. Results are represented as 'photographic' grains per 36 μ^2 of cytoplasm. Grains over nuclei were avoided. In each case, the differences between thyroxine injected and control rats are significant ($P < 0.01$).

Confirming the findings of STENRAM[7], we observed an enlargement in nucleoli of liver cells from thyroxine treated rats. The mean volumes, obtained from measures made in 10 nucleoli in each lot of animals (treated and controls) are 2.1 ± 0.55 μ^3 mean and standard deviation for the control rats, and 5.6 ± 1.15 μ^3 for the thyroxine injected ones.

In spite of the nucleolar hypertrophy produced by thyroxine in liver cells, and the increased uptake of amino acids by nucleus and cytoplasm in this situation, most of the enlarged nucleoli were not labelled (Figure 1).

Many authors agree that the nucleolus is active in incorporating RNA precursors (AMANO and LEBLOND[12]; SIRLIN[13]). However, the results on the uptake of labelled amino acids by the nucleolus are somewhat controversial[14–17].

In contrast to the nucleolus, in both groups of rats, most of the chromatin clumps were labelled, mainly the nucleolar associated chromatin and the nuclear membrane attached chromatin masses. The radioactivity present in nucleolar associated chromatin was particularly easy to see in the cells with thyroxine enlarged nucleoli.

The data available are not enough to interpret fully the effects of thyroxine on protein synthesis in the several cells examined. But whatever mechanisms are involved, our results show that thyroxine injected into suckling rats produces dissimilar effects on amino acid uptake, according to the organ examined.

Résumé. L'administration de thyroxine cause une chute de l'incorporation de la phénylalanine-H^3 et du tryptophane-H^3 par divers organes, excepté le foie. Le cytoplasme et les noyaux des hépatocytes ont presenté une plus grande concentration de substance injectée.

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Riboflavin in Human Milk

MODI and OWEN[1] have observed that the riboflavin in human milk is present as flavin adenine dinucleotide (FAD). GIRI et al.[2] have reported the presence of two enzymes associated with the synthesis of FAD from riboflavin in human milk; one of them formed FAD from riboflavin and the other one synthesized flavin mononucleotide (FMN) from riboflavin. No investigation has so far been made about the nature of FAD or whether it is in the combined or free form. In this communication, the results of experiments carried out to study the amount and nature of FAD in human milk are reported.

Riboflavin was extracted from milk by the method of BESSEY et al.[3]. For chromatographic identification of the

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