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.

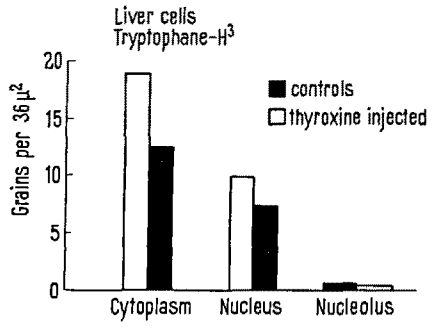

Fig. 1. Shows the effects of thyroxine on the uptake of H^3-labelled tryptophane by liver cell components. The results are represented as silver grains per 36 μ^2 of each structure. The number of grains represented by each bar is the average for 40 cells from 2 rats. The differences in the uptake of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

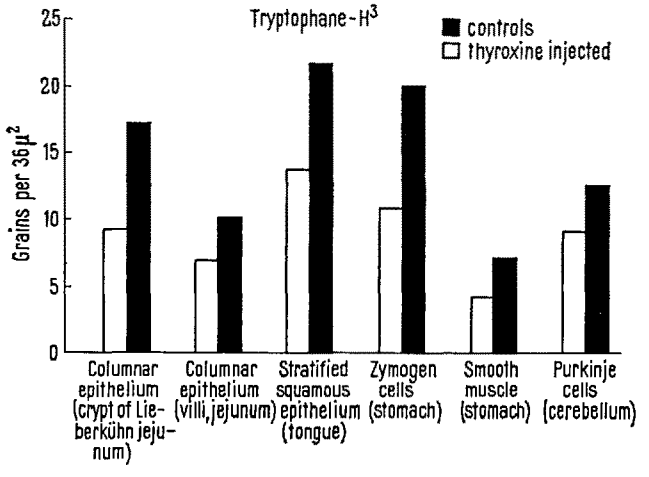

Fig. 2. In several other cell types the effect of thyroxine on amino acid uptake is the reverse of that found in liver. In this figure each bar is the average for 40 cells from 2 rats. Results are represented as 'photographic' grains per 36 μ^2 of cytoplasm. Grains over nuclei were avoided. In each case, 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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Flavin adenine dinucleotide (FAD) hydrolysis in cow and human milk

Time in h	Amount of FAD (μg)		Amount of riboflavin and riboflavin phosphate produced and calculated as riboflavin (μg)		Percentage of FAD destruction according to riboflavin estimation	
	Cow	Human	Cow	Human	Cow	Human
0	10.0	10.0	13.08	10.25	–	–
1			18.91	13.91	76.4	53.0
2			19.00	14.82	78.0	62.0
3			19.00	14.82	78.0	62.0

concentrated material, Butanol/acetic acid/H_2O system of CRAMMER[4] was used. FAD was enzymatically estimated in the D-amino acid oxidase system as described by MANSON and MODI[5]. Under these conditions, 0.5 μg FAD produced an oxygen uptake of 75 μl; on examining 2 ml of whole milk for the presence of free FAD no detectable amount was found to be present. On the other hand, when human milk was boiled for 3 min, the supernatant free from precipitated proteins contained FAD. In analyzing a number of such samples, it was observed that the FAD content varied between 0.15 and 0.4 μg/ml. On paper chromatographic examination, one fluorescent spot corresponding to FAD was seen. From the above observation, it seems that FAD of human milk is bound to one or more of the milk proteins which on heating become denatured and liberate free FAD.

The complete absence of free FAD cannot, however, be assumed, because the method, due to the limitations of the assay system, could be expected to detect only amounts greater than 0.02 μg/ml. In order to explore the possibility of the occurence of free FAD in human milk, the fate of FAD added to raw milk was studied. MANSON and MODI[5] had observed 100% degradation of FAD in cow milk within 1 h. 2 ml samples of cow and human milk were incubated at 37°C with 20 μg FAD for 3 h. The amount of FAD present in trichloroacetic extracts was estimated by the method of BURCH, BESSEY and LOWRY[6]. From the figures listed in the Table, it can be seen that, though less than in cow milk, there was considerable destruction of FAD in human milk. Paper chromatography indicated that FAD was hydrolysed to free riboflavin. This was further confirmed by incubating known amounts of FAD with human milk proteins obtained by ammonium sulphate fractionation. The results indicate that, if 0.2 μg of FAD was incubated with milk protein and the specific protein of D-amino acid oxidase, wide variations in oxygen uptake were obtained depending upon the order in which the reactions were added to the incubation vessel. When FAD and enzyme solution were mixed before the addition of milk fraction, concordant values for the rate of O_2 consumption were always obtained. In this experiment it was observed that milk proteins hydrolyzed the added FAD, thus suppressing the reaction of D-amino acid oxidase.

Preliminary studies have been made to characterize the combined FAD form. Cow milk is a rich source of xanthine oxidase, of which FAD is the prosthetic group. Unlike cow milk, human milk does not contain xanthine oxidase[1], or, if it does contain it, is a very poor source[7]. Therefore, a preliminary study about the occurrence of milk flavoproteins was made. Using the procedure of BAILLE and MORTON[8], reduced diphosphopyridine nucleotide-diaphorase and reduced diphosphopyridine nucleotide cytochrome C reductase in human milk were detected in significant amounts, whereas glycine oxidase and succinic dehydrogenase were not detectable, using the method of RATNER[9] and BONNER[10] respectively.

Zusammenfassung. Riboflavin ist in der Muttermilch in Form von Flavin-adenin-dinucleotid anwesend, was für eine Komplexform spricht. Ferner wurden in der Milch zwei Flavoproteine, die DPN-Cytochrom C-Reduktase und DPN-Diaphorase reduzierten, gefunden.

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Oxygen Isotope Paleotemperature Measurements on Lower Jurassic Belemnoidea from Bamberg (Bavaria, Germany)

Specimens of Belemnoidea were collected from the Trimeusel locality on the Main river near Bamberg (Bavaria)[1]. This is an outcrop of the upper part of Lias epsilon (Toarcian). The oxygen isotope analyses were carried out on CO_2 samples prepared according to the normal techniques[2] and using an Atlas-Werke M86 mass-spectrometer. The standard was CO_2 made from Carrara marble, but the results reported are corrected to relate to

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