

who suffered from dementia. The six other cases showed few or no mental symptoms.

The objection may be raised that any biochemical dissimilarities between normal and neoplastic tissues may result from the disease process and not from differences in cell constitution. Thus the fact that 5-HT was found in smaller quantities in gliomas than in normal brain does not prove that normal glia contain less 5-HT than nerve cells but constitutes suggestive evidence. It must be noted, however, that two instances of high pressor activity have been reported^{6,7} in neoplastic tissues from a child. This is of interest because tumours from young adults or children are likely to be derived from embryonic tissue and are therefore more closely related to true nerve cells.

In this investigation the 5-HT content of both gliomas and meningiomas from adult patients was found to be lower than that of adjacent normal cerebral cortical tissue and the hypothesis that the distribution of 5-HT might be related to a cell type such as the glial cells was therefore not supported^{7,8}.

Résumé. La teneur en 5-HT de tumeurs intra-crâniennes humaines, déterminée par une méthode biologique, est en général plus faible que celle du cortex cérébral adjacent. Cette observation n'est pas en faveur de l'hypothèse que la 5-HT est localisée dans les cellules névrogliques.

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⁸ This work was carried out at the Institute of Neurology, London. The author wishes to thank Mr. W. McKISSOK for the human tissues and Dr. J. MARSHALL for helpful discussions. Messrs. May and Baker supplied the 5-hydroxytryptamine creatinine sulphate and generous financial assistance.

Effect of Lysine and Threonine Deficiency on the Endogenous Cholesterol Content of Rat Plasma and Liver¹

An important stimulus to studies of the relationship between dietary proteins and cholesterol metabolism is the observation that not only the level but also the type of dietary protein is involved in the regulation of the serum cholesterol level in experimental animals (cebus monkey, rat, chicken) maintained on diets containing cholesterol^{2,3}.

More recently, data have been presented which would suggest that in essential amino acids deficiency, especially in the chicken, balance and imbalance may be of importance in the metabolism of endogenous cholesterol^{4,5}.

However, in reviewing animal and clinical studies, it appears that in the absence of cholesterol in the diet, relative deficiencies of lysine^{4,5}, methionine⁴, tryptophan⁴, and leucine⁶ in chickens result in a hypercholesterolemic response, while in adult man fed a rice diet^{6,7} which is lysine- and threonine-limited⁸, and in children with kwashiorkor, a multiple amino acid deficiency disease, the serum cholesterol is low⁹. Further, the amounts of serum cholesterol, according to SINGAL et al.¹⁰, do not differ in rats on amino acid rations deficient in lysine or in threonine from those observed in animals on an adequate amino acid ration.

In the present communication, the effect on the endogenous cholesterol metabolism in the rat, of low protein isonitrogenous diets deficient in or balanced in lysine and threonine is reported.

For this purpose, both liver and plasma cholesterol values are examined.

Methods. Twenty-four weanling male rats of the Wistar strain, weighing 40 ± 2 g were divided into two groups. The composition of the diets is reported in Table I. As is shown, the diets are isonitrogenous and, following ROSEMBERG et al.¹¹, lysine and threonine supplemented to obtain better growth and physiological levels of hepatic lipids.

The animals were weighed weekly during the six weeks experimental period, and the daily food consumption was calculated from weight.

Food and water were given *ad libitum*. At the end of the experimental period, plasma was obtained by using the procedure outlined by HANDLER¹².

Table I. Diets used

Ingredient %	1	2
White polished rice	89	89
Salt mixture ^a	4	4
Corn oil	3	3
Cod liver oil	1.5	1.5
Vitamine mixture ^b	1.5	1.5
Diammonium citrate	1	—
L-Lysine HCl	—	0.425
DL-Threonine	—	0.360

^a K_2HPO_4 322 g, $CaCO_3$ 300 g, NaCl 167 g, $MgSO_4 \cdot 7H_2O$ 102 g, $CaHPO_4 \cdot 2H_2O$ 75 g, $FeC_6H_5O_6 \cdot 6H_2O$ 27.5 g, $MnSO_4 \cdot H_2O$ 5.1 g, KJ 0.8 g, $CuSO_4 \cdot 5H_2O$ 0.3 g, $ZnCl_2$ 0.25 g, $CoCl_2 \cdot 6H_2O$ 0.05 g.

^b At the 1.5% level, the vitamin mixture supplies per 100 g diet: Choline chloride 150 mg—inositol 75 mg—*p*-aminobenzoic acid 75 mg— α -tocopherol acetate 7.5 mg—nicotinic acid 3 mg—riboflavin 1.5 mg—calcium pantothenate 3 mg—thiamine 0.75 mg—pyridoxine hydrochloride 0.75 mg—menadione 0.375 mg—folic acid 0.075 mg—biotin 18.75 μ g—vitamin B_{12} (0.1% triturate) 1.500 mg.

¹ Supported by grant from Consiglio Nazionale delle Ricerche, Roma.

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The rats were then killed by decapitation and the livers removed and stored at -15°C . The plasma cholesterol was determined according to the method of PEARSON et al.¹³ and liver cholesterol, according to that of SPERRY and WEBB¹⁴. Fats were determined by the method outlined by HANANHAN et al.¹⁵.

Results. A comparison of the results obtained using diets with the same nitrogen content, with and without lysine and threonine supplementation, appears in Table II. As shown, the rice diet deficient in lysine (first limiting amino acid) and threonine (second physiologically limiting amino acid) induces, in growing rats, in addition to a lower growth rate, fatty liver of the portal type as seen in kwashiorkor and an increase of total liver cholesterol. In these conditions plasma cholesterol is low. Lysine and threonine supplements are effective in promoting growth and in preventing the accumulation of fats in the liver, and, while the liver cholesterol is lowered, the plasma level is increased.

Our results on plasma cholesterol are in agreement with those found by SCHENDEL and HANSEN in kwashiorkor⁹ and KEMPNER⁶ and OLSON et al. in man⁷.

In regard to SINGAL's¹⁰ result with amino acid ration, our data are in agreement with his findings on liver cholesterol. The failure to find variations in serum cholesterol under those conditions might be ascribed to the use of different diets.

Discussion. Since plasma cholesterol is lower in deficient rats than in those with lysine and threonine, while the opposite is observed regarding liver cholesterol, the phenomenon, as a whole, supports the view that serum lipoproteins transporting cholesterol are not elaborated at normal rates in amino-acid-deficient rats as in children with kwashiorkor. In addition to choline¹², methionine¹⁶, and EFA¹⁷; the amino acids lysine and threonine also seem to be able to control the cholesterol transport from liver to blood in the rat.

If we also consider that the protein level acts on the biosynthesis and catabolism of liver cholesterol^{2,3}, it

seems possible that the variations we observed in the liver may even depend on these mechanisms.

In view of the fact that our results are in conflict with those of JOHNSON et al.⁴ and KOKATNUR et al.⁵, who found that the growth failure in amino acid deficient chickens is associated with an increase in serum cholesterol, it would appear that uricotelic animals react differently to ureotelic animals in essential amino acid deficiency to cholesterolemic response.

Therefore, the rat seems to be a suitable animal for studies of deficiency of, balance of, and imbalance of essential amino acids in cholesterol metabolism, if the results are to be referred to man.

Riassunto. È stato studiato l'effetto di diete ipoproteiche e isoazotate carenti e supplementate in lisina e treonina sul contenuto in colesterolo del fegato e del plasma.

La carenza di aminoacidi essenziali induce negli animali più elevati livelli di colesterolo nel fegato, mentre diminuisce il colesterolo del plasma.

In queste condizioni il ratto dimostra un comportamento simile all'uomo.

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Department of Biological Chemistry, University of Bologna (Italy), December 3, 1962.

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Table II. Effect of supplementation of rice diet with lysine and threonine on growth, total lipid content of the liver, liver and blood plasma cholesterol content (Mean values \pm Standard Deviation)

Diet	Average weight in g	Total liver lipids g/100 g liver	Total plasma cholesterol mg/100 ml	Total liver cholesterol mg/100 g tissue
No. 1	74.5 \pm 12.3	9.49 \pm 1.5	111.4 \pm 14.3	367 \pm 55.8
No. 2	144.8 \pm 27.9	5.93 \pm 0.72	130.3 \pm 12.8 ^a	203 \pm 46.5 ^b

^a Significantly different from values of diet No. 1. $P = < 0.01$.

^b Significantly different from values of diet No. 1. $P = < 0.01$.

Somatic Evoked Responses in Cats during Natural Sleep¹

The aim of the present research is to study the patterns of somatic evoked responses in some central nervous structures of the cat during natural sleep. Such an experimental approach seems justified in view of some recent studies² which have shown that sleep can be subdivided into two fundamental phases: (i) light sleep, which is characterized by EEG slow waves (2-4/sec) intermingled with spindles (8-15/sec), moderate muscular tonus and basal blood pressure level; (ii) deep sleep, which is charac-

terized by EEG low voltage fast activity, complete muscular relaxation and a marked decrease in blood pressure.

Material and Methods. The experiments were carried out on 30 adult, unanaesthetized, freely moving cats, carrying stimulating and recording electrodes previously introduced aseptically during barbiturate anaesthesia. It

¹ Part of these results has been presented at a meeting of the Società Italiana di Biologia Sperimentale held in Genova on July 25th 1962.

² O. CANDIA, E. FAVALE, A. GIUSSANI, and G. F. ROSSI, *Arch. Ital. Biol.* **100**, 216 (1962).