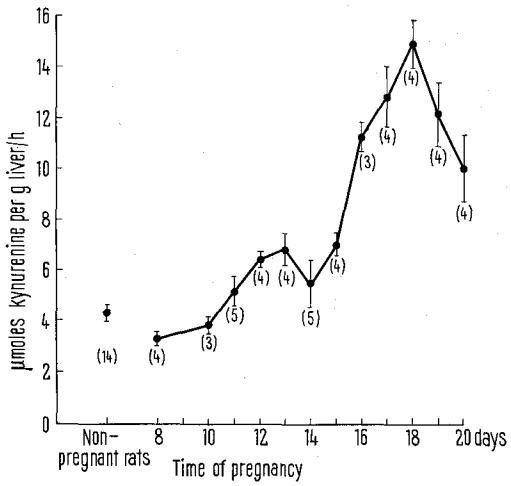

Fig. 1. Variation in liver tryptophan pyrrolase activity from the 8th to 20th day of pregnancy. Values are expressed as the mean ± S.E.M. The number of animals in each group is given in parenthesis.

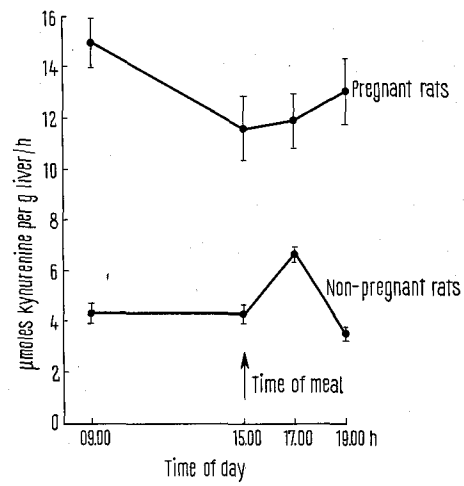

Fig. 2. Variation in liver tryptophan pyrrolase activity in 18-day pregnant and non-pregnant rats after feeding of a single meal at 15.00 h. Values are the mean ± S.E.M. of 14 rats in the case of the non-pregnant rats killed at 09.00 h and of 4 rats in the case of all other groups.

(Figure 1 and Table). The level in non-pregnant rats was however significantly lowered by restricting their food intake ($P < 0.05$), confirming a previous observation of FULLER[13]. Fuller also showed that when normal rats were fed with single daily meals, tryptophan pyrrolase activity increased during the 2–3 h after they had eaten. This effect was also noted in the present work in non-pregnant rats, where a 51% increase in activity occurred 2 h after the meal (Figure 2). However in 18-day pregnant rats, tryptophan pyrrolase was not significantly altered in the immediate post-absorptive period (Figure 2) even although all the food offered was consumed within 1 h. Thus in the pregnant rat, tryptophan pyrrolase level appears to be insensitive to food intake.

The temporal pattern of change in tryptophan pyrrolase activity during pregnancy bears a close similarity to that shown by liver RNA[15]. A placental factor has been shown to be involved in liver RNA metabolism in pregnancy[16] and the possibility therefore exists that the placenta may also have a regulatory effect on tryptophan pyrrolase. The facts that the rise in tryptophan pyrrolase was not evident until the placenta was established and that rat placental endocrine function has been shown to be independent of diet[17] lend some support to this view.

Résumé. Chez la rate, la dose de tryptophan pyrrolase fut augmentée du douxième au quinzième jour de gestation. L'activité de ces rates gravides ne changea pas quand leur nourriture fut modifiée.

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Influence of Dietary Methionine on Rat Growth and Thyroid Activity

COHEN, CHOITZ and BERG[1] reported that the addition of 1.8% methionine to a diet containing 12% casein produced marked growth depression in rats. They also found that while 1.45% cystine added to the diet did not affect the growth rate, the addition of 1.6% homocystine did greatly decrease the growth rate. If high dietary methionine were converted to homocystine at a rate greater than that which could be removed by metabolic processes, the latter's toxicity might decrease the growth rate of the rat in a similar fashion. BENEVENGA and HARPER[2] have reported that feeding DL-homocystine equivalent in sulfur to 3% methionine with a 10% casein diet reduced growth rate of rats about the same extent as occurred with feeding methionine. Both dietary treatments[2] produced pathological lesions in the spleen, pancreas, liver, small intestine and kidney of the rat. Methionine levels in the plasma of rats fed 2% methionine with 18% casein diet were increased[3] from 10 µg/ml to 200 µg/ml by the dietary treatment. The observed pathological changes may be related[4] to methionine's effect on certain tissue enzymes, e.g., as an essential amino acid in structure of tissue protein and as a methylation reagent both effecting protein anabolism. Dietary

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methionine (2%) prevented[5] the increase of liver 3-phosphoglycerate dehydrogenase in rats fed a low protein 2% casein diet.

Since thyroid hormones facilitated protein catabolism in hyperthyroid adults and protein anabolism in growing animals[6], interactions between dietary amino acids and thyroid activity might exist. CHARKEY[7] has reported that addition of methionine to a methionine deficient diet containing iodocasein suppressed the increased oxygen consumption in the chick that was caused by iodocasein. Since the plasma amino acid patterns were directly proportional[8] to the essential amino acid composition of ingested protein, the influence of methionine on rat growth and thyroid activity were determined here by feeding diets containing different levels of added methionine. Thyroid activity was estimated from the conversion of ^{131}I to protein-bound ^{131}I in plasma. Rat growth was measured as the average gain in weight per week.

Methods. Weanling Wistar rats were divided equally with respect to litter and sex into groups of 10 rats each. The weanling rats were fed a basal diet (Table I) in addition to different levels of iodine and DL-methionine or cystine. The excess amino acids were added at the expense of diammonium citrate to keep the total nitrogen content of the diets constant. The iodine content of the basal diet was 19 ppb as determined by the method of Parker[9]. To the diets with iodine levels of 50 and 100 ppb, 2.25% methionine was added to bring the total level of the amino acid to 2.8%. Methionine was also added to diets containing 85 ppb iodine to give final levels of 1.0%, 1.4%, 2.1% and 2.8%. Cystine was added to a diet containing 85 ppb iodine to give a level of 2.14%. The rats were given feed and deionized water ad libitum for 6 weeks. 24 h prior to sacrifice, each rat was injected i.p. with 1 ml of sterile isotonic saline containing 10 μC of carrier free ^{131}I. Upon sacrifice, thyroid glands and heparinized blood samples were removed from each rat. 1 ml of plasma was counted for total ^{131}I content using a probe type scintillation counter. Protein-bound iodine, $PB^{131}I$, was determined by the method of BARKER[10] as modified by COMAR[11].

Results. Decreasing the iodine level in the diet increased the thyroid size per 100 g of body weight and increased thyroid activity. The conversion of ^{131}I to $PB^{131}I$ was greater in the rats on low dietary iodine than on higher levels. This indicated that the thyroid glands of the rats at the low dietary iodine level had become more efficient in forming thyroxine. Thus, these results reflected the iodine-thyroid-pituitary interactions[6]. The growth rate was not changed by varying the levels of iodine in the diet within the range studied.

Thyroid activity was greatly decreased by the addition of methionine to 4 times the normal dietary requirements[12] in the diets containing 50 and 100 ppb iodine, i.e., 2.8% dietary methionine (Table II). The change in thyroid activity was not due to iodine contamination in methionine since iodine analysis showed that methionine

Table I. Composition of rat diet

	% of diet
Defatted wheat germ meal	45.0
Ground corn	44.0
Cotton seed oil	4.0
Mineral mixture[a]	4.0
Vitamin mixture[b]	0.3
DL-Methionine	0.3
Diammonium citrate	2.4
	100.0

[a] Supplied as percent of mineral mix: sodium chloride 4.43, anhydrous magnesium sulfate 6.78, disodium phosphate 8.86, monopotassium phosphate 19.03, potassium chloride 10.41, monobasic calcium phosphate 13.75, calcium lactate 32.46, ferrous ammonium sulfate 3.63, manganese sulfate 0.16, copper sulfate 0.25, and zinc carbonate 0.24.

[b] Composition of vitamin mixture is given in milligrams: riboflavin 500, thiamine hydrochloride 375, niacin 2,500, *i*-inositol 15,000, pyridoxine hydrochloride 500, choline chloride 100,000, calcium pantothenate 2,500, *p*-aminobenzoic acid 15,000, menadione 250, biotin 5, folic acid 5, vitamin B_{12} 1.0, α-tocopherol 25,000, vitamin A acetate 25, and calciferol 2.5.

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Table II. Influence of methionine on growth rate and thyroid function

Diet Supplementation	Growth rate (g/week)	Thyroid size (mg/100 g body wt)	Thyroid activity[a]
50 ppb iodine	28±2[b]	10.6±0.6	97±2
Idem + 2.8% methionine	13±1	8.8±0.4	82±5
Significance	$p<0.001$	$p<0.025$	$p<0.01$
100 ppb iodine	27±2	7.6±0.3	90±4
Idem + 2.8% methionine	15±1	8.4±0.8	68±7
Significance	$p<0.001$	n.s.	$p<0.02$
85 ppb iodine[c]	11±1	7.3±0.7	78±5
Idem + 2.8% methionine[c]	13±1	7.4±0.5	74±8
Significance	n.s.	n.s.	n.s.

[a] plasma $PB^{131}I$/plasma $^{131}I \times 100$. [b] Means ± SE. [c] In this experiment rats in first group were pair fed with those in second group.

contributed only 0.9 ppb iodine to the diet. Increasing the dietary DL-methionine level to 2.8% decreased the growth rate by 50%.

The addition of cystine to the diet levels of sulfur equivalent to that provided by 2.8% methionine did not alter growth rate of thyroid activity when compared to control rats. Therefore, the conversion of methionine to cystine, or the amount of sulfur that methionine added to the diet was not the cause by which methionine decreased thyroid activity.

It appears that methionine first decreased growth through reduction of food consumption and this lack of growth in turn may have resulted in lower thyroid activity. A growth rate of only 19 ± 2 g/week ($p < 0.005$) was obtained in rats fed an 85 ppb iodine diet supplemented with methionine at a 2.1% level (not shown in Table II); no reduction in thyroid activity was observed for rats fed this diet. Moreover, control rats pair fed (no additional methionine) to those given 2.8% methionine (85 ppb iodine in both cases) showed the same decrease in growth rate and in thyroid activity (Table II).

Conclusion. Increasing the methionine level of the diet to 2.8% reduced the growth rate and thyroid activity of the rat. Restriction of the feed intake of normal rats to that consumed by rats fed a diet with 2.8% methionine produced about the same reduction in growth rate and thyroid activity that occurred by feeding 2.8% methionine in the diet. Increasing the dietary cystine level to provide the same amount of sulfur as that in the 2.8% methionine diet did not alter significantly growth rate or thyroid activity. It was concluded that the reduction in thyroid activity caused by feeding excess methionine was related to the reduction in growth rate that was due to poor food consumption.

Résumé. Une diète contenant 2.8% de méthionine a réduit la croissance et l'activité thyroïdienne du rat. La restriction de nourriture appliquée à des rats normaux a donné des résultats comparable. L'augmentation de la cystine dans cette diète pour obtenir le même taux de soufre n'a pas altéré la croissance ou l'activité thyroïdienne comparée à celle des rats normaux. En conclusion, la réduction de l'activité thyroïdienne des rats recevant un excès de méthionine est due à une insuffisance de nutrition.

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In vivo Perfusion of Human Thyroid Tissue with 4-^{14}C-Dehydroepiandrosterone and 7α-^{3}H-Dehydroepiandrosterone Sulfate. Metabolism of Steroid Conjugates. X

As it appears today, most human tissues may possess a certain capacity to metabolize steroids or steroid conjugates. In the course of such investigations the in vivo perfusion of human thyroid tissue was attempted.

In a 41-year-old, euthyroid female patient, undergoing operation due to a large colloidal struma, one half of the struma tissue was removed and the second half perfused with 2.82 µg 4-^{14}C-dehydroepiandrosterone (DHEA) (495,000 cpm ^{14}C) and 0.07 µg 7α-^{3}H-DHEA sulfate (2,810,000 cpm ^{3}H) in 1.0 ml saline via the arteria inferior. From one of the ligated veins blood samples were withdrawn by an inserted cannula. The time of collection and the volume of heparinized plasma, obtained from the various blood samples (T-1 to T-5) are indicated in Table I. In addition also a sample of peripheral blood (PB) was collected. For isolation of ^{14}C- and ^{3}H-labelled free and conjugated steroids, standard procedures were employed[1], depending on the separation of steroid conjugates, solvolysis or enzymatic hydrolysis, and multiple thin layer chromatography of free steroids as well as suitable derivatives. By determination of the specific activity in the course of subsequent thin layer chromatography, eventually after reverse isotope dilution, the identity of numerous isolated compounds could be verified.

From Table I it becomes evident that 56.0 ml of venous effluent from struma tissue contained a total of 26.35% of infused ^{3}H-activity and 27.55% of ^{14}C-activity. Such figures suggest an appreciable loss of activity which may be attributed to a drainage of tissue by smaller blood vessels and a retention of labelled compounds within the tissue. The escape of substrate or metabolites into the general circulation is demonstrated by significant ^{14}C- and ^{3}H-activity in the sample of peripheral plasma. From a delayed appearance of ^{14}C-activity in the venous effluent of perfused tissue, as compared to that of ^{3}H-labelled compounds, a certain retention of free steroids cannot be excluded. Whereas in the first sample (T-1) roughly 14% of injected ^{14}C-activity and 19% of ^{3}H-activity were detected with an isotope ratio of 7.8, the last sample (T-5) yielded only 1.9% of ^{14}C- and 0.8% of ^{3}H-activity with an isotope ratio of 2.3. Appreciable ^{3}H-activity in the fraction of free steroids reveals the presence of steroid sulfatase in struma tissue. On the other hand, the fraction of sulfoconjugated steorids did not exhibit significant ^{14}C-activity, thus demonstrating the absence of steroid sulfokinase. At the same time, the conversion of steroid sulfate to steroid sulfatide – presumably by a diglyceride transferase[2] – was found to be negligible. Likewise, no glucuronosyl transferase seems to occur in human thyroid tissue.

Concerning the metabolism of DHEA and DHEA sulfate in struma tissue, the figures in Table II indicate that 30% of ^{14}C- and 31% of ^{3}H-labelled, isolated free steroids were represented by metabolites of DHEA. In contrast hereto, only 14% of ^{3}H-labelled steroid sulfates consisted of metabolites, suggesting a preferred metabolism of the free compound. In view of the absence of sulfokinase activity, it may be assumed that the various metabolites in the fraction of sulfoconjugates arose by direct conver-

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