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-

[1] G. W. OERTEL, P. KNAPSTEIN and L. TREIBER, Z. physiol. Chem. *345*, 221 (1966).
[2] G. W. OERTEL, Biochem. Z. *339*, 125 (1963).

Table I. Free and conjugated steroids in thyroid venous and peripheral plasma

Sample	Time (min)	ml	cpm ($^3H/^{14}C$)/10 ml							
			Free steroids		Sulfates		Sulfatides		Glucuronosides	
				R		*R*		*R*		*R*
T-1	0–10	10.0	28100	0.41	504000	>100	1670	>100	651	26
			68000		63		0		25	
T-2	10–15	10.0	7850	0.26	87500	>100	584	>100	177	4.4
			29900		20		0		40	
			3810	0.25	40900	>100	423		14	0.9
T-3	15–20	10.0	15500		31		2	>100	16	
T-4	20–25	10.0	2340	0.24	28600	>100	271	>100	47	0.6
			9910		22		0		81	
T-5	25–30	16.0	1510	0.19	19200	>100	384	96	46	0.6
			7880		13		4		74	
PB	9–14	8.8	144	0.96	102	3.9	625	4.8	92	1.0
			160		26		130		94	

R = (cpm ^3H/cpm ^{14}C)

Table II. C_{19}-and C_{19}-steroids in the fractions of free and sulfoconjugated steroids

Steroid	free cpm ($^3H/^{14}C$)	*R*	Sulfate cpm ($^3H/^{14}C$)	*R*
Dehydroepiandrosterone	26400	0.32	428000	>100
	81700		33	
Androstenediol	3160	0.36	24400	>100
	8860		9	
16-OH-Dehydroepiandrosterone	1840	0.35	4170	>100
	5270		0	
Androstenetriol	1920	0.32	8680	>100
	6090		4	
Androstenedione	2610	0.30	18200	>100
	8780		31	
Testosterone	468	0.33	3050	>100
	1410		14	
Androsterone	1350	0.45	6550	>100
	2990		24	
Etiocholanolone	470	0.38	2760	>100
	1230		19	
Estrone	149	0.42	505	>100
	351		0	
Estradiol	30	0.40	403	40
	75		10	
Estriol	170	0.35	308	>100
	485		2	

R = (cpm ^3H/cpm ^{14}C)

sion of DHAE sulfate, e.g. without cleavage of the sulfuric acid ester bond. Among the different metabolites, isolated from the fractions of free and sulfoconjugated steroids, androstenediol (5-androstene-3β, 17β-diol) turned out to be the predominant C_{19}-steroid, followed by androstenedione (4-androstene-3, 17-dione) and androstenetriol (5-androstene-3β, 16α, 17β-triol). Androsterone (3α-hydroxy-5α-androstan-17-one) and etiocholanolone (3α-hydroxy-5β-androstan-17-one), the major metabolites of i.v. administered DHEA in urine, exhibited considerably less radioactivity. Furthermore, it seems to be of interest that also the fraction of estrogens contained significant ^{14}C- and ^3H-activity. Due to limited counting rates, however, their identification relied only on a twofold thin layer chromatography of free estrone (3-hydroxy-1, 3,5-estratrien-17-one), estradiol (1, 3,5-estratriene-3, 17β-diol), and estriol (1, 3,5-estratriene-3,16α, 17β-triol) as well as their acetates. It therefore remains for further experiments to establish the presence of steroid aromatase activity in human thyroid tissue beyond any doubt.

Zusammenfassung. Bei der In vivo-Perfusion menschlichen Schilddrüsen-Strumagewebes mit 4-^{14}C-DHEA und 7α-^3H-DHEA-Sulfat zeigte es sich, dass unter physiologischen Bedingungen eine beachtliche Hydrolyse von Steroid-Sulfat eintrat. Demgegenüber liess sich keine Steroid-Sulfokinase oder Steroid-Glucuronosyl-Transferase nachweisen. Der Anteil der Metaboliten erreichte in der Fraktion der freien Steroide ungefähr 30% isolierter Verbindungen, in der Fraktion der Steroid-Sulfate jedoch nur etwa 14%. Androstendiol erwies sich als wichtigster Metabolit, gefolgt von Androstendion und Androstentriol.

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