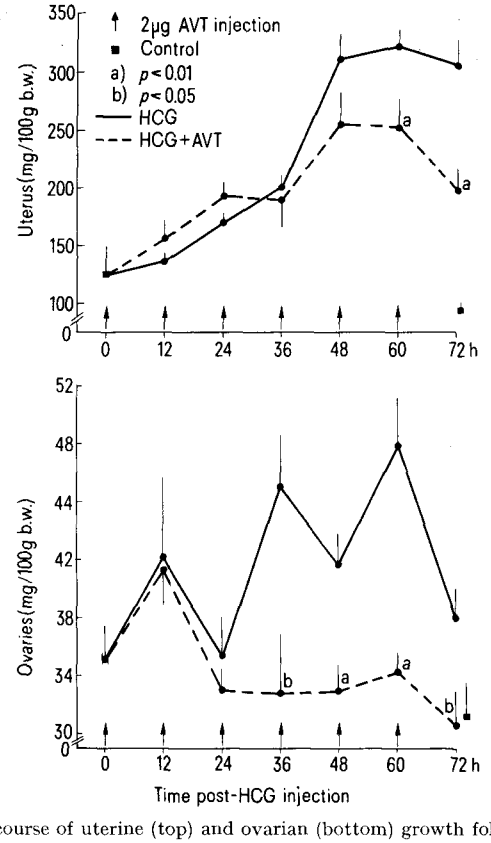
Cahn DTL electrobalance. Results at each timepoint were analyzed by a Student's t-test.

A diurnal rhythm in ovarian weight in mice treated with HCG only was observed during the 3 day course of the experiment (Figure, bottom). AVT treatment blocked this rhythm in ovarian weight during the last 2 days of the study. A significant reduction in ovarian weight was observed at 36 h and at all subsequent timepoints. In the case of the uterus, the initial phase of growth was not blocked by AVT although the latter phase was significantly attenuated (Figure, top). AVT treatment did not cause a reduction in body weight in this experiment since the final body weight of HCG-treated mice was 16.8 $\pm$ 0.6 g while that of the HCG + AVT treated mice was 15.6 $\pm$ 0.6.

A single injection of HCG causes estrogen release and uterine growth in the immature mouse within a few hours[8]. Our results indicate that AVT probably did not block the initial growth of the ovaries and uterus which is due to the peripheral action of HCG. However, endogenous gonadotrophin secretion contributes significantly during the latter stages to HCG stimulation of the ovaries and uterus in mice and rats[9,10]. Thus, the inhibition of ovarian and uterine growth reported here could be the result of the action of AVT on the hypothalamus where it may modify the discharge of gonadotrophin releasing hormones or directly on the pituitary where it may modulate the synthesis and/or release of gonadotrophic hormone. PAVEL et al.[5] favor the central gonadotrophin inhibiting action of AVT in that they found that the injection of the nonapeptide into the 3rd ventricle was more effective in inhibiting compensatory ovarian hypertrophy than AVT administered by other routes.



Time course of uterine (top) and ovarian (bottom) growth following a single injection of 1 IU HCG. AVT was administered every 12 h for a total of 6 injections. Solid blocks indicate untreated control animals necropsied at 0 and at 72 h. Standard errors are indicated. [a] $p < 0.001$; [b] $p < 0.05$ vs HCG.

Other pineal compounds such as melatonin[11], 5-methoxytryptophol[12] and crude pineal extracts[13] also reportedly inhibit HCG-induced stimulation of uterine growth. Whether the actions of these substances are similar to those of AVT remain unknown. Of particular interest is that although a direct effect of the methoxyindoles on the hypothalamo-hypophyseal-gonadal axis cannot be discounted, recent evidence has shown that melatonin injected into the 3rd ventricle of cats released into the cerebrospinal fluid 50% of the AVT normally present in the pineal[14]. Thus, melatonin may inhibit HCG-induced uterine stimulation by promoting the release of endogenous AVT from the pineal gland.

Summary. 21-day-old Swiss-Webster female mice were injected with 1 IU HCG at 09.00 h. Injection of freshly prepared arginine vasotocin (2 µg/0.1 ml/injection) every 12 h inhibited the HCG-induced hypertrophy of the ovaries at 36, 48, 60 and 72 h after HCG-treatment while the uterine weight was depressed at 60 and 72 h.

MARY K. VAUGHAN[15], G. M. VAUGHAN and R. J. REITER[16]

Department of Anatomy, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio (Texas 78284, USA), 24 February 1975.

[8] C. W. EMMENS, P. J. CLARINGBOLD and D. R. LAMOND, Nature, Lond. *180*, 38 (1957).
[9] D. R. LAMOND and C. W. EMMENS, J. Endocr. *18*, 251 (1959).
[10] L. J. HIPKIN, J. Reprod. Fert. *31*, 151 (1972).
[11] A. KONIG and K. WULFF, Acta endocr. Copenh. Suppl. *173*, 150 (1973).
[12] L. J. HIPKIN, J. Endocr. *48*, 287 (1970).
[13] L. J. HIPKIN, Nature, Lond. *228*, 1202 (1970).
[14] S. PAVEL, Nature, Lond. *246*, 183 (1973).
[15] U.S.P.H.S. Postdoctoral Fellow, No. AM-55966.
[16] U.S.P.H.S. Career Development Awardee, No. HD-42398.

Increased Responsiveness of the Thyroid to Thyrotropin by Pretreatment with Thyroid Hormones in Intact Mice

In the MCKENZIE-assay[1,2] for the detection and quantitation of thyrotropin (TSH) and long-acting thyroid stimulator (LATS), iodine-depleted mice are labelled with radioactive iodide and afterwards treated with thyroid hormones, in order to suppress endogenous TSH secretion before stimulators are injected and the change of radioiodine in blood is measured. It has been shown that large suppressive doses of thyroid hormone reduce the response of the animal to TSH and LATS[3,4]. In studying the inhibition of TRH-induced TSH release of the pituitary by L-thyroxine (L-T4), L-triiodothyronine, and

[1] J. M. MCKENZIE, Endocrinology *63*, 372 (1958).
[2] J. M. MCKENZIE and A. WILLIAMSON, J. clin. Endocr. *26*, 518 (1966).
[3] W. H. FLORSHEIM, A. D. WILLIAMS and E. SCHÖNBAUM, Endocrinology *87*, 881 (1970).
[4] Y. SHISHIBA, S. YOSHIMURA and T. SHIMIZU, Endocrinology *95*, 922 (1974).

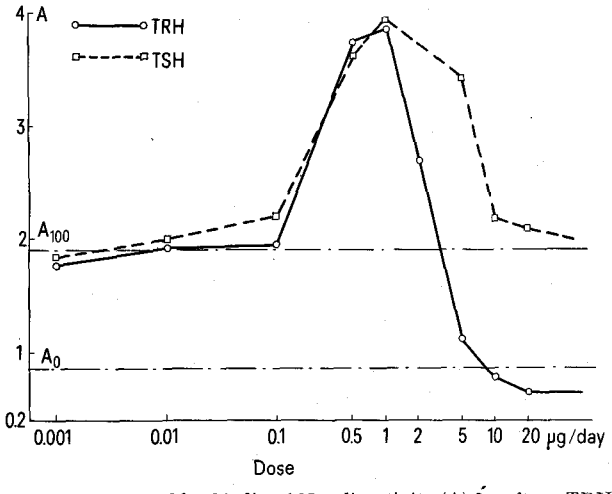
Pretreatment with L-T4 (μg/d)	Response A after		Probability of identity (p)
	500 ng TRH (mean $\pm$ SD)	0.3 mU TSH (mean $\pm$ SD)	
nil	1.93 ± 0.26	1.85 ± 0.24	~ 0.45
0.001	1.80 ± 0.29	1.81 ± 0.26	~ 0.95
0.01	1.94 ± 0.59	2.05 ± 0.33	~ 0.70
0.1	1.95 ± 0.30	2.23 ± 0.38	~ 0.17
0.5	3.75 ± 1.73	3.65 ± 0.58	~ 0.90
1.0	3.88 ± 1.56	3.91 ± 0.72	~ 0.96
2.0	2.63 ± 0.44	–	–
5.0	1.12 ± 0.33	3.44 ± 0.53	$\ll 0.001$
10.0	0.79 ± 0.59	2.19 ± 0.97	~ 0.01
20.0	0.67 ± 0.28	2.13 ± 0.57	< 0.001
100.0	0.70 ± 0.30	2.05 ± 0.42	$\ll 0.001$

their D-isomers in the McKENZIE-mouse we have found that with increasing doses of thyroid hormones the sensitivity of the animal to a constant TRH stimulus first increases before a range is reached, where the suppressive activity is positively correlated with the amount injected daily[5]. The present study is performed in order to find out whether the enhancement of the responsiveness comes from the pituitary or thyroid or both.

As test model we used a modified McKENZIE-bioassay, as previously described[5]. The animals in groups of at least 8 mice were pretreated with daily i.p. doses of L-T4 ranging from zero to 100 μg (see Table) for 3 days. No pretreatment and saline injection yielded a 3-hour response of $A_0 = 0.86 \pm 0.16$. The stimuli of 500 ng TRH and 0.3 mU TSH i.p. were compared as they produce a fairly equivalent response of $A_{100} = 1.93 \pm 0.26$ and $A = 1.85 \pm 0.24$ respectively without thyroxine pretreatment.

The results are shown in the Figure and the Table. There is no change of responsiveness up to daily doses of L-T4 = 0.1 μg. For both stimuli, TSH and TRH, the response curves increase in parallel for L-T4 > 0.1 μg/d up to a peak value around L-T4 = 1.0 μg/d. Beyond that dose both curves decline disparately; for L-T4 = 10.0 μg/d the curves start levelling out at $A = A_{100}$ after TSH and at $A = A_0$ after TRH stimulation.

The data show that there is an enhanced responsiveness of the animals pretreated with doses of L-T4 ranging from 0.1 to 10.0 μg/d, not only after stimulation with TRH but also after TSH. The enhancement cannot be explained by the release of a larger storage of TSH from the pituitary thyrotrophs saved during L-T4 substitution, as the dose-response curve elicited by the application of 0.3 mU TSH is significantly congruent with the curve caused by TRH except in the descending branch (Table). The steeper decline of the TRH curve to the level of unresponsiveness A_0 is brought about by the inhibition of the TSH release from the pituitary by suppressive doses of L-T4[5].



Relative change of blood iodine-125 radioactivity (A) 3 h after a TRH and TSH bolus in mice pretreated with various doses of L-thyroxine as shown on the abscissa.

Consequently it must be concluded that the enhancement originates from the thyroid gland. Further it can be inferred that the range of enhanced responsiveness equals the range of partial daily substitution with thyroid hormones. This warrants the assumption that, in intact animals, exogenous partial substitutive doses of thyroid hormones lead to the largest and fastest daily changes of thyroid hormone levels in blood. In consequence of the daily decrease of thyroid hormone levels, there is an increase of pituitary TSH release stimulating colloid endocytosis[4,6] and inducing de-novo-synthesis of enzymes for thyroid hormone release[7,8]. Thereby the thyroid is enabled to produce an increased response to a TRH-mediated or exogenous TSH stimulus. In a systems theoretical concept, it can be stated that the otherwise highly damped pituitary-thyroid feed-back system is sensitized by partial daily substitution with thyroid hormones to higher frequencies of a TRH or TSH stimulus, implying that the thyroid functions as low-pass filter and that its band-width can thus be extended to higher frequencies by TSH stimulation.

In order to increse the sensitivity of the McKENZIE-bioassay for TSH, LATS, or TRH, pretreatment with partial substitutive doses of thyroid hormone can be used, but a greater variance of the data is expected.

Zusammenfassung. Im McKenzie-Bioassay für TSH, LATS oder TRH steigt die Ansprechbarkeit der Mäuseschilddrüse auf TSH an, wenn die Tiere 3 Tage lang mit Schilddrüsenhormon vorbehandelt werden. Die Mengen von Schilddrüsenhormon sind dabei so zu wählen, dass sie zu einer partiellen Substitution führen.

F. J. SEIF and W. KLINGLER

Medizinische Poliklinik, Universität Tübingen, Liebermeisterstr. 14, D-7400 Tübingen 1 (German Federal Republic), 13 February 1975.

[5] F. J. SEIF, W. KLINGLER, K. ZECH and W. VOELTER, Experientia *31*, in press (1975).
[6] P. G. MALAN, J. STRANG and W. TONG, Endocrinology *95*, 394 (1974).
[7] R. CAVIEDES and J. B. STANBURY, Endocrinology *95*, 447 (1974).
[8] K. YAMAMOTO and L. J. DEGROOT, Endocrinology *95*, 606 (1974).