Enzyme activity increases by 100% in microsomes 3 h after administration of cortisone and appears in the cytosol. 5 h incubation leads to a 10% increase of enzyme in mitochondria also. Prolonged administration of cortisone for 5 days maintains a 100% increased level of enzyme in the microsomes and in cytosol; its level in mitochondria returns to normal. Corticosterone induces enzyme activity in the cytosol; after a 5 day prolonged administration, there is a 60% decrease of enzyme activity in mitochondria and a 100% increase in microsomes. Hydrocortisone-acetate (cortisol) induces a rapid 70% decrease of enzyme level in mitochondria after 5 h, causes its appearance in the cytosol and, after a 5 day prolonged administration, the enzyme level in microsomes increases by about 100%. In the cytosol the enzyme is maintained at a very high level. ACTH at first increases the enzyme level in mitochondria (by about 10%) and causes its appearance in the cytosol. Prolonged administration of the hormone maintains a high level of enzyme in the microsomes (about 100%) and in mitochondria (about 20%). c-AMP causes a rapid 10-fold increase of enzyme activity in the microsomes, appearance of the enzyme in the cytosol with a 20% concomitant decrease in mitochondria. Insulin administered for 3 days decreases the enzyme level in mitochondria by about 90% and in the microsomes by about 70%; it produces a slight appearance of the enzyme in the cytosol (if any).

Discussion. In former studies, summarized in Table I, GDH activity was measured in crude liver homogenates in water after centrifugation at 900 $\times g$. It can be seen that GDH level is controlled by glucocorticoids, the hypophysis, D-aldosterone, and the adrenal glands. Relative to the effect of diet, starvation and diabetes, controversial results were reported. All reported changes in enzyme activity were within the range of 15–20%. In our experiments, the enzyme level is relatively stable in mitochondria. It increases only after a prolonged administration of ACTH, and decreases after the administration of insulin, cortisol and corticosterone. These effects seem to be secondary and associated with the transport of the enzyme from microsomes and with changes in permeability of the mitochondrial membrane. The most dramatic and rapid increases in enzyme level are observed for microsomes after administration of cortisone, and c-AMP with a concomitant appearance of the enzyme in the cytosol. In long-term experiments, corticosterone, cortisone, ACTH and cortisol maintain a high level of enzyme in microsomes and cytosol. Especially interesting is the high level of enzyme in the cytosol following the prolonged administration of cortisol. It was reported that the half-life of specific proteins in ribosomes, nuclei, cytosol and mitochondria (including GDH as a representative enzyme of the soluble mitochondrial fraction) is about 5 days [20, 21]. Thus it seems unlikely that the observed changes in enzyme activity in long term experiments are due also to the differences in the turnover of the enzyme in various subcellular fractions. The results presented suggest that the control of enzyme biosynthesis is located between the nucleus and the microsomes. Cortisol, corticosterone, insulin and ACTH (or other hormones increased by ACTH) may have additional effects on the transport of the enzyme and on the permeability of the mitochondrial membrane.

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On the Time Course of Thyrotropin Suppression by High Doses of Thyroid Hormones[1]

M. HÜFNER and K.-H. GLESS[2]

Medizinische Universitäts-Poliklinik, Hospitalstrasse 3, D–69 Heidelberg (German Federal Republic, BRD),
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Summary. Basal and stimulated TSH decreased progressively. Basal TSH was suppressed below the detection limit of 0.4 μU/ml after 74 h in 2 of the T_3 and all of the T_4 treated individuals. At this time, in both groups 3 individuals could be significantly stimulated by TRH (about 5% of the pretreatment stimulation). There was no significant difference in the time course of suppression obtained by T_3 or T_4, though plasma T_3 levels in the T_4 treated group were considerably lower.

Suppression of basal and TRH-stimulated TSH secretion in thyrotoxicosis or by artificial elevation of plasma thyroid hormone levels is well-documented. The relative contribution of triodothyronine (T_3) and thyroxin (T_4) to this process is not yet established. There are also discrepancies which concern the time course of suppression of the thyrotrope. SNYDER and UTIGER[3] found an almost complete abolishment of the TRH-stimulated TSH secretion by a 4-week treatment with 30 μg T_3 and 120 μg T_4 daily. SHENKMAN et al.[4] saw a complete lack of responsiveness of the thyrotrope to TRH stimulation 1 h after ingestion of 50 μg T_3. AZIZI et al.[5], however, reported recently that the TSH response after TRH injection under these conditions is only minimally depressed compared to basal conditions.

Therefore we have investigated the time course of the basal and TRH-stimulated TSH secretion in normal subjects after ingestion of 50 μg T_3 every 12 h and after administration of a single dose of 3.0 mg T_4 respectively. 100 μg T_3/d is used during the conventional suppression test whereas 3.0 mg T_4 as a single dose has been suggested more recently[6] for the same purpose.

We wanted to study the following questions: 1. How long does it take under these conditions to obtain a complete abolishment of the TSH response after TRH? 2. Is there any difference in the time course of pituitary

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suppression by T_3 and T_4 respectively? 3. Does the suppressive effect correlates more with T_3 than with T_4 plasma concentrations?

Materials and methods. T_3[7] and T_4[8] was measured by radioimmunoassay, the normal range being 0.85–1.72 ng/ml and 3.8–12.0 µg/100 ml, respectively. Thyrotropin (TSH) was determined by radioimmunoassay using the usual double antibody method[9]. Anti-hTSH was purchased from Calbiochem. 125I-TSH, spec. act. 100 mCi/mg, was obtained from Hoechst Inc., Frankfurt. hTSH standard 38/68 was a gift of the British Medical Research Council. Goat anti-rabbit globulin came from Beringwerke AG, Marburg. The sequential saturation technique was used, adding the tracer after 3 days of preincubation. Thyrotoxic patients served as source of TSH-free serum. The normal range was 0–6.0 µU/ml. The sensitivity of the assay was 0.4 µU/ml. All TSH determinations in this study were performed in one assay to exclude interassay variability. The intraassay variation was ± 3.0% corresponding to ± 0.2 µU/ml in the concentration range of 0.4–1.0 µU per ml of plasma.

125 I-T_3 (spec. act. 500 mc/mg) and 125 I-T_4 (spec. act. 100 mCi/mg) from Hoechst Inc.; DASP anti rabbit globulin from Organon, München. TRH was a gift of Hoechst Inc., Frankfurt; L-T_4 1.0 mg tablets were generously supplied by Henning Inc., Berlin.

Clinical studies. One group of 4 healthy volunteers (4 females) was treated with 50 µg T_3 every 12 h for 3 days; another group of 4 persons (3 females, 1 man) got 1 single dose of 3.0 mg T_4. A TRH test[10] (200 µg TRH i.v.) was performed before administration of thyroid hormones and after 10, 26, 50 and 74 h. The second sample (after TRH injection) was taken after 30 min. This TRH dose represents a maximal stimulus and can not be considered as physiological.

Results. Figure 1 summarizes the result of T_3, T_4 and TSH measurements in the 4 subjects treated with 50 µg T_3 every 12 h. As expected T_3 plasma levels are increased considerably within a range of 3.2–10.7 ng/ml. T_3 was administered at 0.5, 12, 24, 36 h, etc. Therefore T_3 concentration is less increased at 10 h than at later periods because it misses the peak level. T_4 plasma concentrations show only minor variations.

In the lower part of the figure, the result of the TSH measurements is summarized. Some basal TSH is detectable in all 4 subjects after 26 h and in 2 even after 74 h (D.K. = 0.85 µU/ml; A.J. = 0.80 µU/ml). However, person D.K. could not be stimulated by TRH. In 3

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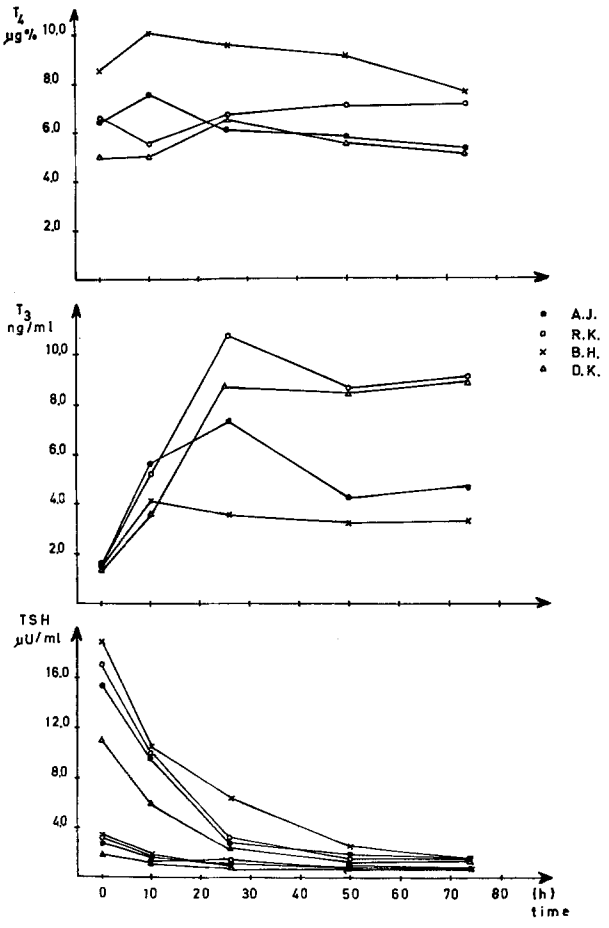

Fig. 1. Measurements of plasma T_4, T_3 and TSH in the T_3 treated group. T_3, T_4 and TSH values are indicated individually for the 4 subjects. The basal and TRH stimulated TSH concentration for each TRH test is demonstrated.

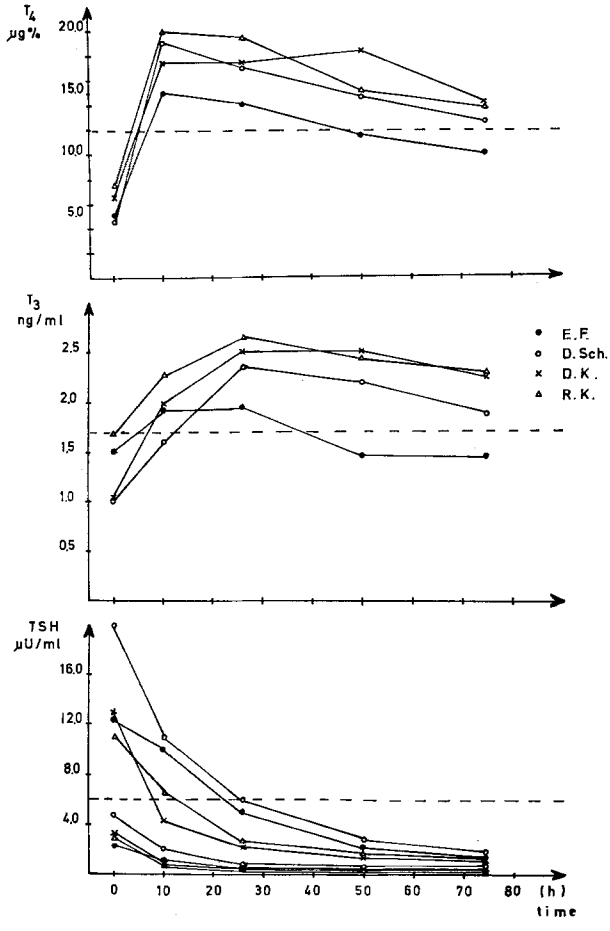

Fig. 2. Measurements of plasma T_4, T_3 and TSH in the T_4 treated group. T_4, T_3, basal and TRH stimulated TSH concentration are indicated individually. The dotted lines indicate the upper normal ranges.

individuals TSH could be significantly stimulated after 74 h. The mean stimulated TSH level was 1.2 μU/ml and the TSH increase was about 5% compared to zero time.

In Figure 2 the results obtained during T_4 suppression are listed. T_4 plasma levels increased to about 300% of the basal level and then slowly decreased according to the slow decay rate of T_4. Plasma T_3 concentrations became only moderately but constantly elevated throughout the time of investigation, except for person E.F. The time course of basal and TRH-stimulated TSH was not significantly different from that observed during T_3 suppression. At 74 h basal TSH was below the detection limit (< 0.4 μU/ml) in all cases, however, in 3 of these individuals a small increase of the plasma TSH concentration after TRH could be obtained. The mean stimulated TSH level was 1.47 μU/ml.

Discussion. Our results clearly show that the suppression of TSH secretion by high doses of thyroid hormones is a slow and gradual process which takes several days to achieve a maximal effect. This is in agreement with a recent report of AZIZI et al.[5] who found only a slight inhibition of TRH-stimulated TSH secretion 1 h after ingestion of 50 μg T_3 and does not confirm earlier results of SHENKMAN et al.[4]. WILBER et al.[11] found a complete inhibition 48 h after administration of a single dose of 150 μg T_4 plus 37.5 μg T_3. Finally, WENZEL et al.[12] obtained only about 30% of the maximal TSH response 24 h after suppression with 50 μg T_3 which corresponds closely to the values reported here. However, they could not detect any inhibition of TSH release within the first 24 h after administration of 1.0 mg T_4. The authors draw the conclusion that there was a qualitative difference between the action of T_3 and T_4 at the pituitary.

We could not detect such a difference; in fact the time course of TSH suppression was almost identical for both hormones in the doses used here. This does not exclude a conversion of T_4 to T_3 in the pituitary cells before initiating the processes connected with the feed-back mechanism; however, the similarity of the time course of basal and stimulated TSH despite the great differences of plasma T_3 and T_4 concentrations is remarkable. One explanation of the difference from WENZEL's work is that the dose of 1.0 mg T_4 is too small to increase T_3 plasma levels enough to suppress pituitary TSH secretion.

SNYDER and UTIGER[3] investigated normal subjects who had been taking 30 μg T_3 and 120 μg T_4 daily for 3–4 weeks. After this time of treatment, they observed a nearly complete suppression of TRH-stimulated TSH secretion, though the dose of thyroid hormones used was considerably lower than in this study. This agrees very well with our experience that it takes several days to produce a maximal effect at the pituitary by a certain dose of thyroid hormones. This occurs despite the fact that the plasma concentrations of T_3 and T_4 already increased above the normal level after 2–3 h. Obviously, besides the dose of hormone applied, the length of time of administration determines the response of the pituitary.

The slow response of the thyrotrope to large elevations of peripheral hormone concentrations make it very unlikely that the short-term regulation of the pituitary-thyroid axis is being effected by peripheral thyroid hormone concentrations.

Finally, we did not detect any major difference in behaviour between basal and TRH-stimulated TSH secretion. Therefore we assume that some basal TSH secretion is present as long as a positive response after TRH injection is obtained. In other words, we conclude from our observations that suppression of TSH release is a quantitative rather than a qualitative process.

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Immunohistochemical Study of the Pars Intermedia of the Mouse Pituitary in Different Experimental Conditions

M. ROUX and M. P. DUBOIS[1]

Laboratoire d'Histologie, Faculté de Médecine A, Route de Maron, F–54500 Vandœuvre-les-Nancy (France); and Station de Physiologie de la Reproduction INRA, F–37380 Nouzilly (France), 6 November 1975.

Summary. α-MSH, β-MSH and ACTH bave been localized in the cells of hypophyseal intermediate lobe by fluorescence histoimmunological technics. Elaboration and excretion of these polypeptides are enhanced after dehydration or adrenalectomy. The most evident variations are seen with α-MSH and ACTH after dehydration, with β-MSH after adrenalectomy.

The functional significance of the pars intermedia of the mammalian pituitary is not well known to date. In previous studies[2,3] we have looked for functional relationships between the intermediate lobe and the hypothalamo-neurohypophyseal complex. In particular we were able to show a relation between the development of the pars intermedia and endurance to thirst in different species of rodents: all species resistant to thirsting and having a sustained hypothalamo-neurosecretory activity, show a voluminous pars intermedia; in the same species, the mouse, a period of neurosecretory hyperactivity during dehydration, corresponds to an involution of the intermediate lobe with signs of greater secretory and excretory activity.

Immunofluorescence technics were used for further precision of the localization and the variations of the secretory production rate. Considering the chemical relationships between melanotrophic and corticotrophic hormones, a comparative study of the localization of anti MSH and anti ACTH antibodies was made in normal, dehydrated and adrenalectomized mice.

Material and methods. 48 male mice of Swiss strain, weighing 25 g, were utilized; 10 of them served as controls,

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