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.

[11] J. WILBER, A. JAFFER, L. JACOBS, R. UTIGER and N. FREINKEL, Horm. Metab. Res. *4*, 508 (1972).
[12] K. W. WENZEL, H. MEINHOLD and H. SCHLEUSENER, Endocrinologia exp. *8*, 159 (1974).

## 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,

[1] Station de Physiologie de la Reproduction INRA, 37380 Nouzilly, France.
[2] M. ROUX, Thèse Sci. Nat., Nancy 1967, No. 277.
[3] M. ROUX, Archs Anat. microsc. Morph. exp. *60*, 107 (1971).

19 were dehydrated by thirsting for 17 days, 27 days or 3 months; 19 bilaterally adrenalectomized animals were killed 7, 15 or 24 days after operation.

The hypophyses were fixed with Stieve's solution, dehydrated by alcohol, embedded in paraffin and cut in sections of 5 μm. The immunofluorescence reactions were performed according to the 'indirect' technic, using for the 2nd time sheep antirabbit γ-globulin coupled with fluoresceine isothiocyanate. The preparations of anti α-MSH, anti β-MSH, anti β(1–24)-ACTH and anti-α(17–39)-ACTH antibodies[4] and the control of their specificity were exposed previously[5–7]. Furthermore, immunocytological tests were made on mice hypophyseal slides to search for an inhibition of the antibody reaction with several peptides studied.

*Results.* 1. The 4 antibodies are fixed uniformly in all cells of the intermediate lobe except in the epithelial cells bordering the hypophyseal cleft. In the distal lobe, the reactions to the various antibodies are very different: no significant response is observed with anti α-MSH antibody. A few cells of the lateral regions of the pars distalis are fluorescent with anti β-MSH antibody. Numerous cells, dispersed in the whole pars distalis, respond positively to anti β(1–24)- and anti α(17–39)-ACTH antibodies.

2. In dehydrated animals and after 17 days of thirsting, we found very significant variations in fluorescence intensity in the pars intermedia (Table I): after application of anti α-MSH and anti β(1–24)-ACTH antibodies, fluorescence is very much reduced or absent; after 27 days of experimentation or more, the intensity of the reacion again becomes similar to that of the controls. No variation is observed with anti β-MSH antibody. In the pars distalis, immunofluorescence reactions with different antibodies remain constant during different experimental conditions.

3. In adrenalectomized animals, only one variation was noted in the pars intermedia after immunoreaction: the drop in fluorescence intensity with anti β-MSH antibody application 15 days after adrenalectomy (Table II). The number of pars distalis cells reacting to the different antibodies after adrenalectomy increases; their fluorescence becomes more intense.

*Discussion.* 1. Specificity of the immunologic reactions. The tests made to control the antibodies' specificity allow us to exclude cross reactions[5,6]. The inhibition reactions realized on mice hypophyseal slides with synthetic α-MSH, β-MSH, β(1–24)-ACTH and α(17–39)-ACTH towards the different antibodies, furnish a good presumption in favour of the immunologic specificity of these antibodies to the hormonal polypeptides of the mouse adenohypophysis[8]. The different behaviour of the antibodies utilized towards pars distalis and pars intermedia confirms this specificity.

2. Interpretation of results. In the mouse, α-MSH is elaborated only in the pars intermedia; β-MSH and ACTH are detected in the pars distalis and pars intermedia; this fact has been observed in other species[6–9].

The variations presented by the immunofluorescence reactions seem to be interesting, but their interpretation is difficult; indeed ,absence of variation in fluorescence intensity due to the fixation of an antibody does not exclude changes in secretory and excretory rates of the elaborated product, but indicates the maintenance of a balance between these two states. The comparison of these observations with the results of other technics allows some conclusions.

Thus, in the dehydrated mouse, it is during the period from 10 to 20 days of dehydration, when the variations of hypothalamo-neurosecretory activity and the changes of volume and cytology in the pars intermedia are most pronounced, that α-MSH and ACTH excretion is most intense; indeed, diminution of fluorescence intensity can only be the result of excretory hyperactivity, because radioactive aminoacid incorporation clearly shows the increase of synthetic activities in the pars intermedia at that time[3].

The relationships between pars intermedia and adrenal cortex have been demonstrated by numerous authors[10]; it is well known that after adrenalectomy the intermediate cells are hyperactive. In our adrenalectomized animals, the variations in fluorescence intensity due to anti α-MSH and anti ACTH antibodies are not very significant in the intermediate lobe; but there seems to be a great excretion of β-MSH 14 to 18 days after operation.

In *Peromyscus maniculatus* BRONSON[11] shows a plasmatic MSH increase after adrenalectomy. In the pars distalis an increase in fluorescence intensity after adrenalectomy was observed by us as well as by GOSBEE et al.[12] who also measure an increase of ACTH rate in this hypophyseal lobe[13].

Table I. Immunofluorescence reactions observed in the pars intermedia of control animals ($D_0$) and after 17 ($D_{17}$) and 27 days ($D_{27}$) of dehydration

| Reaction to antibodies | $D_0$ | $D_{17}$ | $D_{27}$ |
|---|---|---|---|
| Anti α-MSH | +++ | ± | +++ |
| Anti β-MSH | +++ | +++ | +++ |
| Anti β(1–24)-ACTH | +++ | + | +++ |

Table II. Immunofluorescence reactions observed in the pars intermedia of control animals ($D_0$) and 7 days ($D_7$), 15 days ($D_{15}$), 24 days ($D_{24}$) after adrenalectomy

| Reaction to antibodies | $D_0$ | $D_7$ | $D_{15}$ | $D_{24}$ |
|---|---|---|---|---|
| Anti α-MSH | +++ | +++ | +++ | +++ |
| Anti β-MSH | +++ | +++ | + | +++ |
| Anti β-(1-24) α-(17-39) ACTH | +++ | +++ | +++ | +++ |

*Conclusion.* During dehydration and after adrenalectomy in the mouse, the intermediate lobe shows a state of hyperactivity parallel to that of the neurosecretory hypothalamus, with greater elaboration and excretion of MSH and ACTH type polypeptides. The different modalities in the behaviour of these polypeptides, shown by histoimmunological technics, in particular for α-MSH and β-MSH, suggest an original role for each of them.

[4] The synthetic antigens were kindly offered by the CIBA-GEIGY Society, Basel.

[5] M. P. DUBOIS, Ann. Biol. anim. *11*, 589 (1971).
[6] M. P. DUBOIS, Z. Zellforsch. *125*, 200 (1972).
[7] Y. STEPHAN and M. P. DUBOIS, Z. Zellforsch. *133*, 353 (1972).
[8] M. ROUX and M. P. DUBOIS, Bul. Ass. Anat. *58*, 661 (1974).
[9] M. P. DUBOIS, Bul. Ass. Anat. *57*, 63 (1973).
[10] Y. KOBAYASHI, Z. Zellforsch. *68*, 115 (1965).
[11] F. A. BRONSON, B. E. ELEFTHERIOU and H. E. DEZELL, Proc. Soc. exp. Biol. Med. *130*, 527 (1969).
[12] J. L. GOSBEE, J. KRAICER, A. J. KASTIN and A. V. SCHALLY, Endocrinology *86*, 560 (1970).
[13] J. KRAICER, J. L. GOSBEE and S. A. BENCOSME, Neuroendocrinology *11*, 156 (1973).