was observed in the secretory epithelium and, to a lesser extent, in the connective tissue stroma of the gland. Lastly, ^{125}I-HGH did not localize in any of the organs of the intact and/or hypophysectomized male rat.

HGH is known to act as a synergist with other hormones and to play a supportative role in many biologic phenomena[9]. The present investigation (autoradiograph) suggests that growth hormone may be an active participant in the functioning and maintenance of the mouse SV secretory epithelium. Previous studies have implicated androgen (testosterone) as a prime suspect for the synergistic action of HGH in the mouse[3]. Currently, it is uncertain whether the observed HGH stimulation of the SV could be attributed to the prolactin or the growth stimulating activity of the hormone preparation.

This study has demonstrated radio-uptake inhibition in the SV when specific antiserum was administered prior to the radiohormone injection. This uptake inhibition may have resulted either in steric blockage at the HGH cell receptor site or inability of the immune complex (HGH: anti-HGH) to penetrate the target cell membrane. Whatever the cause, the immune aggregates do not accumulate in the SV and are probably eliminated by the reticuloendothelial tissues, i.e., the liver. It becomes obvious why antibodies to HGH detected during longterm treatment for hypopituitary dwarfism contributes to growth inhibition in children.

Finally, it is of endocrinologic and systematic interest that cell receptor sites for HGH are present in the mouse but have not been detected in the rat. If indeed the stimulation could be attributed to a prolactin effect of the radiohormone, then a different mechanism or system of cell receptors for HGH must be present in the rat as compared to the mouse. This is not unexpected as many differences in reproductive physiology appear to exist between these 2 rodents[10].

Zusammenfassung. Bei männlichen Mäusen wurde die Bindung von ^{125}I-HGH untersucht. Im Vergleich zur Bindung an andere Gewebe derselben Tiere konnte eine zellspezifische Bindung im Bereich der Samenblasen festgestellt werden. Die Befunde werden in Hinblick auf eine Steroid-ergänzende Tätigkeit des HGH diskutiert.

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28 December 1972.

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Epiphyseal-Hypothalamic Interaction. An in vitro Study with Some Sheep Pineal Fractions

Evaluation of the hypophysiotropic activity was carried out using our usual test (MOSZKOWSKA et al.[1]). In this test, the secretion of pituitary gonadotropins from pituitaries incubated in vitro in the presence of a cortical extract (controls) is compared with the secretion of gonadotropins from pituitaries incubated in the presence of a hypothalamic extract. Using this test we were able to evaluate the action of various pineal fractions on the hypothalamic-hypophysiotropic activity by comparing the gonadotropin releasing factor content of hypothalami incubated alone and in the presence of the pineal fractions.

In previous studies on the hypothalamic-hypophysiotropic activity in the rat, we have verified that the pineal Sphadex G-25 fraction F3 is capable of inhibiting this activity (MOSZKOWSKA et al.[1–3]). New information on the effect of the Sphadex G-25 fraction F3 was obtained from in vitro experiments using the mouse hypothalmus.

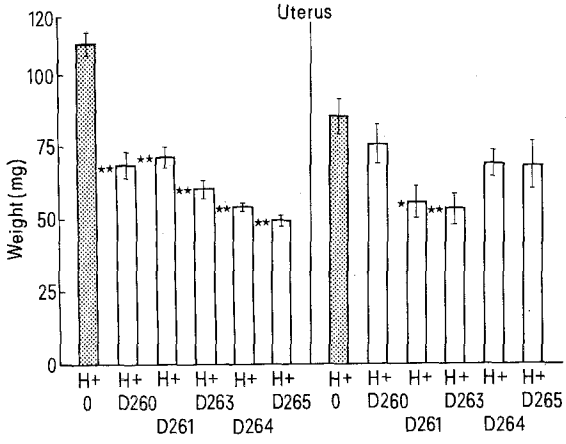

Fig. 1. Weights of the uterus of mice after injection of the incubation liquid (left) and extract (right) of mice hypothalamus incubated with and without a sheep pineal fraction UM-2R. D 260, D 261, D 263, D 264, D 265 are the codes of 5 different Sephadex G 25 columns from which UM-2R fractions are prepared. H, hypothalamus.

In continuation of these results, we carried out in vitro experiments with pineal fractions obtained by Sephadex G-25 filtration of an aqueous pineal extract followed by ultrafiltration of the low molecular weight Sephadex G-25 fractions, on the Amicon membranes UM-2 and UM-05 (EBELS and BENSON[4]).

Methods of extraction and separation of UM-2R and UM-05R. The frozen sheep pineal glands were homogenized in distilled water, filtered on a Sphadex G-25 column, equilibrated and eluated with distilled water. The extraction and separation was carried out in darkness and at 2 °C. The low molecular fraction thus obtained underwent a double ultrafiltration through 2 Amicon diaflomembranes. The first filtration on the membrane UM-2 gave a residue UM-2R and a filtrate. This filtrate, after a further filtration on UM-05, gave a second residue UM-05R and again a filtrate. The two residues were studied in vitro, and will be referred to in the following sections as fractions UM-2R and UM-05R.

From information obtained from the Amicon catalogue, the substance of the residues obtained from UM-2R have molecular weights greater than 1000, while those obtained from UM-05 have molecular weights between 500 and 1000. For details see EBELS and BENSON[4].

Bioassay. For the study of each pineal fraction 6 male mice hypothalami were incubated for $^1/_2$ h in a Krebs

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Ringer solution at 37 °C, aerated with 95% O_2 and 5% CO_2 with a pineal fraction, and 6 male mice hypothalami incubated alone served as a control.

The incubation liquid was used for the incubation with 3 male mice anterior hypophyses for 3 h under the same experimental conditions, to determine the gonadotropin releasing activity. After centrifugation of this second incubation liquid, the supernatant was injected s.c. in 5 injections into 6 immature 21-day-old female Swiss mice of 7–8 g body weight which had been sensitized just before the first injection with 0.25 IU of human chorionic gonadotrophin (HCG). Autopsy was carried out 18 h after the last injection. We compared the average value of the ovary weights and the average value of the uterine weights of the groups. Standard errors of the means were calculated.

The incubated hypothalami, with and without a pineal fraction, were extracted to determine their content of gonadotropin releasing activity by incubating the lyophilized hypothalamic extract with 3 mice anterior hypophyses as described before. For details of the method see MOSZKOWSKA et al.[1].

Results with fraction F3. (Original Sephadex G-25 F3 extracted with pyridine acetate, see[2]). We found that the hypothalami of male mice incubated in the presence of the pineal extract secreted less hypophysiotropic hormone than did those hypothalami incubated alone in Krebs-Ringer solution. In addition, the hypothalami incubated in the presence of that Sephadex G-25 fraction F3 had a higher content of hypophysiotropic factor(s) than the control hypothalami. In conclusion, it clearly appears that in the case of the Sephadex G-25 fraction F3, its action on the hypothalamic hypophysiotropic activity is to inhibit the secretion of hypophysiotropic hormones in rat[1] and mice.

Results with fraction UM-2R. Hypothalami incubated in the presence of UM-2R showed a highly significant decrease in the secretion of hypophysiotropic hormone compared with the controls. In addition these hypothalami showed a certain decrease in the hypophysiotropic factors content, which contrasts with the situation we found in the case of hypothalami incubated with our original Sephadex G-25 fraction F3 (Figure 1). We can only define the molecular weight of the substances in UM-2R as being greater than 1000. It does not seem possible, therefore, to compare the inhibiting activity in this fraction with that in our original Sephadex G-25 fraction F3.

Results with the fraction UM-05R. The hypothalami incubated with UM-05R showed an increased hypophysiotropic activity, since both the incubation medium and the hypothalami ectracts tested following incubation with UM-05R stimulated an increased secretion of pituitary gonadotropin compared with the secretion stimulated by the incubation medium and the hypothalamic extract from control experiments (Figure 2). It seems, therefore, that we are in the presence of a factor which stimulates hypothalamic activity, and which has a molecular weight between 500 and 1000. Thus, we may conclude that the pineal contains active principles, other than melatonin[1], capable of acting via the hypothalamus. It must be noted, however, that BENSON et al.[5] and EBELS and BENSON[4], using a bioassay based on the compensatory ovarian hypertrophy in unilaterally ovariectomized adult mice, have reported that the pineal fraction UM-05R contains an inhibiting activity. With UM-2R they unable to prevent the compensatory hypertrophy. It is therefore evident that with these 2 pineal fractions one must envisage in vivo in addition to in vitro experiments.

Résumé. On a étudié sur la souris trois fractions épiphysaires différentes: les fractions Sephadex G-25 F3, UM-2R, UM-05R. La fraction épiphysaire Sephadex G-25 F3 inhibe l'activité hypothalamique hypophysiotrope en empêchant son excrétion. La fraction UM-2R inhibe aussi bien l'excrétion que la synthèse des facteurs hypophysiotropes. La fraction UM-05R se révèle capable de stimuler l'activité hypothalamique hypophysiotrope.

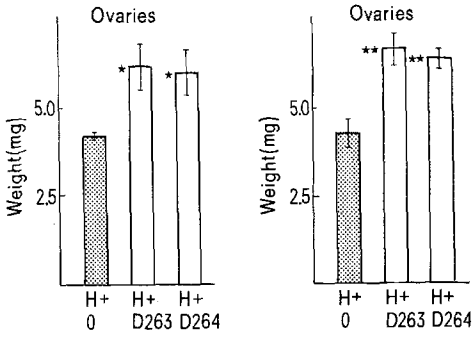

Fig. 2. Weights of the ovaries of mice after injection of the incubation liquid (left) and extract (right) of mice hypothalamus incubated with and without a sheep pineal fraction UM-05R. D 263, D 264 are the codes of two different Sephadex G 25 columns from which UM-05R are prepared. H, hypothalamus. * $p < 5\%$. ** $p < 1\%$.

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[6] Acknowledgment: Our thanks are due to Miss. A. E. M. BRESSER and Mrs. S. ROSEAU for skilful technical assistance.

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Distribution of Vitamin D_3: Evidence of Accumulation in Renal Proximal Tubuli and Thyroid Parafollicular Cells

The conversion of vitamin D_3 to the active metabolites, 25-hydroxycholecalciferol (25-HCC) and 1,25-dihydroxycholecalciferol (1,25-DHCC), has been shown to take place in liver and kidney respectively[1,2]. These processes can explain the time lag between the administration of vitamin D_3 and its hypercalcaemic effect, proposed to be mediated through an action on intestine and bone[3–5]. In the present investigation we have used whole-body