Effects of Methylthiouracil or Thyroidectomy on Activation of Pituitary Acid Phosphatases *in vitro* by Whole Hypothalamic Extract

IN EARLIER experiments¹ it was shown that incubation of whole rat pituitaries *in vitro* in whole aqueous hypothalamic extract increased pituitary acid phosphatase activity. In further experiments^{2,3} evidence has been submitted in support of the view that a relationship exists between activation of pituitary acid phosphatases *in vivo* and formation of the thyreotropic hormone and that the presumed hypothalamic humoral factor activating pituitary acid phosphatases *in vitro* is related to thyroid-stimulating hormone secretion. The present communication gives the results of experiments investigating the extent to which the influence of hypothalamic extract on acid phosphatases in the rat pituitary *in vitro* can be modified by previous administration of methylthiouracil to hypothalamus donor rats or by previous thyroidectomy of these animals.

In the first experiment 40 fresh pituitaries from albino rats (descendants of the Wistar strain) were divided into five groups and incubated for one hour at $37 \pm 0.1^\circ \text{C}$. in the following media: (1) Krebs-Ringer phosphate with 300 mgm. per cent glucose; (2) the same medium plus extract from 2/3 of one control rat hypothalamus/c.c.; (3) the same amount of hypothalamic extract from rats to which 0.2 per

cent methylthiouracil was administered in food 16 days before they were killed; (4) hypothalamic extract from rats to which 0.5 per cent dried thyroid ('Thyreoidin' SPOFA) was administered in food for a period of nine days before they were killed; (5) hypothalamic extract from rats to which both methylthiouracil and dried thyroid were administered. The results of biochemical determination of acid phosphatase activity in the individual groups of pituitaries are given in Table 1.

In the second experiment 40 fresh rat pituitaries were again divided into five groups and incubated in the following media: (1) Krebs-Ringer phosphate with 300 mgm. per cent glucose; (2) extract of 2/3 control rat hypothalamus/c.c.; (3) hypothalamic extract from rats subjected 12 days previously to thyroidectomy; (4) hypothalamic extract from rats to which 0.5 per cent dried thyroid was administered in food for 12 days before they were killed; (5) hypothalamic extract from rats subjected to both treatments (thyroidectomy + 'Thyreoidin'). The results of the determination of acid phosphatase activity are given in Table 2.

The results show that: (1) incubation in control hypothalamic extract increases pituitary acid phosphatase activity *in vitro*; (2) previous administration of methylthiouracil to hypothalamus donor rats or previous thyroidectomy of these animals inhibits this increase; (3) the administration of dried thyroid alone does not affect activity under the given conditions; (4) the administration of dried thyroid counteracts the inhibition of activation observed after methylthiouracil or thyroidectomy alone. In our view the above findings are evidence of the existence of a hypothalamic humoral factor activating simultaneously pituitary acid phosphatases and thyroid-stimulating hormone secretion. The decrease which occurs in the concentration of this factor in the hypothalamus after methylthiouracil or thyroidectomy could be due to its being washed out to portal system to increase thyroid-stimulating hormone secretion. The administration of dried thyroid caused no marked change in the activating effect of hypothalamic extract; in other experiments⁴, however, more prolonged administration of dried thyroid slightly enhanced its effect.

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Comparison of individual groups in *t*-test: 2:3 = $P > 0.01$; 2:5 = $P = 0.05-0.02$.

TABLE 2.

Group	1 medium only	2 control hypothal.	3 hypothal. thyroidect.	4 hypothal. 'Thyreoidin'	5 hypothal. thyroidect. + 'Thyreoidin'
No. of pituitaries	8	8	8	8	8
Mean weight of pituitaries (mgm.)	5.7	5.2	5.8	5.4	5.4
Mgm. of hypothalamic tissue/cc. incubations medium	0	100	100	95	100
Mean activity of acid phosphatase in the pituitaries K.A.U./gm. $\pm \sigma_M$	1.95 $\pm$ 0.138	2.93 $\pm$ 0.205	2.11 $\pm$ 0.279	2.67 $\pm$ 0.259	2.69 $\pm$ 0.121

Comparison of groups No. 2 and 3 in *t*-test = $P = 0.02$.

- ¹ Charvat, J., Schreiber, V. and Kmentova, V., *Nature*, **182**, 62 (1958).
- ² Schreiber, V. and Kmentova, V., *Physiol. Bohemoslov.* (in the press).
- ³ Schreiber, V. and Kmentova, V., *Acta Biologica Hung.* **9**, 285 (1959).
- ⁴ Schreiber, V. and Kmentova, V., *Endokrinologie*, **38**, 69 (1959).
- ⁵ Schreiber, V. and Kmentova, V., *Folia Biologica* **5**, 272 (1959).

Inhibition of Skeletal Formation in the Chick Embryo following Administration of Tetracycline

It has been shown that tetracyclines form complexes with metallic ions^{1,2}, that they become incorporated into bones of young mice³, and also that they are retained in bones of several other species for a considerable period following administration⁴⁻⁵. We have recently demonstrated⁶ that tetracycline inhibits skeletal formation in the sand dollar (*Echinarachnius parma*) embryo. The above observations prompted us to extend our previous studies and test the effect of the administration of