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Failure of Hypothalamic Tissue to Reinitiate Thyrotropin Production By Mouse Pituitaries in Tissue Culture.* (23321)

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Guillemin and Rosenberg(1) reported that pituitary explants, which normally lost their ability to produce corticotropin rapidly in tissue culture, could be stimulated to reinitiate corticotropin production by the addition of hypothalamic explants. The stimulating potency resided in the median eminence, a region which previously had been shown to be involved in control of corticotropin production in the intact animal(2).

The importance of hypothalamic centers in regulating production of thyrotropin by the pituitary has been established(3,4,5), but the mechanism of control has not. Greer(3) has postulated the existence of 2 thyrotropins, a "metabolic" and a "growth" factor, and the production of the latter appears to depend upon both pituitary and hypothalamic centers. We therefore thought it of interest to utilize Guillemin and Rosenberg's technic to see whether evidence for a direct stimulation by hypothalamic tissue of hypophyseal thyrotropin production could be obtained.

Materials and methods. Pituitaries were taken from adult male Swiss albino mice under sterile conditions. The tissues were washed in Hanks' balanced salt solution and quartered with a sharp scalpel. Four quartered pituitaries were placed in the lower third of 16 x 150 mm test tubes which had been coated with 3 drops of chicken plasma. Following coagulation of the plasma, 1.0 ml of nutrient fluid was added, the tubes stoppered and incubated in a Roller Drum at 36°. Thirty-two such tubes were prepared in this study. The tissues were observed daily for growth and the nutrient medium was changed twice a week. The removed medium was kept at −35° prior to analysis for thyrotropin. Hypothalamic explants were added to 20 of the tubes on the twelfth day of *in vitro* growth and imbedded in a fresh plasma clot. Two entire mouse hypothalami, extending from the optic chiasm to the mammillary bodies were minced into 1 mm^3 fragments and added to each tube containing 4 pituitaries. The nutrient fluid consisted of 20% Beef Serum, 10% Chicken Embryo Extract, 20% Hank's

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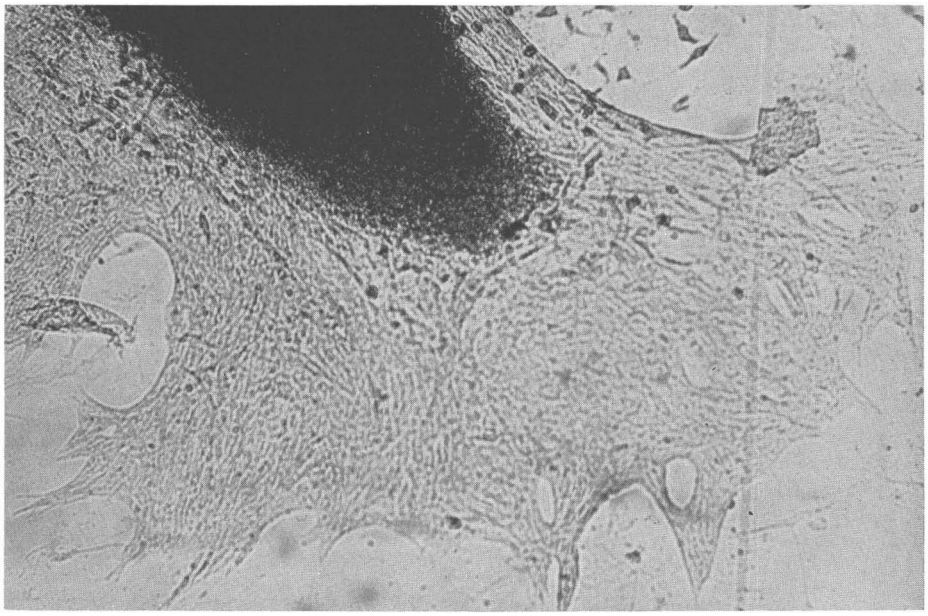

FIG. 1. Photomicrograph of typical pituitary explant after 6 days of *in vitro* growth. Unstained preparation (× 100).

Balanced Salt Solution containing trypsin inhibitor and 50% "199" Solution(6). Phenol Red was used as a pH indicator and each ml of medium contained 500 U of penicillin and 150 μg of streptomycin. The adequacy of these tissue culture conditions was confirmed by testing some of the incubation fluids for corticotropin content by means of Hodge's assay(7) in young DOCA-treated rats.‡ Our results were very similar to those published by Guillemin and Rosenberg(1). But, due to the small number of assays performed, increase in corticotropin content of the media after addition of hypothalamic explants, though marked, was significant only at the 5% level of confidence. Thyrotropin analysis was performed by 2 unrelated assays: the assay of Florsheim *et al.*(8) based on *in vitro* stimulation of phospholipid labelling in beef thyroid slices, and a modification of the chick assay of Gilliland and Strudwick(9). The modifications of the latter method consisted of adding 0.15% propylthiouracil(10) and 1% thyroid powder to the diet of the chicks subsequent to the initial 24 hour period allowed for uptake and binding of I^{131} in the thyroid gland. Ten white Leghorn cockerels were used per sample, each receiving 1.0 ml of test solution in 2 subcutaneous injections.

Results. All pituitary explants grew very well, as shown in Fig. 1, and there was no evidence of bacterial contamination at any time. Hypothalamic tissue grew much more slowly than pituitary, but there appeared to be no interference with the normal growth of both tissues in mixed cultures. Tissues grew for approximately 30 days before becoming granular in appearance. Tissue culture fluid analyses showed a rapidly decreasing rate of thyrotropin output by the pituitary explants. No thyrotropin could be detected in any of the nutrient fluids beyond the second change by either assay. The concentration in the first change of medium averaged about 80 mU per tube, in the second about 20 mU and thereafter no activity was detectable even when injected into chicks at a level of 2 ml per chick. Since the minimum amount of thyrotropin which can be detected reliably by the assay procedures used is 3 mU, it appears that less than 3 mU was liberated into the tissue fluid per 4 pituitaries in 3-4 days. This amount did not increase after addition of hypothalamic explants. Hypothalamus growing

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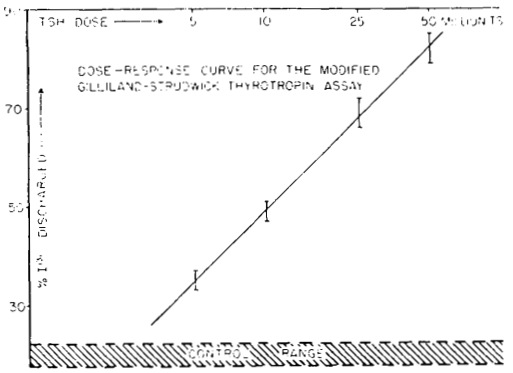

FIG. 2. Dose-response curve for modified Gilliland-Strudwick chick assay showing means and stand. errors of response at 4 levels of thyrotropin.

alone in tissue culture also produced no detectable thyrotropin, as was to be expected.

A few of the samples were also analyzed by the chick assay of Greenspan(11) and found to be devoid of activity. However, since only 0.2 ml of the test solution can be injected by the intracardiac route employed in this method, the use of this assay did not extend the detectable limit of thyrotropin concentration. Since the method of Greenspan is less accurate than the other 2 methods, we abandoned its use after only a few assays.

Cultures were checked for growth and thyrotropin production for 25 to 32 days. At the end of this period, the tissue began to appear granular. The remaining tissue and plasma clot were therefore removed from the tubes, homogenized in saline and analyzed for thyrotropin content in chicks. They contained no detectable amounts of the hormone.

It was established by chick assay that a commercial thyrotropin preparation (Parke, Davis "50P4") could be recovered quantitatively after being in contact for one week with growing explants of pituitary and hypothalamus. We also showed that less than 10% of pituitary thyrotropin content is lost from the gland during the explanting procedures.

The chick assay for thyrotropin as used in our laboratory was useful in the range of 3 to 50 mU of USP thyrotropin reference substance (Fig. 2). The Index of Precision (λ), calculated by Emmens' method(12) was 0.23 and was very reproducible with chicks obtained from the same source. The sensitivity and precision of the chick assay were very similar to those of the thyroid slice assay employed.

Discussion. With the 2 assay procedures employed we were unable to detect the production of more thyrotropin by mouse pituitary explants than that originally present in the glands. The elution of this material occurred during the first 2 changes of the incubation medium, and we recovered approximately 25 mU per gland, which is in fair agreement with the values for mouse pituitary thyrotropin content given by Bates(10). Since the growing tissue did not metabolize exogenous thyrotropin and since the entire hormone content of the original gland was recovered during the first week of incubation, it is unlikely that newly formed thyrotropin was lost to analysis by metabolic degradation. The thyrotropin produced, if any, during a week of growth in tissue culture thus was less than 4% of the original content of the glands, even after addition of hypothalamus explants.

The assays employed were chosen to reflect different aspects of thyrotropin action. The Gilliland-Strudwick assay is clearly a measure of "metabolic" thyrotropin, since it measures the discharge of labelled thyroid hormone from the target gland. The assay based on the phospholipid labelling in surviving beef thyroid slices was chosen because it proved completely unresponsive to all goitrogens including propylthiouracil, thiocyanate and iodide, and thus seemed to measure an aspect of thyrotropin action unrelated to the effects ascribed to the metabolic factor. However, no new synthesis of any thyrotropin could be detected by this method. It appears, therefore, that intervention of the hypothalamus in thyrotropin synthesis is not as direct as it has been shown to be for corticotropin production, and no hypothalamic neurohumor could be demonstrated by the technics used in this study.

Summary. Contrary to Guillemin's findings regarding the reinitiation of corticotropin production by pituitary explants in the presence of hypothalamic tissue, no thyrotropin

was produced by mouse pituitary explants even after addition of growing hypothalamic tissue. Since the assay procedures employed would have detected 3 mU of thyrotropin, less than 4% of the original thyrotropin content of the explants was produced per week.

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Alteration of Hepatic Storage of Radiolabeled Vit. B_{12}.* (23322)

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The liver is the most important storage organ for vit. B_{12}. After oral or parenteral administration of radiocobaltlabeled cyanocobalamin, surface counting over various sites has shown that the liver accumulates most of the radioactivity(1-4). The liver also retains the radioactivity with great avidity. Indeed, from 86 to 94% of the maximal activity was found after 3 months(1), and 50% after one year(5). Moreover, the plateaus of liver radioactivity in 4 control subjects were unaffected by single, intramuscular doses of 1 mg of "cold" vit. B_{12} given on the seventh day (6).

The objective of the present investigation was to observe the effect of a large series of 1 mg parenteral doses of "cold" vit. B_{12} on hepatic storage of radioactivity after orally administered radiolabeled cyanocobalamin.

Materials and methods. The Co^{60} labeled vit. B_{12} used in these experiments had a specific activity of 1082 μc per mg. A test dose of 0.46 μg vitamin containing 0.5 μc Co^{60} was administered to 2 healthy female volunteers after an overnight fast in approximately 100 ml of water and breakfast was withheld for 2 hours. Plasma radioactivity(7) as well as a 24 hour urinary excretion study(8) started at 24 hours showed that they both absorbed the vitamin normally. External monitoring over the organ areas was carried out according to the method of Glass(1) employing a NaI (thallium activated) probe scintillation counter with a crystal 1½ inches in diameter and 1 inch thick and with an aperture of 2½ inches in diameter. Uptake of radioactivity over the liver, spleen and precordium was determined 9-13 days after administration of the test dose and at intervals up to 7½ months after the tests. The radioactivities recorded between one and 2 weeks after the tests were taken as the 100% values. One of the subjects was given a series of one hundred 1 mg parenteral injections of nonradioactive cyanocobalamin over the ensuing 7 months, while the other subject served as a control. Twenty-four hour urine collections were made

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