8354 C

Elaboration of Hormones by Pituitary Cells Growing in vitro.

EVELYN ANDERSON AND WEBB HAYMAKER. (Introduced by Wilder Penfield.)

From the Montreal Neurological Institute, McGill University, Montreal, Canada.

The purpose of this study is to determine whether pituitary cells proliferating *in vitro* elaborate hormones. The pituitary gland was removed from 8-day-old rats and placed in Tyrode's solution. By aid of the dissecting microscope the anterior lobe was separated from the posterior. Each was cut into fragments about 0.2 sq. mm. in size. Anterior lobes of the pituitary were then planted in lots of 5 glands in a Carrel D-3 flask whereas posterior lobes were planted in lots of 10 each. The amount of the various constituents of the medium was as follows: rat plasma, 0.6 cc.; Tyrode's solution, 0.3 cc.; and chick embryonic tissue juice, 0.3 cc. The cultures were washed with salt solution daily and the liquefied areas of the coagulum were patched with 0.1 cc. plasma and 0.1 cc. embryonic tissue juice. The cultures were incubated at a temperature of 38.5°C. and were allowed to grow for 6 days. By that time there was extensive proliferation of epithelial cells of both anterior and posterior lobe explants, an increase of as much as 8 times the original diameter of the explant. On the contrary, there was no evidence of growth of pars nervosa elements.

On the sixth day the coagulum was removed from each flask. That containing the anterior pituitary cultures was broken up in 8 cc. of Tyrode's solution and 1 cc. of absolute ethyl alcohol, added as a preservative. The coagulum containing the posterior lobe cultures was ground in sand. This material was then extracted with 0.25% solution of acetic acid and placed on a boiling water bath for 5 minutes. Both anterior and posterior lobe preparations were stored in the refrigerator at 4°C. and were used within 30 days.

For control material an equivalent number of pituitary glands from rats of the same age were set aside without being cultured. The material was preserved in the same way as that which was cultured.

In assaying the posterior lobe extracted material for hormones the test object for the detection of the melanophore-expanding principle was the frog. For determining the oxytocic and antidiuretic content, the virgin guinea pig uterus and bladder-fistula dog, respectively, were used. Presence of anterior lobe hormones was determined by injection into the hypophysectomized rat.

Only the melanophore-expanding principle was shown to have increased with growth of the posterior lobe *in vitro*. This conclusion was arrived at by comparing the melanophore-expanding effect of cultured posterior lobe material with that of an equivalent number of non-cultured posterior lobes, both treated in a like manner as described above. The frogs were first bleached by being placed in intense light for 40 minutes. The extracted pituitaries in various doses were then injected in the neighborhood of the lymph sac, 8 frogs being used in testing for melanophore-expansion in each of 7 batches. Each batch consisted of a 6-day growth of 10 posterior pituitary glands taken from 8-day-old rats. Of the 8 frogs, 2 received 0.05 unit of pituitrin (Frosst), 2 received the extract of cultured pituitary originating from ¼ of a posterior lobe, 2 received the extract of ¼ of a posterior lobe which had not been cultured and the remaining 2 received no injection. In addition, graded doses of extracts of each lot of cultured pituitary material were injected into a series of 8 frogs in order to determine the minimum effective dose of each lot; this was likewise done with the non-cultured extracted material. The change in the shade of the frogs was noted every 10 minutes for an hour or until the skin of the injected frogs resembled the bleached shade of the non-injected control frogs. The volume of the cultured and non-cultured material injected into the frogs was the same in each case, a sufficient dilution being made with Tyrode's solution to bring it to 0.5 cc.

The results are shown in Table I. The doses tested are expressed in terms of the amount of original pituitary tissue administered per 30 gm. of frog body weight. It was found that 0.05 unit of pituitrin had turned the bleached skin of the frog very dark 40 minutes after injection. When the extract of ¼ of a non-cultured posterior lobe was injected, the melanophore-expanding effect was considerably less (slightly visible, +) than that produced by the injection of an extract of ¼ cultured posterior lobe (very dark, ++++). The latter produced a melanophore-expanding effect roughly equivalent to that of 0.05 unit of pituitrin. The end point (the dilution below which there was no melanophore-expanding effect) in the case of the cultured material was reached with injection of 0.012 of the original lobe whereas the end point with injection of the non-cultured material was 0.10 of the original lobe. Thus, there was approximately an eightfold increase in melanophore-expanding principle with growth *in vitro*.

Subculture of posterior lobe was found to contain melanophore-expanding principle roughly equivalent to that of cultures not trans-

TABLE I.

Material	Amt. Injected per 30 Gm. of Frog Wt.	Melanophore-Expanding Effect on Skin of Frogs After 40 Min.
Pituitrin	.05 unit	Very dark, ++++
Non-cultured post. lobe	.25 lobe	Slightly visible, +
" " "	.12 "	Very slightly visible
" " "	.10 "	" " "
" " "	.05 "	No effect
Cultured post. lobe		
Lot 40	.25 lobe	Very dark, ++++
	.10 "	Dark, +++
	.025 "	Slightly visible, +
	.012 "	Very slightly visible
	.006 "	No effect
" 41	.25 "	Dark, +++
" 42	.10 "	Very dark, ++++
	.025 "	Slightly visible, +
	.012 "	Very slightly visible
	.006 "	No effect
" 50	.10 "	Dark, +++
" 50a	.12 "	" ++
" 64	.08 "	" +++
" 80	.25 " *	Very dark, ++++
	.10 "	Dark, +++
	.05 "	Slightly visible, +
	.012 "	Very slightly visible
	.006 "	No effect
Subcultured post. lobe		
†Lot 81 (once subcultured)	.50 lobe	Very dark, ++++
	.25 "	Dark, +++
†Lot 82 (twice subcultured)	.50 "	Very dark, ++++
	.25 "	Dark, +++
	.10 "	Slightly visible, +

*After 100 min. frogs receiving 0.25 posterior lobe of Lot 80 were darker than those receiving 0.05 unit of pituitrin.

†The amount injected is approximately that recorded.

ferred. In transferring the cultures the original explanted fragments of tissue were discarded, so that the transferred material was made up solely of cells which had proliferated *in vitro*. The cultures extracted after one transfer were grown for 9 days; those extracted after 2 transfers were grown 12 days.

In testing of the oxytocic principle the dilution of the extracts appeared to be too great to give a satisfactory test. The test for the antidiuretic principle was more conclusive. An extract containing 1½ posterior lobes, whether cultured or non-cultured, when injected

into a bladder-fistula dog excreting 3.0 cc. of urine per minute resulted in a suppression of urine to 0.4 cc. per minute, the duration of the antidiuretic effect being 25 minutes. Three batches of cultured posterior lobe and 3 of non-cultured were thus tested; results were practically identical.

In regard to the effect of injecting anterior pituitary cultures into hypophysectomized rats, it was found that 60 anterior lobes which had been grown for 6 days, and in which the amount of tissue had increased considerably, produced definite hormonal restorative effects upon the thyroid, adrenals and ovaries of the hypophysectomized rat. This effect, however, was no greater than that produced by an extract of an equivalent number of non-cultured pituitaries. The lack of a more delicate test for assaying anterior lobe hormones was a handicap in this study.

Conclusion. With growth *in vitro,* pars intermedia cells of the posterior lobe of the pituitary retain their power to elaborate melanophore-expanding principle. Under the conditions of experiment no discernible production of hormones took place with growth of anterior lobe cell *in vitro.*

We wish to thank Dr. K. I. Melville for his very kind assistance in the assay of posterior lobe hormone.

8355 P

Effect of Thyroidectomy and Thyroid Feeding on the Estrus Cycle in the Rat.

B. FREEDMAN, L. M. WRIGHT AND B. WEBSTER.

From the Barbara Henry Research Laboratory of the New York Hospital and the Department of Medicine, Cornell University Medical College, New York.

Many investigators have reported both anatomical and physiological disturbances in the reproductive system following ablation of the thyroid gland.[1-5] In the adult female rat the most striking functional change is an increase in the length and irregularity of cycles.[4, 5] It seemed of interest to determine to what extent the nor-

[1] Hofmeister, F., *Beitr. zu Klin. Chir.*, 1894, **11**, 441.

[2] Kunde, M., Carlson, A. J., and Proud, T., *Am. J. Physiol.*, 1929, **88**, 747.

[3] Tatum, A. L., *J. Exp. Med.*, 1913, **28**, 500.

[4] Lee, M., *Endocrinology*, 1925, **9**, 410.

[5] Bokelmann, D., and Sheringer, W., *Arch. f. Gynak.*, 1932, **151**, 190.