Distribution of Growth Hormone Among Cell Fractions Isolated from Pituitary Gland. (23683)

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By the technic of differential centrifugation, tissue homogenates can be fractionated to obtain a rough separation of cellular elements. This technic has now been applied to pituitary homogenates, with a view to ascertaining the distribution of growth hormone (GH) among the isolated fractions, *viz.* nuclear fraction (nuclei contaminated with some cytoplasmic material), mitochondrial fraction ('large granules'), 'fluffy layer' (material overlying the sedimented mitochondria), microsomal fraction ('small granules'), and supernatant fraction ('cell sap'). Since it was essential that fresh material be used for this fractionation, the experiments were performed with freshly excised rat pituitary glands.

Materials and methods. All isolative procedures were carried out at 0-2°C. In each of the 2 experiments, the pituitary tissue (approx. 0.5 g) obtained from 100 young albino rats was homogenized in 0.25 M sucrose solution with a Potter-type homogenizer. The debris obtained on centrifugation (600 *g* for 10 min.) was re-homogenized and recentrifuged, and the sedimented material ('nuclear' fraction) treated as described below. The combined supernatant fluids (approx. 5 ml) were centrifuged to sediment the mitochondrial fraction (12,000 *g* for 15 min.), from which the loose 'fluffy layer' was separated. These 2 fractions were washed once. The supernatant fluid and washings were combined, and centrifuged (20,000 *g* for 90 min.) to give the microsomal fraction and the supernatant fraction. Electron microscopy (kindly carried out by Mr. M. S. C. Birbeck) showed that mitochondria were present in the 'mitochondrial fraction' and absent from the 'microsomal fraction', but each fraction contained some particles which could not readily be identified. Each fraction was dialyzed over night against water containing a trace of ammonium carbonate, the alkaline pH thus maintained being likely to minimize inactivation of the GH. The dialyzed suspensions were finally lyophilized. The fractions were suspended in saline and injected subcutaneously into rats in eight divided doses over a period of 4 days(1). On the fifth day the rats were autopsied, the tibia preserved in 10% formalin and stained by the silver nitrate method. The tibia were measured by taking 10 measurements along the epiphyseal line to arrive at the mean width. All the fractions from each experiment were tested simultaneously. With a standard preparation of bovine GH, tested under similar conditions, a ten-fold increase in dose gave a net increase of 107 μ ($\lambda = 0.241$) in epiphyseal width; this value was used in the calculation of relative potencies. The mean epiphyseal widths in rats given the pituitary fractions were in all instances greater than those in control rats treated only with saline.

Results. Table I shows the yields of the dried fractions, and the amounts of activity in the particulate fractions relative to the amount in the supernatant fraction.

It is evident that a substantial proportion of the activity was present in the supernatant fraction, but that appreciable activity was present in each of the particulate fractions. The dry weight of the supernatant fraction being relatively high, the particulate fractions were in general richer in GH, in terms of dry weight, than the supernatant fraction. It cannot, of course, be assumed that the potency values obtained from the tibia test are an absolute index in the amount of GH present, since it is known that ACTH and thyrotrophin, which were presumably present in some of the fractions examined, may modify the effectiveness of GH in this test.

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TABLE I. Growth-Promoting Potency of Rat-Pituitary Fractions.

Fraction	Yield of dried fraction (mg/g pituitary tissue)		Activity of fraction, in arbitrary units (supernatant fraction = 100)*			
			Activity/mg fraction		Total amt of activity	
	Exp. A	Exp. B	Exp. A	Exp. B	Exp. A	Exp. B
'Nuclear'	11	22	110	30	16	14
Mitochondrial	17	35	270	55	62	39
'Fluffy layer'	24	8	110	120	36	86
Microsomal	26	20	130	170	46	71
Supernatant	74	49	(100)	(100)	(100)	(100)

* Total amt of activity/g of original pituitary tissue was equivalent to 2.4 mg (Exp. A) or 1.5 mg (Exp. B) of bovine GH, batch 22KR2.

Ziegler and Melchior(2) have now made a similar comparison of fractions isolated from rat pituitary tissue; a high proportion of the GH activity was found in the supernatant fraction, essentially as in the present study. Assays for gonadotrophic activity, on rat-pituitary fractions analogous to those now isolated, suggest that, at least in the case of follicle-stimulating hormone, activity may not be consistently localized in a single fraction (2,3,4). However, it is difficult to reconcile the present results with those recently reported by Brown and Hess(5), who concluded after careful study that GH was located mainly in the mitochondrial fraction. It is difficult to accept the suggestion of these authors that the occurrence of high hormonal activity in the supernatant, as in the experiments of Ziegler and Melchior(2), indicates the use of severe conditions; in the present study the glands were fresh and were homogenized gently. A more probable explanation is that bovine pituitaries, as used by Brown and Hess, may differ from rat pituitaries with respect to the intracellular localization of GH, the GH in the supernatant fraction being conceivably in transit from secretory particles to the cell exterior.

Summary. Fractions isolated from rat pituitary homogenates by differential centrifugation have been assayed for growth-promoting activity by the tibia test. The supernatant fraction contained a relatively high proportion of the activity, but no sharp localization was evident.

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1. Segaloff, A., Komrad, E. L., Flores, A., Segaloff, A., Hardesty, Mary, *Endo.*, 1955, v57, 527.

2. Ziegler, D. M., Melchior, J. B., *J. Biol. Chem.*, 1956, v222, 721.

3. Catchpole, H. R., *Fed. Proc.*, 1948, v7, 19.

4. McShan, W. H., Rozlich, R., Meyer, R. K., *Endo.*, 1953, v52, 215.

5. Brown, J. H. M., Hess, M., *Am. J. Physiol.*, 1957, v188, 25.

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