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Separation of Thyrotropic and Interstitial Cell Stimulating (Luteinizing) Hormones of Ant. Pituitary.

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Introduction. Preliminary chemical and physiological studies of the relationship between the thyrotropic and interstitial cell-stimulating (luteinizing) factors of the anterior pituitary indicated the possibility that these two principles are either identical or very closely related in their chemical aspects.¹ We would like to emphasize that we did not regard the evidence thus far obtained by us as proving definitely the identity of the two factors.

In the course of further chemical fractionation of our preparations we have obtained results which do not support the view of the identity of the two principles. We found that the interstitial cell-stimulating hormone can largely be freed from thyrotropic activity when precipitated repeatedly between 0.25 and 0.35 saturation of ammonium sulfate and that the thyrotropic potency is precipitated mainly between 0.35 and 0.5 salt saturation.

Methods of Assay. The interstitial cell-stimulating activity of the various fractions was determined in hypophysectomized immature female rats according to the method previously described.² The thyrotropic effect was assayed in 4-day-old chicks according to the procedure of Smelser^{3, 4} as well as in hypophysectomized immature rats, and was determined by the histological stimulation of the thyroids rather than by the weight increase of that organ. It has been found that chick thyroids are more sensitive to thyrotropic hormone than the thyroids of hypophysectomized rats.⁵ Comparison has been made of the minimal effective dose for interstitial cell stimulation in hypophysectomized rats and for stimulation of the thyroids in chicks.

Experimental. The starting material, obtained from sheep pituitary according to the procedure of Jensen, Simpson, Tolksdorf and

¹ Jensen, H., Tolksdorf, Sibylle, *J. Biol. Chem.*, 1940, **133**, proceedings, p. 49.

² Jensen, H., Simpson, Miriam E., Tolksdorf, Sibylle, and Evans, Herbert M., *Endocrinology*, 1939, **25**, 57.

³ Smelser, G. K., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **37**, 388.

⁴ Smelser, G. K., *Endocrinology*, 1938, **23**, 429.

⁵ Frankel-Conrat, Jane, Frankel-Conrat, Heinz, Simpson, Miriam E., and Evans, Herbert M., *J. Biol. Chem.*, 1940, **135**, 199.

Evans,² was repeatedly precipitated with ammonium sulfate between 0.25 and 0.5 saturation and was found to have a comparatively high content of interstitial cell-stimulating and thyrotropic activity. Further fractionation with ammonium sulfate was carried out at a pH of 5 to 6 and a protein concentration of approximately 1%. An interstitial cell-stimulating fraction was obtained by repeated precipitation between 0.25 and 0.35 saturation as described by Jensen, Tolksdorf and Bamman.⁶ This preparation was found to contain between 100 and 200 interstitial cell-stimulating units per mg and a thyrotropic potency up to 10%.*

From the supernatants of the precipitate obtained between 0.25 and 0.35 ammonium sulfate saturation a fraction was isolated by repeated precipitation between 0.35 and 0.5 ammonium sulfate saturation which contained approximately equal amounts of interstitial cell-stimulating and thyrotropic potency. Traces of follicle-stimulating hormone were also found to be present. The minimal effective doses for interstitial cell-stimulating and thyrotropic potency found for representative samples of both fractions are illustrated in Table I.

Discussion. The experiments reported indicate the separate existence of an interstitial cell-stimulating and a thyrotropic hormone in sheep pituitary as demonstrated by their slightly different solubility in the presence of ammonium sulfate. These findings are in agreement with the observations recently reported from other laboratories. Chow, Greep and van Dyke⁷ found that crystalline pepsin destroyed the thyrotropic hormone without seriously inactivating the interstitial cell-stimulating hormone. Fevold, Lee, Hisaw and Cohn⁸ reported that the luteinizing (interstitial cell-stimulating)

TABLE I.
Minimal Effective Doses for Interstitial Cell-Stimulating and Thyrotropic Potency.

Preparation	Hypophysectomized rats		Chicks
	Ovarian IT*	Thyroids	Thyroids
.25-.35 SAS†	10 γ	800 γ	160 γ
.35-.5 SAS†	50 γ	200 γ	40 γ

*IT—interstitial tissue.

†SAS—saturation of ammonium sulfate.

⁶ Jensen, H., Tolksdorf, Sibyle, and Bamman, Frieda, *J. Biol. Chem.*, 1940, **135**, 791.

* A purified interstitial cell-stimulating hormone preparation kindly supplied by Dr. H. M. Evans gave similar results.

⁷ Chow, B. F., Greep, R. O., and van Dyke, H. B., *J. Endocrinology*, 1939, **1**, 440.

⁸ Fevold, H. L., Lee, Milton, Hisaw, F. L., and Cohn, E. J., *Endocrinology*, 1940, **26**, 999.

hormone is largely precipitated from sheep pituitary extracts at 2.0 M concentration of ammonium sulfate and the thyrotropic hormone at 2.4 M. Evans and his collaborators⁵ have been able to obtain a thyrotropic fraction from anterior beef pituitary[†] by ammonium sulfate precipitation between 0.3 and 0.5 saturation. Further purification yielded a thyrotropic preparation containing 10% of interstitial cell-stimulating hormone.

Summary. The view of the identity of the interstitial cell-stimulating and thyrotropic pituitary factors could not be substantiated by the results obtained on further chemical fractionation of such preparations.

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Spontaneous and Reflex Emotional Responses Differentiated by Lesions in the Diencephalon.

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The diencephalic areas have been invoked as important subcortical centers having to do with the emotions.^{1, 2, 3} The emotional reactions of animals with hypothalamic lesions have not been differentiated as spontaneous and reflex phenomena. The former appear without provocation and vary somewhat between different subjects. The reflex emotions occur only after stimulation of one or another sensory modality. The monkey is an excellent animal for study of spontaneous emotionality. He threatens, grimaces, wiggles his ears, undergoes pilo-erection, and vocalizes either in anger or in fear. When approached he may attempt to run away or permits his anger to carry him into definite aggressions. Occasionally, in blind rage, he dashes himself against the mesh of the cage as if he meant to rend it apart. Cats show a good deal less of spontaneous emotional display than do monkeys.

† It is generally accepted that anterior beef and hog pituitaries contain more thyrotropic hormone than sheep pituitary.

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¹ Grinker, Roy R., *Psychosomatic Med.*, 1939, **1**, 19.

² Ingram, W. R., *Psychosomatic Med.*, 1939, **1**, 48.

³ Kabat, H., Anson, B. H., Magoun, H. W., and Ranson, S. W., *Am. J. Physiol.*, 1935, **112**, 214.