No satisfactory explanation for this interesting finding has, as yet, been given. The tendency towards low impedance skin values in mental disease may be a symptom of deficiency in growth hormone affecting directly or indirectly both the epidermis and the central nervous system. It is proposed, therefore, to study the therapeutic effect of growth hormone and other growth stimulating substances[6] in mental patients particularly where low skin impedance readings are obtained.

10542 P

Preparation of Follicle-Stimulating Extracts by the Use of Trypsin.*

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In a previous publication we have reported that the luteinizing activity in sheep pituitary gonadotropic extracts is destroyed by trypsin.[1] This has been confirmed by Chen and Van Dyke.[2] Our procedure for destroying the luteinizing activity has been utilized, therefore, in developing a convenient method for obtaining follicle-stimulating preparations.

The method of preparation is as follows: Acetone-dried sheep pituitary powder (100 g) was shaken with 1 liter of water and 0.5 cc of toluene for 12 hours and centrifuged. The extraction was repeated twice. The activity was recovered from the supernatant liquids by precipitation with acetone and centrifugation, after which the precipitate was suspended in 400 cc water, shaken and supercentrifuged.

The supercentrifuged supernatant liquid was treated at 37°C for 3.5 hours at pH 8 with 40 mg of trypsin† per gram of original pituitary powder and centrifuged. The precipitate was discarded. The clear supernatant liquid was placed in 50 cc centrifuge tubes and

6 Various authors in the Symposium on Growth, *Cold Spring Harbor Symp.*, 1934, **2**.

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1 McShan, W. H., and Meyer, R. K., *J. Biol. Chem.*, 1938, **126**, 361.

2 Chen, G., and Van Dyke, H. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 172.

† The trypsin used was samples No. 360427 and No. 390120 prepared by Fairchild Bros. and Foster, New York.

heated at 75°C for 20 minutes or until a precipitate formed which was eliminated by centrifugation. This step served to free the supernatant liquid of trypsin as determined by the method of Anson and Mirsky,[3] but with little if any loss in follicle-stimulating activity. A third inactive precipitate was formed by placing the heated supernatant liquid in cellophane tubes and dialyzing against 0.05 M sodium acetate buffer of pH 4. This precipitate was removed by centrifugation and the pH of the aqueous follicle-stimulating extract was adjusted to neutrality by dialysis against 0.05 N phosphate buffer. Certain of the extracts were used in this form for animal experimentation and others were sterilized by Seitz filtration for clinical use. There is apparently no significant change in the activity of the preparations after Seitz filtration (Table I), and no local or systemic reactions occurred when they were injected subcutaneously into human beings.

The nitrogen content of the follicle-stimulating preparations was 0.048% as determined by the micro-Kjeldahl method, and they gave a pink biuret test. The protein content of the aqueous preparations averaged 0.3% when calculated from the nitrogen content, or 6 mg per 0.5 g of original pituitary powder. The relation of the total nitrogen to the carbohydrate content of these preparations is given in another report.[4]

The data given in Table I show that when extracts prepared by the method just described were injected into normal 21-day-old female rats twice daily for 4.5 days, ovaries were obtained which

TABLE I.
Assay of Follicle-stimulating Preparations Made by Digestion of Sheep Pituitary Extract with Trypsin.

No. of Extract	Dose Trypsin, mg	No. of Rats†	Ovarian Response	
			Wt, mg	Qualitative
100WS	—	10	115	Many corpora lutea
FSH10WS	20	4	41	All follicles
FSH100WS	"	3	44	" "
FSH100WS*	"	3	39	" "
FSH50CS	"	3	35	" "
FSH50CS*	"	3	51	" "
FSH101WS	"	3	40	" "
FSH102WS	"	3	60	" "
FSH103WS	"	3	48	" "
FSH103WS*	"	3	43	" "

*After Seitz filtration.
† Each rat received 500 mg equivalent of dry pituitary powder.

[3] Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, 1933-34, **17**, 151.

[4] McShan, W. H., and Meyer, R. K., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 701.

contained only follicles. The uteri of these rats were distended with fluid in most cases while the vaginæ of approximately two-thirds of the rats were not open. The supercentrifuged aqueous extract before digestion with trypsin, however, produced ovaries which contained many corpora lutea together with undistended uteri and open vaginæ in all rats. These follicle-stimulating preparations were found to be free also of lactogenic and thyrotropic activities as indicated by the pigeon and young chick tests respectively.

Summary. A convenient method for the preparation of follicle-stimulating extracts from sheep pituitary powder by use of trypsin is described briefly. These preparations produced follicles only, when tested on normal female rats for 4.5 days, and were free of lactogenic and thyrotropic activities.

10543 P

Carbohydrate Properties of Pituitary Follicle-Stimulating and Luteinizing Preparations.*

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We reported in a previous publication that the follicle-stimulating activity of sheep pituitary extract is destroyed by certain amylolytic enzymes, and on the basis of this evidence we suggested that the follicle-stimulating activity might be due to, or dependent upon, a carbohydrate grouping.[1] Such a point of view logically led to an examination of the carbohydrate properties of our follicle-stimulating and luteinizing hormone preparations. Anticipation of a part of our findings by Evans, *et al.*,[2] prompts us to publish a brief report of certain of our results at this time.

Follicle-stimulating preparations made by the trypsin method[3] and luteinizing extracts which were strong in thyrotropic activity were hydrolyzed with hydrochloric acid and the total reducing action of

* Supported in part by a grant from the Wisconsin Alumni Research Foundation.

1 McShan, W. H., and Meyer, R. K., *J. Biol. Chem.*, 1938, **126**, 361.

2 Evans, H. M., Frankel-Conrat, H., Simpson, M. E., and Li, C. H., *Science*, 1939, **89**, 249.

3 McShan, W. H., and Meyer, R. K., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 699.