not indicate refection. Greying has not been observed in the female rats, but 5 males, averaging 394 g in weight, showed definite greying at 163 days of age after being kept on the diet since weaning. The high mortality of the young may have been due partly to injuries received from the wire floors during the first few days. Diet R-1 permits some degree of reproductive success, but is obviously not as good as a "normal" diet of natural foods. It is of interest, however, to record the rearing of healthy representatives of a second generation of rats on a diet supplemented with the B-vitamins in a synthetic form.

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Purification of Follicle-Stimulating Hormone (FSH) of the Anterior Pituitary.*

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Purification of the FSH has been achieved by the following steps: (1) extraction of acetone desiccated sheep pituitaries with 40% alcohol; (2) precipitation from 50% acetone at pH 4.5; (3) ammonium sulphate (A.S.) fractionation in which the hormone is reprecipitated several times between 0.5 and 0.67 SAS; (4) precipitation from 33% acetone at pH 4.8; (5) precipitation from 67% alcohol; (6) removal of less active material by saturation with NaCl at pH 4.1.

Details of this procedure have been published.[1] The product contained 1 unit in 6 γ (for method of assay and definition of unit, see below). It has recently been found that fractionation of very dilute FSH solutions (0.075%) with A.S. generally leads to doubling of hormonal activity in a fraction precipitating between 0.6 and 0.7 saturation with A.S. (the protein concentration at this stage being

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1 Fraenkel-Conrat, H., Simpson, M. E., and Evans, H. M., *Anales de la facultad de Medicina, Montevideo*, 1940, XXV.

TABLE I.
Standardization in Hypophysectomized Rats of an FSH Preparation Containing 1 Unit in 3 Gamma.

Total dose, γ/R	No. of rats	Ovaries		Estrous uterus No. of rats
		Wt, mg	Follicles (Histology*)	
18	8	39	many medium large	4
15	8	30	medium large	1
12	3	33	small, medium, few large	0
9	3	25	small, medium	1
6	3	22	" "	0
5	4	22	" "	0
4	6	15	few small medium	0
3	9	14	many small, few small medium	0
Controls	100	11	small only	0

*Interstitial tissue is deficient in all cases.

about 0.02%). Fractions precipitating below 0.6 and above 0.7 saturation show potencies similar to or lower than those of the material before fractionation. By this method preparations can be obtained which contain 1 unit in 2.5-3 γ (or 300-400 units per mg).

When assayed in a 3-day test in immature female rats, such FSH fractions:

1. Injected subcutaneously into hypophysectomized animals cause follicular development associated with increase in ovarian weights. The definition of the FSH unit is based on this effect; 1 unit represents the minimal total dose which will cause the resumption of follicular growth with antrum formation (Table I). There is no alteration of the interstitial tissue which consists of so-called "deficiency cells" as in uninjected hypophysectomized controls of the same postoperative period.

2. When the injections are made intraperitoneally into hypophysectomized rats, 15 FSH units will bring about beginning luteinization of follicles whereas repair of the interstitial tissue begins only on injection of 40 units and is not complete until 200 units are injected. Subcutaneous injections produce these secondary effects only at somewhat higher dose levels.

3. On subcutaneous injection into normal immature rats, 3-4 units are required to cause a detectable ovarian or uterine stimulation; on intraperitoneal injection about 6 units show an effect.

4. In both normal and hypophysectomized rats about 4-6 times the minimal dose causes the appearance of estrous uteri. In both types of rats, ovarian weights rarely exceed 60 mg.

5. On subcutaneous injection of FSH simultaneously with chorionic gonadotropin, "augmentation" is observed, *i. e.*, ovarian weights are increased considerably beyond what would be expected

as an additive effect of the two hormones. This effect is used as an alternate assay method. One hypophysectomized rat unit was found to cause 100% increase in the ovarian weights of normal rats over those produced by a standard amount of chorionic gonadotropin (8 R.U.); this was defined as 1 Augmentation Unit (Table II). Some degree of augmentation can be obtained regardless of whether the two hormones are injected mixed or at different sites of the body and the phenomenon is therefore not due to delay of absorption or similar nonspecific means of augmenting gonadotropic reactions.

6. On intraperitoneal injection in normal rats low levels of FSH (1-6 units) decrease the effect of other gonadotropins, *e. g.*, that of pregnant mare serum.[2] This antagonistic effect is also given by 1 unit of the other pituitary gonadotropin, ICSH. It appears, however, that it represents an independent property of each pituitary gonadotropin, since antagonism can be elicited with amounts of FSH which are very much smaller than those necessary to cause interstitial cell stimulating effects.

The physico-chemical properties of the most active FSH preparations correspond well to those ascribed by others to less active

TABLE II.

Standardization of Purified FSH Preparation in Normal Immature Female Rats. By "augmentation" of effect of a standard amount of chorionic gonadotropin (C. G.).

	Fraction					
	IV F102A I		IV F102B II		IV F102E II	
Total dose, γ/rat	No. of rats	Wt of ovaries mg	No. of rats	Wt of ovaries mg	No. of rats	Wt of ovaries mg
12					6	126
10	6	100				
9					9	107
8	3	113	3	149	9	93
6	3	118	6	114	18	99
5	9	112	6	118	9	87
4	9	85	6	98	15	79
3	6	70	6	88	18	73
2.5	6	66	6	60		
2.3					6	57
2.0			6	42	3	60
Control (C.G. alone)	12	32	18	34	36	36
1 unit (= 100% augmentation of the effect of C.G. alone)	2.5 γ		2.7 γ		3 γ	

2 Fraenkel-Conrat, H., Simpson, M. E., Li, C. H., and Evans, H. M., *Anales de la facultad de Medicina, Montevideo,* 1940, XXV.

preparations.[3, 4, 5] A preliminary electrophoresis experiment has shown FSH to consist mainly of one component.

Summary. The method of preparation of FSH in highly potent form is outlined. Some of the biological properties of this fraction are described.

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Eating Habit and Fasting Metabolism of Rats.

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The rate of fasting metabolism depends on the level of previous food intake. In order to measure the influence on the metabolic rate of any condition which lowers the appetite one must therefore reduce the food intake of controls to that of the experimental animals in order to eliminate the influence of food level. The rats whose food intake is thus reduced but whose appetite is not affected may eat their daily ration at once while their pair mates with their decreased appetite on unlimited food supply extend their food intake over the whole day.

Table I shows the feeding habit of 18 rats during 12 days. The amounts eaten during the various daily periods are expressed in percent of the daily intake. The behavior of 9 rats that had pre-

TABLE I.
Eating Habit of Rats.
Means of 9 rats.

Nutritive condition of rats	Food eaten per day per rat g	Food eaten in % of daily intake in following periods: 4-8 p.m. %	8-12 p.m. %	0-8 a.m. %	8-12 a.m. %	0-4 p.m. %
A. Mean of 12 days:						
1. Previously on unlimited food	14.9	16	23	47	10	4
2. " " restricted "	15.2	26	25	30	15	4
B. 1st day after on day's fast:						
1. Previously on unlimited food	15	60	7	20	0	13
2. " " restricted "	24	71	8	4	4	13

[3] Greep, R. O., van Dyke, H. B., and Chow, B. F., *J. Biol. Chem.*, 1940, **133**, 289.

[4] Fevold, H. L., Lee, M., Hisaw, F. L., and Cohn, E. J., *Endocrinology*, 1940, **26**, 999.

[5] Jensen, H., Tolksdorf, S., and Bamman, F., *J. Biol. Chem.*, 1940, **135**, 791.