been observed by Schacher, *et al.*,[7] and Nelson[8] has found estrin toxic in hypophysectomized male rats.

Large doses of pituitary implants are therefore perhaps toxic rather than life-sustaining because of the high estrogen production induced. In any case the beneficial effect of pituitary therapy is not because of its estrin stimulating effects. Emery and Schwabe's interpretation that pituitary stimulation was effective because of its luteinizing effect was satisfactory for their data and for most of ours on the rat, but the non-effect of progesterone and pregnancy urine extract administration as seen here and in work on dogs,[2] and the effectiveness of pituitary extracts in castrate and male cats,[2] point to the inadequacy of this explanation for all conditions in various species.

9520

Comparison of Pituitary Gonadotropic Extract and Prolan on Ovarian and Uterine Response in Immature Rats.

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Aschheim and Zondek[1] emphasized the similarity of prolan to anterior pituitary implants or extract in its effects on the genital tract of immature mammals. Later work of other authors demonstrated several important differences, which have been summarized in recent reviews.[2, 3] Evans and Simpson[4] and Evans, Meyer, and Simpson[5] observed that prolan, in contrast to anterior pituitary extract, did not cause increasing ovarian hypertrophy with markedly increasing dosage. Similar observations were reported on placental extract by Collip, *et al.*,[6] and on extract from blood of pregnant

7 Schacher, J., Browne, J. S. L., and Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 488.

8 Nelson, W. O., *Cold Spring Harbor Symp. Quant. Biol.*, 1937, **5**. In press.

1 Aschheim, S., and Zondek, B., *Klin. Wschr.*, 1927, **6**, 1322.

2 Evans, H. M., *J. A. M. A.*, 1935, **104**, 464.

3 van Dyke, H. B., *The Physiology and Pharmacology of the Pituitary Body*, Chicago, 1936.

4 Evans, H. M., and Simpson, M. E., *Am. J. Physiol.*, 1929, **89**, 381.

5 Evans, H. M., Meyer, K., and Simpson, M. E., *Am. J. Physiol.*, 1932, **100**, 141.

6 Collip, J. B., Thomson, D. L., McPhail, M. K., and Williamson, J. E., *Canad. M. A. J.*, 1931, **24**, 201.

women by Fluhmann.[7, 8] The latter also drew attention to the greater uterine hypertrophy in rats treated with prolan. Lipschutz[9] stressed the difference in luteinizing capacity between pregnancy urine and pituitary implants. This communication presents data on ovarian and uterine weights in response to varying doses of pituitary gonadotropic and pregnancy urine extracts, which can be fitted into rational curves. These define accurately the relationship of weight response to dosage and may serve as standard curves for bioassay.

Pituitary gonadotropic extract was prepared from acetone-dried sheep pituitary powder by the method of Wallen-Lawrence and van Dyke.[10] Further purification was carried out by fractional precipitation with alcohol and chilling, somewhat similar to the technic of Wallen-Lawrence.[11] The precipitate obtained by adding ethanol to 70% concentration and chilling at —5°C. was found to be the most potent fraction in causing ovarian hypertrophy, and was, therefore, used throughout the experiment. Prolan was prepared from pregnancy urine by adsorption with benzoic acid as described by Katzman and Doisy.[12] The crude extract was purified by precipitation by ethanol at 75% concentration. Colorimetric determination[13] on 5 mg. samples of this powder showed no detectable trace of sex hormones.

Female albino rats 21 to 22 days old from the same breed and fed on improved stock diets[14] were used. The pituitary extract was dissolved in 0.2 N acetate buffer (pH 5.3) and prolan in distilled water. Each rat received subcutaneously 3 injections of 0.5 cc. daily for 4 days, making a total of 6 cc. which contained the desired total dose of either preparation. Daily examination was made to detect vaginal opening. The rats were sacrificed approximately 120 hours after the first injection. The ovaries were carefully dissected. The uterus was cut at the level of cervix and stripped free from adventitious tissues and tubes, and fluid inside the lumen pressed out. Weighing was done on a torsion balance. The organs were sectioned for microscopic study. Controls were taken from the littermates of treated animals, and killed at the same time. Altogether

7 Fluhmann, C. F., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1193.

8 Fluhmann, C. F., *Endocrinology*, 1933, **17**, 550.

9 Lipschutz, A., *Endocrinology*, 1935, **19**, 42.

10 Wallen-Lawrence, Z., and van Dyke, H. B., *J. Pharm. and Exp. Ther.*, 1933, **47**, 163.

11 Wallen-Lawrence, Z., *J. Pharm. and Exp. Ther.*, 1934, **51**, 263.

12 Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1934, **106**, 125.

13 Wu, H., and Chou, C. Y., *Chinese J. Physiol.*, 1937, **11**, 409.

14 Wu, H., Wan, S., and Chen, T. T., *Chinese J. Physiol.*, 1932, **6**, 295.

TABLE I.
Pituitary Gonadotropic Extract.

Dosage (mg.)	Control	.05	.10	.15	.20	.25	.50	.75	1.00
No. of animals	20	8	8	10	9	15	16	15	11
Ovarian wt. (pair)									
Mean (mg.)	13.55	14.31	14.04	13.96	16.06	16.91	28.17	58.03	73.51
Standard deviation	3.02	1.95	2.51	3.03	5.40	4.44	16.40	30.30	31.80
" " of mean	0.68	0.69	0.89	0.96	1.80	1.15	4.10	7.83	9.58
Uterine wt.									
Aver. (mg.)	26.10	28.50	30.38	35.80	58.94	55.93	68.60	69.41	78.98
Standard deviation	5.38	9.87	4.86	8.14	17.11	25.10	15.56	12.28	13.20
" " of mean	1.20	3.50	1.73	2.58	5.70	6.49	3.89	3.18	3.98

TABLE II.
Prolan.

Dosage (mg.)	Control	.01	.015	.02	.04	.08
No. of animals	20	10	10	10	10	10
Ovarian wt. (pair)						
Mean (mg.)	13.55	12.90	14.75	18.60	25.95	34.20
Standard deviation	3.02	2.71	3.88	3.10	6.34	6.35
" " of mean	0.68	0.86	1.23	0.98	2.01	2.10
Uterine wt.						
Mean (mg.)	26.10	55.70	78.10	90.00	87.90	101.65
Standard deviation	5.38	37.61	26.61	24.40	27.90	16.72
" " of mean	1.20	11.92	8.43	7.72	8.83	5.29

162 rats were included in the present experiment; 20 for control, 92 receiving pituitary extract at 8 dosage levels and 50 receiving prolan at 5 dosage levels.

The results set forth in Tables I and II are plotted in Figs. 1 and 2 for pituitary extract and prolan respectively. The trend of weight response of ovaries and uterus to increasing dosage of the 2 gonadotropic hormones corresponds satisfactorily to logistic curves. These are rational curves useful in elucidating many biochemical phenomena,[15] and have the general equation:

$$y - d = \frac{k}{1 + e^{a - bx}}$$

where y and x are variables, and d, k, a and b are constants. In our case, y represents ovarian or uterine weight, and x, dosage. The quantity d is the lower asymptote, the lower limiting value of the

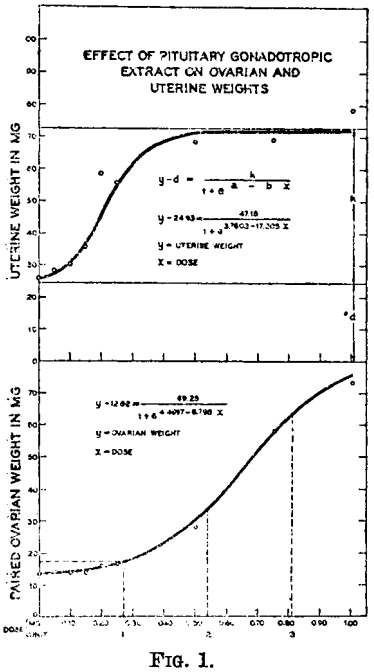

FIG. 1.

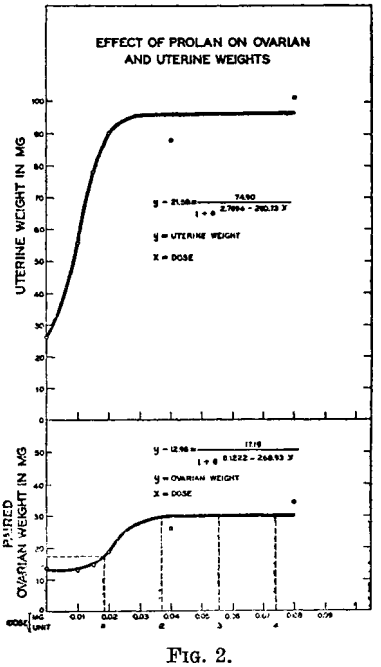

FIG. 2.

average ovarian or uterine weight of untreated rats, while k is the average maximum increase of the organ weight attainable with increasing dosage under the specified circumstances, thus $k + d$ giving the upper asymptote, the upper limiting value of the organ weight of

[15] Reed, L. J., and Berkson, J., *Phy. Chem.*, 1929, **33**, 760.

treated rats. The midpoint of the curve, namely $k/2 + d$, is the point of inflection where the organ weight changes at maximum rate. The numerical values of the constants are computed by the 4-point method,[16] and given with the respective curves.

In the curves the maximum attainable weight of paired ovaries or uterus $(k + d)$ differs according to the type of extract given. With ovaries, this value is much greater for the pituitary extract-treated animals (81.10 mg.) than for prolan-treated ones (30.15 mg.), though the highest dose of pituitary extract used has not clearly established the maximum. This difference is a numerical expression of the striking contrast, observed by previous workers, between the tremendous increase in ovarian weight produced by pituitary extracts and the slight hypertrophy with prolan. On the other hand, the maximum uterine response is somewhat greater to prolan (96.48 mg.) than to pituitary extract (72.11 mg.), as observed by Fluhmann.[7]

For comparison and bioassay it is necessary to convert the weight of both pituitary extract and prolan to units of potency. A unit may logically be defined as that amount of either extract which causes a significant increase in the *mean weight* of paired ovaries of a group of animals over the control. Statistically, a difference between 2 means is considered significant when it is 3 times the square root of the sum of the squares of the standard deviations of the 2 means. When the standard deviation of the mean of the control group alone is taken, we consider an increase as significant when the mean weight of paired ovaries of the treated group exceeds the control weight by 4 times the square root of twice the square of the standard deviation of the mean of the control group. Thus in the case of paired ovaries, we have $13.55 + 4\sqrt{2 \times (0.68)^2} = 13.55 + 3.85 = 17.40$ mg., this being taken as the mean ovarian weight corresponding to one unit. Reading from the respective curves gives 0.27 mg. of pituitary extract and 0.0185 mg. of prolan as equivalent to one unit.

In terms of units as defined the ovarian weight reaches the maximum more quickly in response to prolan (2 units) than to pituitary extracts (4 units). This is also true of uterine weight. The increment of ovarian weight per unit of pituitary extract at the steep portion of the curve is much greater than that with prolan, but the corresponding increment of uterine weight is smaller.

As recorded in Table III, the 2 gonadotropic extracts differ in their capacity to provoke vaginal opening and corpus luteum for-

[16] Pearl, R., and Reed, L. J., *Proc. Nat. Acad. Sc.*, 1920, **6**, 275.

TABLE III.
Frequencies of Opening of Vagina and Formation of Corpus Luteum.

Pituitary gonadotropic extract						Prolan					
Dosage mg.	Total No. of rats	Opening of vagina		Corpus luteum		Dosage mg.	Total No. of rats	Opening of vagina		Corpus luteum	
		No.	%	No.	%			No.	%	No.	%
Control	20	0	0	0	0	Control	20	0	0	0	0
.05	8	1	12.5	0	0	.01	10	0	0	3	30
.10	8	1	12.5	0	0	.015	10	5	50	8	80
.15	10	2	20.0	2	20.0	.02	10	9	90	9	90
.20	9	4	44.4	1	11.1	.04	10	10	100	10	100
.25	15	9	60.0	2	13.3	.08	10	10	100	10	100
.50	16	15	93.8	8	50.0						
.75	15	15	100.0	14	93.3						
1.00	11	11	100.0	9	81.8						

mation. Prolan has greater luteinizing activity, while the pituitary extract tends to cause earlier opening of the vagina.

From the above observations, the ovarian weight curve seems more satisfactory for assay of pituitary gonadotropic extract than of prolan. However, the applicability of the curves for this purpose remains to be demonstrated. The great variability of uterine response renders its curve unsatisfactory for assay.

Summary. This study demonstrates that the function of both ovarian and uterine weight response to pituitary extract and to prolan follows the same type of curve, namely, logistic curves. However, they differ from each other markedly in the numerical values of their constants, among which the value of $d + k$ is the most noteworthy. This defines the average maximum weight response attainable under the specified conditions. It is much higher in the case of ovaries, but lower in the case of uterus when pituitary extract is given, compared with prolan. The ovarian weight curve with pituitary extract may be of interest in assay.

We wish to express our thanks to Dr. H. B. van Dyke for suggestions in preparing pituitary gonadotropic extracts, to Dr. I. C. Yuan and Mr. C. S. Hsieh for help in mathematical analysis, and to Mr. C. Y. Chou for the chemical determination of sex hormones in prolan.

9521 P

Growth of Ultracentrifuged Cells in Tissue Culture.*

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It has recently been shown that the eggs of *Ascaris suum* and *A. megalocephala* (Beams and King[1, 2]) Fucus eggs (Beams[3]), cancer cells of rat (Guyer and Claus[4]) and the cells of the adrenal cortex

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1 Beams, H. W., and King, R. L., *Science*, 1936, **84**, 138.

2 Beams, H. W., and King, R. L., *Biol. Bull.* In press.

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4 Guyer, M. F., and Claus, P. E., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 468.