TABLE I.
Effect of Varying the Interval Between Hypophysectomy and Gonadotrophin Injection on Ovulation in the Rat.

Hrs from hypophysectomy to injection	No. of rats hypophysectomized	No. of rats ovulated
0	6	6
2	6	5
4	9	8
6	8	7
8	12	11
10	14	6
12	8	1
19	6	0
24	5	0

duct, newly formed corpora lutea, and preovulatory follicles.

Results and Discussion. The results as summarized in Table I show that in a high percentage of the animals tested the mature follicles retain their ability to ovulate in response to an intravenous injection of chorionic gonadotrophin provided the injection is made within 8 hours after the hypophysectomy. If the interval between hypophysectomy and injection is extended to 10 or more hours, the percentage of animals which will respond declines rapidly with no animals responding after 12 hours.

The data show that the pituitary is necessary for maintaining the mature follicles found in early proestrus so that they will ovulate in response to a gonadotrophic stimulus. They also indicate that after hypophysectomy the amount of the pituitary hormones responsible for the maintenance drops rapidly to a level in the blood below that required to keep the follicles in a condition where they can be ovulated.

It was found that the follicles in the ovaries of the rats injected 8 to 12 hours following hypophysectomy which failed to ovulate often showed a marked degree of luteinization, but when the interval was extended to 19 hours or more luteinization did not occur.

Summary. After an interval of about 10 hours following hypophysectomy in early proestrus, the mature follicles in the ovary of the rat become refractory to a single intravenous injection of human chorionic gonadotrophin but the ability of the follicle to luteinize is retained for a longer time.

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Statistical Evaluation of Growth Curves.

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In an article with the above title which appeared in this journal,[1] Weil compares the growth curves of groups of experimental animals subject to different treatments. Five treatments are compared against each other and a control, 30 rats are used for each treatment and the weight of each rat is determined at weekly intervals. Weil proposes a method of analysis in which he constructs a frequency distribution for each treatment taking all the weights of the rats over the period of the experiment and then applies the chi-square test to examine differences between those frequency distributions for the various treatments.

This is an oversimplified method of analysis and not a valid one. The fallacy lies in the tacit assumption that all the observations within each of the frequency distributions are independent. Although the 30 rats in each group are independent, the repeat weighings on each rat are certainly not so. In practically all biological work, the main source of variability lies between animals;

1 Weil, C. S., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 468.

repeat determinations per animal will result in more precise measurement for the individual animals, but will not reduce the effect of the basic variability between the animals. Applying the chi-square test in the way proposed by Weil may grossly overestimate the significance of the differences between the treatments.

There are valid methods of comparing growth curves of the type considered by Weil. These are indicated in broad outline in this note and a more detailed account with numerical examples will be published elsewhere.

In his paper, Weil considers the t-test applied to the weights after a given time, *e.g.* weights after 12 weeks. Provided the apportionment of the animals between the groups has been carried out in a strictly random manner, this method of test is valid. It may not be the best since it does not make use of the earlier weighings. Some improvement would probably result if the final weights are corrected for the variations in the initial weight of the rat (or any other characteristic which may be correlated with the final weight). The method to use here is the covariance method discussed by Fisher.[2]

A more complete method would be to fit regression lines, such as by the method of least squares, to the growth curve of each rat using, if required, a simple transformation to the time scale and weight scale in order to produce a simple curve. We may apply the method of the previous paragraph to estimates of the weight of rat obtained from the fitted lines at any given time. We could go further if desired and assess the significance of the differences between the constants of the fitted curves. For example, in the case of the slope of the curve, we adjust this slope (if required) for the estimated initial weight of each rat and calculate the significance of the effect of treatments on the adjusted slopes by Fisher's method already referred to. If the growth curves have been transformed into straight lines, this would represent a complete analysis.

Sufficiently approximate results may often be obtained from curves fitted by eye. We could compare any property of the growth curves between groups, *e.g.*, rate of growth at any given time, increase in weight between any given times, etc.

When the growth curves cannot be conveniently transformed into straight lines, these methods may have the disadvantage of not measuring the overall differences between the growth curves. A satisfactory method would be to read off from the fitted curves (by eye or by calculation) the estimated weights at the initial time and at two other times chosen to give a fairly adequate description of the growth curves. We then apply the method of "discriminant function analysis"[2] to these pairs of weights. This gives the maximum discrimination between the treatments and at the same time, furnishes a satisfactory test of the significance of the differences between the treatments. When it is desirable to correct for variations in the initial weight of the rats, we can apply this correction and the discriminant function analysis simultaneously.

With, if necessary, the use of a simple transformation for either or both of rat weight and time, it is nearly always possible to give an adequate description of the growth curves from 2 or 3 points. In rare cases a fourth point might be necessary. The discriminant function analysis can be extended quite readily to accommodate another point.

[2] Fisher, R. A., *Statistical Methods for Research Workers*, Ninth Edition, Oliver & Boyd, Ltd., Edinburgh and London, 1944.