STUDIES ON THE BIOASSAY OF PREGNANT MARE SERUM GONADOTROPHIC HORMONES USING IMMATURE FEMALE MICE

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FOUR FIGURES

Details of the responses of immature female mice after administration of pregnant mare serum gonadotrophic hormones (PMS) have never been reported, so authors have attempted to analyze the increment of the weights of sexual organs statistically for the purpose of bioassay.

In these experiments, authors used the ddN-strain of mice, which were bred and supplied by the Central Laboratory for Experimental Animals (CLEA), because this strain has been widely used in the field of bacteriology and experimental chemotherapy of carcinoma in Japan.

MATERIALS AND METHODS

PMS: Antex (Leo) was used as the PMS preparation and was dissolved in distilled water immediately before injection.

Animals: Female mice of the ddN strain were supplied from the Central Laboratory for Experimental Animals the day after weaning. They were about twenty-one days of age and weighed from 9 to 14.5 g.

Techniques: The day after receiving the animals, 0.1 cc. of PMS solutions (containing 2, 6, 20, 40, 60, 200 I.U. respectively) were administered by intramuscular injections to the hind legs. These mice were fed carefully with the compressed diets for mice (Oriental and CLEA) and given tap water to drink *ad libitum*.

The room temperature was carefully controlled between 23 and 26°C.

The assay method employed was similar to the immature rat ovarian weight increasing method described in the "British Pharmacopoea" (1953). After six days of injections, the mice were killed with illuminating gas and both ovaries and uterus from each mouse removed carefully. Both ovaries were decapsulated and cut off from the Fallopian tubes. The uteri were dissected at the utero-cervix and at the salpingo uterine junctions and fixed in Bouin's solution overnight and immersed in 70% alcohol for two hours.

The ovaries and uteri were then freed from any remaining hilum, and in the case of the uteri, the uterine fluids were oozed out on filter paper, and weighed on a torsion balance after drying for one minute on filter paper. The ovaries were weighed on a microtorsion balance, also after drying for one minute on filter paper. The uterine weights and the combined weights of both ovaries from each mouse were recorded.

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In the unstimulated animals of this age, the average weight of a pair of ovaries was 1.58 mg. and the average weight of the uteri was 6.43 mg.

RESULTS AND DISCUSSION

In order to analyze the experimental results, statistically, authors chose both ovarian weight and uterine weight as the index of the response of PMS. For the purpose of quantitative assay, at first authors attempted to determine the dose-response relations.

Figure 1 shows the results of Experiment No. 11 which induced from Table I. The regression lines between logarithm dose and organ weights are indicated,

Fig. 1. Relations between the logarithm dose of PMS and the organ weights of ovaries and uterus. The regression lines (OV for ovarian weight, and UT for uterine weight) and their fiducial intervals are also indicated (at 5% level of significance).

Table I. Effect of PMS on the female reproductive organ weights
(Experiment No. 11)

Organs	Ovaries					Uterus				
Dose in I.U.	2	6	20	60	200	2	6	20	60	200
Individual response	2.45	2.40	4.55	4.95	8.15	11.9	14.5	22.5	30.9	33.5
in mg.	2.05	3.00	5.05	7.00	9.25	11.9	24.1	23.0	34.0	33.8
	2.85	2.15	6.10	5.45	6.75	17.3	18.5	22.7	27.9	39.4
	2.00	3.75	3.60	5.00	8.05	9.9	20.6	21.1	22.0	28.3
	3.60	2.95	4.30	6.10	5.80	11.2	14.0	23.2	37.0	35.7
	1.55	2.95	4.75	6.55	10.15	9.8	20.1	20.4	29.7	25.5
	2.50	3.05	5.55	6.55	5.90	12.1	12.7	27.6	30.3	33.9
	1.55	5.95	6.10	7.45	6.75	8.8	12.8	31.5	31.2	27.3
	1.55	3.45	3.85	5.80	6.75	14.5	13.9	21.0	38.2	29.2
	2.15			4.80	8.65	19.4			35.6	31.7
				4.45	9.10				33.3	39.1
Average*	2.25	3.29	4.87	5.83	7.75	12.68	16.80	23.67	31.83	32.49
	±1.48	±2.50	±2.09	±2.19	±3.24	±7.75	±9.50	±8.32	±10.20	±10.24

* Mean $\pm$ S.E. (standard error) $\times t$

and the fiducial intervals, are also represented.

Comparing these two regression lines, we might assume that the dose-response

Fig. 2. Relations between the logarithm dose of PMS and the cubic roots of ovarian weights. The regression line and its fiducial intervals are also indicated (at 5% level of significance)

curve for uterine weight is better than the dose-response curve for ovarian weight because of its steepness. However, it is not satisfactory with respect to the three points described below:

(1) As regards the fiducial intervals, the uterine weights are larger than those of the ovarian weights.

(2) At a dose of about 60 I.U., the response appears to reach maximum, and the response curve becomes flat.

(3) In respect of the common sense of endocrinology, the ovarian functions in female animals are controlled by the gonadotrophic hormones, so the uterine functions are influenced secondarily by the secreted hormones from stimulated ovaries. Hence, it would be desirable to choose the ovarian responses as the index of PMS assay.

Fig. 3. Relations between the logarithm dose of PMS and the cubic roots of uterine weights. The regression line, and its fiducial intervals (of 5% level of significance) are also indicated.

Figures 2 and 3 show the regression lines between logarithm dose and metameters which are transformed to the cubic roots of ovarian weights and uterine weights of Experiment No. 11, respectively.

This transformation to cubic root curve is described by Ito, Tamaoki, and Egusa (1952) who used the cubic roots of prostates and seminal vesicles, instead of the original organ weights as the response of androgen on the immature castrated

Table II. Effect of PMS on the cubic roots of (ovarian weight $\times 10,000$) and (uterine weight $\times 1,000$)

No. of experiments	10	17	18	28	29
$\sqrt[3]{10,000 \times (OV)}$					
2 I.U.	28.92 $\pm$ 5.51* (8)	31.84 $\pm$ 5.26 (17)		29.73 $\pm$ 5.60 (7)	26.55 $\pm$ 4.03 (10)
6 I.U.	31.51 $\pm$ 7.20 (9)				
20 I.U.	33.29 $\pm$ 7.52 (9)		ovarian weight were not measured	36.05 $\pm$ 6.81 (6)	33.45 $\pm$ 4.17 (9)
40 I.U.		35.05 $\pm$ 6.13 (18)			
60 I.U.	35.72 $\pm$ 7.56 (9)				
200 I.U.	38.60 $\pm$ 12.28 (8)	41.55 $\pm$ 7.82 (17)		42.72 $\pm$ 6.02 (7)	40.73 $\pm$ 6.11 (10)
$\sqrt[3]{1,000 \times (UT)}$					
2 I.U.	24.01 $\pm$ 3.67	24.31 $\pm$ 3.96	24.99 $\pm$ 5.70 (12)	23.84 $\pm$ 0.59	23.24 $\pm$ 2.49
6 I.U.	25.02 $\pm$ 6.32		26.63 $\pm$ 5.40 (14)		
20 I.U.	30.69 $\pm$ 4.40		29.86 $\pm$ 5.44 (14)	33.60 $\pm$ 0.42	31.90 $\pm$ 4.68
40 I.U.		33.50 $\pm$ 4.24	31.51 $\pm$ 3.76 (14)		
60 I.U.	31.42 $\pm$ 4.17				
200 I.U.	32.84 $\pm$ 4.02	33.52 $\pm$ 3.18		36.28 $\pm$ 3.06	34.52 $\pm$ 2.67

* Mean $\pm$ S.E. (standard error) $\times t$

Number of animals used in each dose shows in parenthesis

male rat. Their analysis indicated it might be possible to apply this use of cubic roots to quantitative assay methods.

Thus, in other experiments, observed values of ovarian weight and uterine weight transformed to their cubic roots as shown in Table II.

Table III. Summary of the equations of regression curves which indicate the relations between logarithm dose and metameters

Experimental No.	Name of colony	Experimental periods	Metameters (y)	
			$\sqrt[3]{10,000}$ (Ovarian Weight)	$\sqrt[3]{1,000}$ (Uterine Weight)
10	G ₂	27/X-1/XI (1954)	$y = 4.62X + 27.69$ $\lambda = 0.74$	*
11	A ₃	10/XI-15/XI (1954)	$y = 7.14X + 26.18$ $\lambda = 0.36$	$y = 5.71X + 21.24$ $\lambda = 0.32$
17	G ₃	23/XII-28/XII (1954)	$y = 4.92X + 30.19$ $\lambda = 0.63$	*
18	A ₄	6/I-11/I (1955)		$y = 5.22X + 23.06$ $\lambda = 0.45$
28	F ₅	2/VI-7/VI (1955)	$y = 6.43X + 27.69$ $\lambda = 0.45$	*
29	A ₆	23/VI-28/VI (1955)	$y = 7.10X + 24.39$ $\lambda = 0.48$	*

λ Shows the index of precision.

* The linearity is not significant (see, Fig. 4).

When transformed to their cubic roots, the dose-response curves of the ovarian weight become more steep, but in the case of the uterine weight, the steepness of the line decreases somewhat and steepness appears only within limited dose ranges (between 2 I.U. and 20 or 60 I.U.) just as in the case of the original values.

Comparing the fiducial intervals the transformed values for ovarian weight are less than those for uterine weight.

The equations of the regression lines are summarized in Table III.

The reproducibility of the regression coefficients was as shown in Table III and Figures 4 and 5, even when the experiments were conducted in different seasons.

Fig. 4. Regression lines between logarithm dose of PMS and metameters. (cubic roots of 10,000 times of ovarian weight). Experiments 10 and 17 are used with Colony G (dotted lines) and other experiments are used with Colony A and F.

Fig. 5. Influence of PMS on the dose-response curve of the uterine weight transformed metameters (cubic roots of 1,000 times of uterine weight) (Dotted lines). Two straight lines show the regression lines of equations (in Experiments No. 11 and No. 18)

No. 10	□ ----- □	No. 18	+ ——— +
No. 11	○ ——— ○	No. 28	△ ----- △
No. 17	● ----- ●	No. 29	▲ ----- ▲

But, the regression coefficient would seem to be constant, characteristically depending on the breeding colony.*

In the case of the ovarian weight transformed metameter, the regression coefficients of Colonies A and F show 7.14, 6.43, and 7.10. The fiducial limits of these three regression coefficients fall in the same range. On the other hand, the regression coefficients of Colony G are 4.62 and 4.92 and their fiducial limits fall in the same range.

*The ddN strain of mice would be divided to two sub-strains (Private communications to the authors from Dr. T. Nomura of the Central Laboratory for Experimental Animals).

The regression coefficients of the above described two groups are significantly different. (All the statistical calculations were done at the 5% level of significance.)

By transforming either ovarian weight or uterine weight to the cubic roots, the results of the linear relationship between the logarithm dose and the metameter are reproducible, so it will be useful to assay PMS with immature female mice of ddN strain quantitatively.

Hitherto, there have been numerous assay methods of the gonadotrophic hormones, using female mice. They are based on the phenomena of the congestion of ovaries (Lloyd, *et al.*, 1949), ovulation (Ladman, *et al.*, 1953), luteinization (Hamburger, *et al.*, 1937) and vaginal opening, and all of these methods are classified in *quantal* (all or none type) response in statistics.

As *quantitative* (graded) response, the uterine-weight increasing method (Klinefelter, *et al.*, 1943) is the only one known.

Hamburger (1950), in describing his work, reported that the dose-response curve of the ovarian weight of the immature mice showed steepness between 2 I.U. and 10 I.U. and stated that he expected to use it for the assay method, but he did not write further details.

SUMMARY

1) The effects of PMS on female sexual organ weights of immature mice were studied.

2) In statistical analysis, we chose the cubic roots of both ovarian weights and uterine weight as the metameter of the response.

The relationships between logarithm dose and metameter was found to be linear and the reproducibility was confirmed.

These results will be valuable for the purpose of quantitative assay.

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