

## A Basis for Comparing the Potency of Gonadotrophins. (20017)

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In an earlier report from this laboratory (1) on inducing ovulation in the diestrous mouse by a single subcutaneous injection of chorionic gonadotrophin, it was suggested that this method might be useful in testing the potency of various gonadotrophins. Such information should be of value in interpreting ovarian responses to these hormones, and should also serve as a basis for comparing the various kinds of units now assigned to gonadotrophins.

To test the validity of this suggestion, bioassays were made of 3 types of commercial gonadotrophins: anterior pituitary extract from the horse,\* pregnant mare's serum,<sup>†</sup> and chorionic gonadotrophin from human pregnancy urine.<sup>†</sup> Minimal ovulating doses were established for each gonadotrophin.

*Procedure.* Mature female albino mice of the Rockland strain were injected during diestrus, and the reproductive tracts were examined for evidence of ovulation 18 hours later, as has been explained in several previous reports. During the course of this experiment, it was found that mice weighing at least 20 g yielded more consistent results than mice weighing less than that amount. It is quite possible that the estrous cycle had not yet begun in these lighter animals, even though the vaginal orifice was patent in each. For the purpose of this study, the minimal ovulating unit has been defined as that amount of gonadotrophin which will induce ovulation in 70% of 10 or more diestrous mice. This unit is more reliable for a bioassay of this type than the 50% response formerly suggested in the 1943 report.

*Results with chorionic gonadotrophin.* In preliminary attempts to establish a tentative

\* Equiphysin, an extract from anterior pituitary of the horse, was kindly furnished by the G. T. Harvey Co., Saratoga Springs, N. Y.

<sup>†</sup> Generous supplies of Anteron (pregnant mare's serum) and Pranturon (extract from human pregnancy urine) were furnished by Schering Corp., Bloomfield, N. J.

TABLE I. Comparison of Capacities of Gonadotrophins to Induce Ovulation.

| No. of mice inj.           | Wt* | Dosage    | % ovulating | No. of ova |
|----------------------------|-----|-----------|-------------|------------|
| Chorionic gonadotrophin    |     |           |             |            |
| 17                         | +   | 1.4 I.U.  | 100         | 1-13       |
| 12                         | +   | 1.2       | 83          | 1-14       |
| 9                          | —   | 1.2       | 44          | 1-5        |
| 15                         | +   | 1         | 73          | 1-16       |
| 18                         | —   | 1         | 44          | 1-7        |
| Pregnant mare's serum      |     |           |             |            |
| 12                         | +   | 5 I.U.    | 100         | 1-18       |
| 7                          | +   | 4         | 43          | 6-15       |
| 11                         | +   | 3         | 36          | 1-9        |
| Anterior pituitary extract |     |           |             |            |
| 14                         | +   | .4 F.H.U. | 100         | 4-14       |
| 9                          | +   | .25       | 88          | 1-11       |
| 12                         | +   | .20       | 41          | 1-6        |

\* + = >20 g; — = <20 g.

minimal ovulating dose, mice weighing at least 20 g were injected with either 0.8 I.U. or 0.9 I.U. of the pregnancy urine extract. These amounts proved to be subthreshold doses. 1. *Results with 1.0 I.U.* Eleven (73%) of the 15 mice weighing more than 20 g ovulated. Only 8 (44%) of the 18 mice weighing less than 20 g ovulated. 2. *Results with 1.2 I.U.* Twenty-one animals were used, 9 of which weighed less than 20 g and 12 weighed more than that amount. Of the lighter animals, only 4 (44%) ovulated. Of the 12 mice weighing more than 20 g, ovulation took place in 10 (83%). 3. *Results with 1.4 I.U.* All 17 (100%) of the treated animals weighing more than 20 g ovulated.

*Results with pregnant mare's serum.* All of the mice used in these tests weighed more than 20 g. None of the animals injected with 2.0 I.U. ovulated. 1. *Results with 3.0 I.U.* Four (36%) of the 11 mice in this series ovulated. 2. *Results with 4.0 I.U.* Seven mice were used in this group, of which only 3 (43%) responded. 3. *Results with 5.0 I.U.* This amount of pregnant mare's serum induced ovulation in all of the 12 mice (100%). Superovulation occurred in 2 animals, 18 ova being found in one and 15 ova in another.

*Results with anterior pituitary gonadotrophin.* The mice in this series also weighed over 20 g. Numerous animals, not included here, were used to determine an approximate minimal ovulating dose. 1. *Results with 0.2 Fevold-Hisaw Unit (F.H.U.).* Five (41%) of the 12 mice ovulated. 2. *Results with 0.25 F.H.U.* This dosage caused 8 of the 9 animals (88%) to respond. 3. *Results with 0.4 F.H.U.* Ovulation took place in all of the 14 mice (100%).

*Discussion.* Although the physiological action of both the chorionic gonadotrophin and the pregnant mare's serum is measured in terms of the International Unit, yet the capacity of each to induce ovulation in the diestrous mouse, unit for unit, is certainly not the same. The minimal ovulating unit was found to be only 1 I.U. for the chorionic gonadotrophin, but it was approximately 5 I.U. for the pregnant mare's serum. The minimal ovulating unit of the anterior pituitary extract, measured in terms of Fevold-Hisaw Units, was found to be only 0.25 F.H.U.

Experimenters are using these 3 types of gonadotrophins with 2 distinctly different bases of standardization. Even the chorionic gonadotrophin and the pregnant mare's serum are likely to yield confusing results when used in equal dosages in terms of International Units. Since the main function of the ovary is to produce ova, it would seem logical to assume that a test for the potency of a gonadotrophin should properly be in terms of its capacity to induce ovulation. If mouse-ovulating units are used, then the ovulating potency of the various gonadotrophins will be comparable. Application of these results shows that nearly 5 times as much pregnant mare's serum as chorionic gonadotrophin was necessary to produce equivalent results, and that as little as 0.25 Fevold-Hisaw Unit of the anterior pituitary extract was as potent as the other gonadotrophic substances. This method, which is completed within 18 hours, gives a clear picture of ovarian activity and does not

depend upon the interpretation of data gathered after a series of injections. In the latter procedure, the question arises whether the corpora lutea, seen under such conditions, are the result of the first or of a later injection. As has been stressed before, the reaction of the ovary within the first 24 hours after an injection of a gonadotrophin is of great significance in understanding subsequent responses of that organ.

Again we wish to emphasize the importance of using mice weighing at least 20 g. In one series, even more consistent results were obtained by using animals weighing 22 g. That there are major differences in ovarian responses in those mice that weighed 17 g to 19 g and those that weighed at least 20 g is shown by the series of mice injected with chorionic gonadotrophin. In the 1.2 I.U. series, of those weighing at least 20 g, 83% ovulated, but only 54% of the lighter animals responded. Similarly, in the 1.0 I.U. series, ovulation occurred in 73% of the heavier mice, whereas only 44% of the lighter animals ovulated.

*Summary.* 1. Three types of gonadotrophins were compared on the basis of the capacity of each to induce ovulation 18 hours after a single subcutaneous injection of a minimal ovulating unit of the extract into mature diestrous mice weighing at least 20 g. The minimal ovulating dose is defined as the smallest amount of gonadotrophin which will induce ovulation in 70% of a group of 10 or more mice similarly treated. 2. The minimal ovulating dose for the chorionic gonadotrophin was found to be 1.0 I.U.; for the pregnant mare's serum, approximately 5.0 I.U.; for the extract of the anterior pituitary of the horse, 0.25 Fevold-Hisaw Unit. 3. This bioassay method is of definite value in comparing the potency of various gonadotrophins.

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1. Burdick, H. O., Watson, H., Ciampa, V., and Ciampa, T., *Endocrinology*, 1943, v33, 1.

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