

- C. S., *N. Eng. J. Med.*, 1952, v246, 124.
7. Bessman, S. P., and Bessman, A. N., *J. Clin. Invest.*, 1955, v34, 622.
- 8a. Geiger, A., and Yamasaki, S., *Fed. Proc.*, 1956, v15, 71.
- 8b. Bessman, S. P., *AAAS Symposium on Development of Nervous Control*, 1956, Dec. 31.
9. Wooley, D. W., and Shaw, E., *Proc. Nat. Acad. Sci.*, 1954, v40, 228.
10. Gaddum, J. H., *Ciba Found. Symp. on Hypertension*, Boston, 1954, p75.
11. Shore, P. A., Silver, S. A., and Brodie, B. B., *Science*, 1955, v122, 284.
12. Udenfriend, S., Bogdanski, D., Weissbach, H., and Brodie, B., *Fed. Proc.*, 1956, v15, 493.
13. Udenfriend, S., Weissbach, H., and Bogdanski, D. F., *J. B. C.*, 1957, v224, 803.
14. Udenfriend, S., personal communication.
15. Haverback, B., Sjoerdsma, A., and Terry, L. L., *N. E. J. Med.*, 1956, v255, 270.

---

Received May 13, 1957. P.S.E.B.M., 1957, v95.

### Ovarian Weight and Responsiveness to Gonadotrophin Throughout the Estrous Cycle of Mice. (23268)

JAMES A. GREEN

*Department of Anatomy, University of N. Carolina, Chapel Hill, and Biology Division, Oak Ridge National Laboratory,\* Tenn.*

Information about ovarian weight and capacity to respond to gonadotrophin at each stage of the estrous cycle is necessary for investigation of the effects of gonadotrophin on the ovary of the intact adult female. Astwood(1) reported the average uterine weight during estrous cycle of the rat, but data on weight of ovary during the cycle are not available for either rat or mouse. Folley and Malpress(2) stated that the stage of the estrous cycle had no effect on response of the cow ovary to exogenous gonadotrophin. Rowson (3) and Willett *et al.*(4) reported that cows gave a greater ovulatory response when gonadotrophin was administered during the follicular phase of the cycle. The purpose of this investigation was to determine if the weight of the mouse ovary and its capacity to respond to a constant level of equine serum gonadotrophin fluctuated during the estrous cycle.

**Methods.** Virgin female mice of the C57BL/6JAX strain, 90 to 120 days of age were used. To determine the stage of the estrous cycle, a vaginal smear was taken the day the mouse was put on experiment. The smears were air-dried and stained with 0.5% methylene blue in saline solution; the stage

of the cycle was designated according to the scheme of Allen(5). Routine daily vaginal smearing prior to use of animals was not done because this tended to upset the cycle in a high percentage of the mice. *Average ovarian and uterine weights during the estrous cycle.* For this phase of the investigation, 74 mice were chosen at random from the colony and vaginal smears were made. They were sacrificed and the right ovary and uterine horn excised, dissected free of fat, and weighed on a torsion balance. The average weight data obtained are summarized in Table I. With the exception of late metestrus ( $M_2$ ) and proestrus (P), the average and median ovarian weights were similar throughout the cycle. The values for those 2 stages were not reliable because the number of mice was not sufficient. Uterine weights shown here are at variance with similar data that Astwood compiled for the rat(1). The rat data showed a peak uterine weight at proestrus, and a decline that began at estrus and continued until it reached its lowest ebb on the first day of diestrus. The failure to decline rapidly at estrus (O) and early metestrus ( $M_1$ ) reported here may represent a fundamental difference in the ovarian hormone secretion pattern of rat *vs.* mouse. In a group of 60 mice from 5 to 11 months of age, the average uterine weights at

---

\* Operated by Union Carbide Nuclear Co. for the U. S. Atomic Energy Commission.

TABLE I. Ovarian and Uterine Weights at Each Stage of the Estrous Cycle.

|                             | O    | M <sub>1</sub> | Stage of cycle |      | P    | All  |
|-----------------------------|------|----------------|----------------|------|------|------|
|                             |      |                | M <sub>2</sub> | D    |      |      |
| No. of mice                 | 14   | 21             | 3              | 33   | 3    | 74   |
| Avg wt of right ovary (mg)  | 3.6  | 4.0            | 2.9            | 3.9  | 3.5  | 3.8  |
| Median of right ovary wts   | 3.8  | 3.9            |                | 3.9  |      |      |
| Avg wt of right uterus (mg) | 45.8 | 45.2           | 31.1           | 30.8 | 26.3 | 37.6 |

each stage of the cycle were almost identical to those shown here. The number of mice taken at estrus and early metestrus may seem high for a random sample, but it has been reported that the period of vaginal cornification persists as long as the period of diestrus in C57BL mice(6).

*Results. Response to gonadotrophin injected at different stages of estrous cycle.* A procedure was designed in which each animal was used as its own control to conserve mice and to attempt to overcome the variability encountered in mature mice. The animals were anesthetized with nembutal and the entire right half of the reproductive tract was exteriorized through a ventral midline incision; the right uterine horn was cut just above the cervix and the right reproductive tract was removed by blunt dissection of the mesenteries. The right ovary and uterine horn were trimmed free of fat and weighed separately on a torsion balance. The body wall was then sutured and the animal was given a subcutaneous injection of 20 i.u. of equine serum gonadotrophin<sup>†</sup> (PMS), contained in 0.25 ml of distilled water. Seventy-two hours later the animals were sacrificed and the left ovary and uterine horn removed and weighed. The differences in weight between the right and left sides were taken as the measure of the degree of stimulation.

Fifteen control mice were treated in the manner just outlined except that distilled water was substituted for the PMS solution. Seventy-two hours after removal of the right side of the reproductive tract, the average weight of the left ovaries had declined approximately 25%; the amount of decline could not be related to the stage of the es-

trous cycle during which the operation was performed.

Table II is a summary of ovarian and uterine weights before and after gonadotrophin administered during the estrous cycle. There appeared to be no difference in ovarian weight response to PMS during estrus, early metestrus, and diestrus. Too few mice were treated during proestrus and late metestrus to furnish a basis for any conclusions.

Many workers merely divide vaginal smears into two categories, one called "estrous smears" containing epithelial cells only (this would include O, M<sub>1</sub>, and P by Allen's scheme); the other category usually called "diestrus" containing epithelial cells and polymorph leucocytes (Allen's M<sub>2</sub> and D). On the right side of Table II the results are tabulated by this scheme, and again there appeared to be no difference in the capacity of the ovaries of either group to respond to PMS.

The uterine weight before PMS varied with the stage of the cycle. However, the stage of the cycle had little if any effect on the average uterine weight after PMS.

*Discussion.* The average weight of the mouse ovary did not fluctuate during estrus, early metestrus, and diestrus; but the cyclic fluctuation of the uterus adequately demonstrated that ovarian hormone secretion was changing with the cycle. Thus, in the mouse, ovarian weight is not a valid quantitative criterion of secretion. It is possible that the weight of the mature follicles and their fluid are about the same as that of the corpora lutea, and in the mouse a shift from follicular to luteal phase would not be accompanied by any significant ovarian weight change.

The ovarian weight increase after gonadotrophic stimulation was about the same when the stimulus was begun in estrus, early metestrus, and diestrus. This indicated that the capacity of the ovary to respond to gonado-

<sup>†</sup> The equine serum gonadotrophin "Equinex" was supplied in generous amounts by Dr. John B. Jewell of Ayerst Laboratories, Inc.

TABLE II. Weight Responses of the Ovary and Uterus to 20 i.u. of PMS at Each Stage of the Estrous Cycle.

|                                   | Vaginal smear |                |                |      |      | Epithelial            |                      | All stages |
|-----------------------------------|---------------|----------------|----------------|------|------|-----------------------|----------------------|------------|
|                                   | O             | M <sub>1</sub> | M <sub>2</sub> | D    | P    | Epithelial cells only | cells and leucocytes |            |
| No. of mice                       | 9             | 14             | 2              | 15   | 3    | 26                    | 17                   | 43         |
| Avg wt of right ovary (mg)        | 3.6           | 3.8            | 3.2            | 3.6  | 3.5  | 3.7                   | 3.5                  | 3.6        |
| " " " left " "                    | 6.2           | 6.6            | 4.8            | 6.4  | 7.4  | 6.7                   | 6.2                  | 6.4        |
| Increase of ovarian wt (%)        | 73            | 75             | 50             | 79   | 108  | 78                    | 76                   | 77         |
| Avg wt of right uterine horn (mg) | 46.0          | 44.9           | 36.7           | 31.0 | 26.3 | 43.1                  | 35.9                 | 38.9       |
| " " " left " "                    | 43.1          | 51.1           | 36.5           | 43.5 | 42.0 | 47.3                  | 42.6                 | 45.5       |

trophin is not influenced by the stage of the cycle and hence that ovarian responsiveness is not a factor involved in the cyclic phenomena. The similarity of uterine weights after stimulation at different stages of the cycle suggests that ovarian hormone secretion capacity is not influenced by the stage of the cycle either.

These data indicate that adult animals of the same age can be used to bioassay gonadotrophin by ovarian weight increase if great accuracy is not necessary. Furthermore, the stage of the cycle can be disregarded as a factor that influences the outcome of the assay.

**Summary.** 1) The average weight of the right ovary of C57BL/6JAX mice 90 to 120 days old was about the same in mice killed in estrus, early metestrus, and diestrus. The average uterine weight varied according to

the stage of the cycle. 2) The average ovarian weight increase after 20 i.u. of equine serum gonadotrophin was the same when the gonadotrophin was administered in estrus, early metestrus, and diestrus. Also the average uterine weight after ovarian stimulation was about the same, regardless of the stage of the cycle in which the stimulus was begun.

1. Astwood, E. B., *Am. J. Physiol.*, 1939, v126, 162.
2. Folley, S. J., and Malpress, F. H., *Proc. Roy. Soc., London*, s. B, 1944, v132, 164.
3. Rowson, L. E., *J. Endocrinol.*, 1951, v7, 260.
4. Willett, E. L., McShan, W. H., and Meyer, R. K., *PROC. SOC. EXP. BIOL. AND MED.*, 1952, v79, 396.
5. Allen, E., *Am. J. Anat.*, 1922, v30, 297.
6. Burns, E. L., Moskop, M., Suntzoff, V., and Leob, L., *Am. J. Cancer*, 1936, v26, 56.

Received May 14, 1957. P.S.E.B.M., 1957, v95.

### Radioactivity in the Uteri of Rats Following Injection of Estrone-16-C<sup>14</sup>\* (23269)

IRA RINGLER†

*Biological Laboratories, Harvard University, Cambridge, Mass.*

The distribution and localization of gonadal steroids appear to be more closely related to their metabolism and excretion than to organs having sites of physiological action (1,2,3,4). In this report an attempt has been

made to define the nature of the radioactivity in the uteri of rats injected with estrone-16-C<sup>14</sup>.

**Materials and methods.** Adult ovariectomized rats (7 days) were injected subcutaneously with 5  $\mu$ g of estrone-16-C<sup>14</sup> (12,000 c.p.m.) daily for 3 days. Uterine luminal fluid was withdrawn from the distended uteri

\* This study was supported by a grant to Dr. F. L. Hisaw from the U.S.P.H.S.

† Present address: Am. Cyanamid Co., Pearl River, N. Y.

‡ Charles E. Frosst and Co., Montreal, Canada.