

## Gonadotrophic Hormone Secretion in Immature Hypophysectomized Parabiotic Rats.\*

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Following gonadectomy of one parabiotic rat of adult pairs, the other partner develops constant vaginal estrus if it is a female.<sup>1,2</sup> Likewise constant estrus appears in the hypophysectomized partner of adult hypophysectomized parabiotic rats following gonadectomy of the other parabiont.<sup>2-5</sup> In order to gain information concerning the kind and relative amount of gonadotrophic hormone released into the blood following gonadectomy in immature rats, experiments were designed in which parabiotic rats of both sexes were hypophysectomized and at the same time their parabiotic mates were gonadectomized. The gonad response of the hypophysectomized partner was used as the end-point in determining the nature of the pituitary secretion.

**Materials and Methods.** Littermate rats weighing 70 g or more were united in parabiosis at 31 to 33 days of age. The operative technic was that described by Bunster and Meyer,<sup>6</sup> except that metal skin clips were used instead of silk sutures in closing the skin incisions. On the 6th day after the animals were united, the left partner was hypophysectomized and the right partner was gonadectomized. All operations were performed under sterile conditions and under ether anaesthesia.

The majority of the animals were autop-

sied on the 11th day following the hypophysectomy-gonadectomy operation. The remainder were killed between the 3rd and 53rd days following this operation. At autopsy the gonads and accessories were removed and weighed. The ovaries were examined by the use of transmitted light to determine the qualitative response and the sella turcica was examined under binoculars and checked with the operation notes for completeness of the hypophysectomy.

**Results and Discussion.** The data reported in Table I give the weight and qualitative

TABLE I.  
Ovarian Response of Hypophysectomized-Castrated Parabiotic Rats.

| Sex of pairs | No. days hypophysectomized | Hypophysectomized partner |                      |
|--------------|----------------------------|---------------------------|----------------------|
|              |                            | Ovarian wt, mg*           | Qualitative response |
| ♀ ♀          | 3                          | 29                        | Follicles            |
|              | 5                          | 26                        | "                    |
|              | 6                          | 32                        | "                    |
|              | 7                          | 26                        | "                    |
|              | 7                          | 33                        | "                    |
|              | 7                          | 15                        | "                    |
|              | 9                          | 24                        | "                    |
|              | 9                          | 36                        | "                    |
|              | 10                         | 32                        | "                    |
|              | 10                         | 11                        | "                    |
|              | 13                         | 13                        | "                    |
|              | 17                         | 11                        | "                    |
| ♀ ♂          | 4                          | 26                        | "                    |
|              | 8                          | 31                        | "                    |
|              | 10                         | 50                        | "                    |
|              | 10                         | 23                        | "                    |
|              | 10                         | 37                        | "                    |
|              | 10                         | 40                        | "                    |
|              | 10                         | 30                        | "                    |
|              | 53                         | 59                        | "                    |

\* The average ovarian weight of single hypophysectomized control rats after being hypophysectomized for 10 days was 4.0 mg.

response of the ovaries of the hypophysectomized female following gonadectomy of its female or male partner. It will be noted that within 3 days following gonadectomy sufficient gonadotrophic hormone appeared in

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<sup>1</sup> Hill, R. T., *J. Exp. Zool.*, 1932, **63**, 203.

<sup>2</sup> Meyer, R. K., and Biddulph, C., *Am. J. Physiol.*, 1941, **134**, 141.

<sup>3</sup> Witschi, E., and Levine, W. T., *Proc. Soc. Exp. Biol. and Med.*, 1934, **32**, 101.

<sup>4</sup> Möller-Christensen, E., *Acta Path. et Microbiol. Scand.* (Suppl.), 1935, **22**, 1.

<sup>5</sup> DuShane, G. P., Levine, W. T., Pfeiffer, C. A., and Witschi, E., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 339.

<sup>6</sup> Bunster, E., and Meyer, R. K., *Anat. Rec.*, 1933, **57**, 339.

the bloodstream to give a substantial ovarian response in the hypophysectomized female. This indicates that the pituitary gland increases its output of gonadotrophic hormone very soon after the gonads are removed. Regardless of the sex of the gonadectomized animal follicles only were present in the ovaries of the hypophysectomized female, at least for a period of 53 days following gonad removal. From this evidence it follows that FSH was released from the anterior pituitary gland of the gonadectomized rat and that it passed via the circulation to the hypophysectomized parabiont where the ovaries were either maintained at their pre-hypophysectomy level or were stimulated to develop follicles and to increase in size.

The question arose as to whether or not the ovaries contained only follicles because FSH was the only gonadotrophic hormone released from the pituitary gland or whether this was due to a refractory or unresponsive state of the ovaries of the hypophysectomized partner to LH. That the former is more likely true is indicated by the presence of corpora lutea in the ovaries of the hypophysectomized partner of one pair (not included in Table I) in which a fragment of pituitary gland remained in the sella turcica at the time of autopsy, the supposition being that the LH producing the luteinization came from this pituitary fragment. That the ovaries of the hypophysectomized partner were not refractory to LH is indicated by the results of experiments reported previously in adult hypophysectomized pairs<sup>2</sup> and in the accompanying paper,<sup>7</sup> which show that LH injected into a normal or a gonadectomized parabiont produces luteinization in the ovaries of its hypophysectomized mate.

From the above evidence it appears that LH is not released from the pituitary gland of a gonadectomized parabiotic rat during the early stages of castration, or if it is released, the concentration does not reach a sufficiently high level in the nongonadectomized partner to produce luteinization of the stimulated follicles of that rat. It would

seem then that the luteinization of the ovaries of the intact parabiont which follows gonadectomy of the other rat, which has been reported in previous papers from this laboratory<sup>8-11</sup> and by others, is produced by LH from the pituitary gland of the intact rat. Evidently the stimulus effecting release of LH is the estrogen produced by the large stimulated ovaries of the intact rat, the FSH originating from the pituitary gland of the gonadectomized rat. However, it should be noted that the injection of estrogen into adult parabiotic rats does not cause the release of LH from the castrate pituitary gland.<sup>2</sup>

Hellbaum and Greep<sup>12</sup> have reported that the pituitary gland of normal adult female rats contains relatively large amounts of LH, and that of normal males is relatively deficient in this factor. Following castration the presence of LH in the pituitary of male rats becomes definitely evident about 20 days after gonadectomy as judged by assay of their pituitary glands. One of our female-male pairs was allowed to live for 53 days, but during this time only follicular stimulation was evident in the ovaries, which indicates that LH was not released by the pituitary gland, or if it was released the concentration did not reach a high enough level to produce luteinization.

Table II presents the results obtained when the hypophysectomized and gonadectomized parabionts were both males. It will be observed that there is considerable variation among the pairs in the weight of the testes, seminal vesicles and prostate of the hypophysectomized partner. Comparison of average weights of these structures with the corresponding average weights of normal animals indicates that there was some decline

<sup>8</sup> Meyer, R. K., and Hertz, R., *Am. J. Physiol.*, 1937, **120**, 232.

<sup>9</sup> Hertz, R., and Meyer, R. K., *Endocrinology*, 1937, **21**, 756.

<sup>10</sup> Biddulph, C., Meyer, R. K., and Gumbreck, L. G., *Endocrinology*, 1940, **26**, 280.

<sup>11</sup> Biddulph, C., Meyer, R. K., and Gumbreck, L. G., *J. Exp. Zool.*, 1941, **88**, 17.

<sup>12</sup> Hellbaum, A. A., and Greep, R. O., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 902.

<sup>7</sup> Meyer, R. K., Biddulph, C., and McShan, W. H., *Proc. Soc. Exp. Biol. and Med.*, 1946, **63**, 95.

TABLE II.  
Weights Obtained in Hypophysectomized-Castrated Male Parabiotic Rats.

| Sex of pairs | No. days hypophysectomized | Hypophysectomized partner* (mg) |             |        |         | Castrated partner (mg) |        |        |
|--------------|----------------------------|---------------------------------|-------------|--------|---------|------------------------|--------|--------|
|              |                            | Testes                          | Sem. Vesic. | Prost. | Adren.† | Sem. Vesic.            | Prost. | Adren. |
| ♂ ♂          | 10                         | 1733                            | 130         | 177    | 17      | 10                     | 22     | 28     |
|              | 10                         | 1760                            | 92          | 122    | 12      | 8                      | 8      | 30     |
|              | 10                         | 1212                            | 20          | 51     | 19      | 7                      | 17     | 39     |
|              | 10                         | 1438                            | 162         | 172    | 20      | 12                     | 26     | 25     |
|              | 10                         | 1516                            | 129         | 144    | 24      | 9                      | 27     | 26     |
|              | 10                         | 1900                            | 251         | 166    | 15      | 12                     | 19     | 30     |
|              | 10                         | 382                             | 8           | 22     | 13      | 7                      | 15     | 25     |
|              | 10                         | 1611                            | 25          | 64     | 15      | 10                     | 12     | 29     |
|              | 10                         | 1256                            | 185         | 178    | 12      | 10                     | 13     | 32     |
|              | 10                         | 1538                            | 164         | 175    | 20      | —                      | —      | 35     |
|              | 10                         | 1943                            | 280         | 284    | 17      | 7                      | 21     | 34     |
|              | 10                         | 1885                            | 160         | 282    | 16      | 13                     | 25     | 32     |
|              | 10                         | 1357                            | 14          | 32     | 14      | 13                     | 34     | 33     |
|              | 11                         | 645                             | 13          | 22     | 16      | 8                      | 14     | 39     |
|              | 11                         | 2141                            | 375         | 313    | 12      | 9                      | 16     | 25     |
|              | Averages                   | 1474                            | 133         | 147    | 16      | 9                      | 18     | 31     |

\* The testis, seminal vesicle and prostate weights of 7 normal animals of the same age were 2042, 101, and 170 mg, respectively

† The average adrenal weight of single hypophysectomized control rats of the same age was 10 mg.

in testis weight of the hypophysectomized partner, but that the accessory weights of this rat were approximately the same as those of normal controls. Usually when there was a marked decline in testis weight in a given rat the accessory gland weights likewise declined, which shows that the endocrine function of the testes decreases when the testes are not maintained by gonadotrophic hormones. It should be emphasized that in every case the testes of the hypophysectomized partner, regardless of their size and the size of the accessories, remained in the scrotum throughout the experiment. This is in agreement with the results of other workers.

The data obtained when the hypophysectomized partner was a female indicates that only FSH passed from the castrated male to the hypophysectomized partner. From the fact that neither the testes nor the accessories of the hypophysectomized male underwent the typical regressive changes of hypophysectomy, it is suggested that (1) endogenous FSH in the amounts present following gonad removal is concerned with the maintenance of the testes in the scrotum, for it seems logical to assume that FSH also

passed from the gonadectomized male to the hypophysectomized male in these experiments, or (2) that small amounts of LH were also released from the pituitary gland following gonadectomy of the male and that these small amounts were sufficient to stimulate the interstitial cells of the testes to produce androgen, but were not enough to produce luteinization of the ovaries. The experiments reported in this paper on female-male pairs confirm the results of Greep<sup>13</sup> on parabiotic triplets which demonstrated that the gonadotrophins secreted following gonadectomy of a male produce only follicles in the ovary and stimulate the testes and accessory glands of the male.

Cutuly, *et al.*<sup>14</sup> have reported experiments with adult hypophysectomized-castrated male parabiotic rats in which varying degrees of gonadal and accessory maintenance in the hypophysectomized partner were obtained. Our results in immature animals are essentially the same as those reported by

<sup>13</sup> Greep, R. O., *Proc. Soc. Exp. Biol. and Med.*, 1940, **44**, 214.

<sup>14</sup> Cutuly, E., McCullagh, D. R., and Cutuly, E. C., *Endocrinology*, 1937, **21**, 241.

these workers in adult animals.

The variation in ovarian, testis and accessory weights in the hypophysectomized partner may be a reflection of the degree to which the pituitary gland undergoes castration changes following gonad removal. We have previously reported<sup>11</sup> that approximately 11% of a large number of control parabiotic rats failed to undergo the typical ovarian enlargement following gonadectomy of one parabiont. The percentage of rats failing to show the typical castration changes in this study when the recipient parabiont was hypophysectomized is slightly higher (17%), which indicates that the gonads of hypophysectomized rats respond less readily to gonadotrophic stimulation than those of normal rats.

*Summary.* Using immature hypophyse-

tomized-gonadectomized parabiotic rats it has been demonstrated that following gonad removal in both sexes follicular stimulation only is obtained in the ovaries of the hypophysectomized parabiont for as long as 53 days following the operation. If the hypophysectomized partner is a male, the testes are maintained in the scrotum and their average weight is somewhat less than that of normals, whereas the weight of the accessory glands is approximately the same as that of normal animals.

Approximately 17% of the pairs failed to show any gonadal stimulation following the hypophysectomy-gonadectomy procedure. This percentage failure is slightly greater than that occurring in nonhypophysectomized pairs.

15508

### Luteinization of the Ovaries of Immature Hypophysectomized Parabiotic Rats with Gonadotrophic Hormone Preparations.\*

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It has been shown that following hypophysectomy of an immature female parabiotic rat and gonadectomy of its male or female partner only follicular stimulation is obtained in the ovaries of the hypophysectomized parabiont.<sup>1,2</sup> The question arose as to whether or not the ovaries of the hypophysectomized rat failed to become luteinized because they were unresponsive to luteinizing hormone (LH), or whether the condition of follicular stimulation was the result of a failure of LH to either be released from the castrate pituitary gland or to reach the ovaries in sufficient quantities to produce luteinization. In an effort to

gain information on this question, one parabiont of a group of parabiotic rats was hypophysectomized and the intact rat was injected with gonadotrophic hormone preparations and the response determined in the ovaries of the hypophysectomized rat.

*Materials and Methods.* Littermate rats weighing 70 g or more were united in parabiosis at 31 to 33 days of age. The operative technic was that of Bunster and Meyer<sup>3</sup> except that metal skin clips were used instead of silk sutures in closing the skin incisions. On the 6th day following parabiotic union of the animals one partner was hypophysectomized and the other was either gonadectomized or left intact. All operations were performed under sterile conditions and under ether anesthesia.

Within a few hours after the operation,

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<sup>1</sup> Biddulph, C., and Meyer, R. K., *Proc. Soc. Exp. Biol. and Med.*, 1946, **63**, 92.

<sup>2</sup> Greep, R. O., *Proc. Soc. Exp. Biol. and Med.*, 1940, **44**, 214.

<sup>3</sup> Bunster, E., and Meyer, R. K., *Anat. Rec.*, 1933, **57**, 339.