

reverse procedure was applied, that is, transplantation of a piece of skin from a non-reactive area on the abdomen to a denuded site within the genital area; treatment in this animal left the transplant a pale island of skin in brilliantly colored surroundings.

In the male, the left abdominal sympathetic trunk was severed below the fifth lumbar ganglion. Treatment with oestrogenic hormone elicited a temporary but definitely greater degree of swelling in the left scrotal area than in the corresponding area on the opposite side. This was soon obscured, however, by the increasing oedema of the genital reaction as a whole. A complementary type of experiment has already been recorded by Zuckerman,<sup>2</sup> who was able to inhibit the genital response by section of the sacral nerves in the cauda equina.

*Summary.* In the sex skin reactions of *M. mulatta* there are no qualitative differences of response to the keto- and trihydroxy-forms of oestrogenic hormone. Although the skin reactions are to a degree under the influence of the autonomic nervous system, the characteristic phenomena are probably determined by inherent structural peculiarities of the genital skin itself.

#### 8446 C

### Oestrogenic Hormone and the Mechanism of Corpus Luteum Formation in the Rabbit.

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Hohlweg's experiment,<sup>1</sup> in which corpus luteum formation was induced in immature "40 to 50 gm. rats" by the administration of single large doses of dihydroxyoestrin benzoate (Progynon-B), constitutes the crucial evidence in support of the currently favored view that oestrogenic hormone stimulates the output of luteinizing hormone by the anterior pituitary. Attempts to duplicate Hohlweg's attractive experiment in this laboratory have unfortunately been unsuccessful. Single subcutaneous doses of as much as 2000  $\gamma$  of crystalline oestrone (Folliculine-Girard) in oil have been given to each of 10 female rats, weighing from 40 to 50 gm.—this weight corresponding to an age of 24 days in our colony. At autopsy one

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<sup>2</sup> Zuckerman, S., *Proc. Roy. Soc. Lond.*, 1935, **118**, 22.

<sup>1</sup> Hohlweg, W., *Klin. Wchnschr.*, 1934, **13**, 92.

week later the ovaries of these animals were found to be still immature. There was no histologic evidence of granulosa luteinization. The weights of the ovaries (averaging 12.9 mg. per rat) and of the pituitaries were not significantly altered in comparison with those of uninjected control rats. Barring any theoretical difference in the biologic activity of oestrone and dihydroxyoestrin benzoate, the conflicting results may be due to differences in age-weight relationships in the two colonies. In this connection, Selye *et al.*<sup>2</sup> have already indicated that the effects of massive oestrogenic hormone doses in rats are first observed with regularity at or near puberty, irrespective of the absolute weight of the test animal.

In order to examine the question further the oestic rabbit was thought to be a desirable test object. As the rabbit exhibits no spontaneous ovulatory cycle, adult does of this species which have been well fed and isolated for 3 or more weeks should, in the majority of instances, have ovaries free of functioning corpora lutea and containing numerous growing and mature follicles. A number of does with a known record of litter-bearing were accordingly isolated, and after the lapse of one month, were inspected by laparotomy for the condition of the ovaries. By eliminating any animals exhibiting hemorrhagic follicular atresia, 12 suitable animals with grossly healthy and mature follicles were found for experiment. Allowing 3 days for recovery of pre-operative weight, these animals were given crystalline oestrone in the following manner: Four received 500  $\gamma$  in corn oil intramuscularly once daily for 8 days; 4 were given 2 intramuscular injections of 4000  $\gamma$  each in oil at intervals of 3 days; 4 received a single intravenous injection of 2000  $\gamma$  partly dissolved and partly suspended in a 5% alcohol-saline solution. Biopsy of the uterus and one ovary was performed at the end of 48 hours in one of the second group of animals. The uterus was moist, intensely swollen and cyanotic at this time, and averaged 12 mm. in cross-sectional diameter. Histologically the ovarian medulla and the stroma of the uterine mucosa were intensely engorged, but otherwise there were no exceptional features to be noted in the minute anatomy of the ovarian follicles and interstitial tissue. The remaining animals were examined by laparotomy on the ninth day of the experiment, at which time biopsy specimens of the uterus were taken in half of the animals of each group. The uteri of all animals were still markedly turgid at this time, but in only one instance was corpus luteum formation observed. In this instance the corpora

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<sup>2</sup> Selye, H., Collip, J. B., and Thomson, D. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1377.

resembled in all characteristics normal corpora of about the fourth day of pregnancy. The uterine mucosa, however, was not progestational in type, but showed hydrops similar to that observed in the "endometrial mole" of Selye, Harlow and McKeown.<sup>3</sup> It is believed that this animal may have been accidentally stimulated sexually during handling, this rather than any direct effects of oestrone upon the hypophysis accounting for the occurrence of ovulation.

In order to extend the experiment on a comparable basis with the experiment of Hohlweg as to dose per unit of body weight, 2 additional 2½ kilo oestric does were each given intramuscular injections of 15,000  $\gamma$  of oestrone in oil (subdivided into convenient but simultaneously administered portions), and reexamined 72 hours later. In neither case had ovulation or corpus luteum formation occurred by that time.

The original group of 12 rabbits were allowed 3 days in which to recover from the second laparotomy, and on the 12th day following the initial oestrone injections were mated with each of several healthy males. All but 2 does copulated with one or more of the bucks. Two days later the does were killed. In all but the 2 does which had refused to copulate there was abundant fresh apical corpus luteum formation, these bodies corresponding both grossly and histologically to normal corpora of comparable age. The progestational development of the uterine mucosa, however, was interfered with in varying degrees, from that of complete inhibition as noted by Hisaw and Leonard,<sup>4</sup> to that approaching the complex glandular proliferation of the typical reaction.

The weight of the pituitary gland (stalk included) in the 14 rabbits averaged 38 mg., and with one exception did not exceed 42 mg. This exception was noted in one of the 2 animals killed 72 hours after receiving 15,000  $\gamma$  of oestrone; here the gland weighed 72 mg. The average pituitary weight of 21 non-pregnant adult does of the same stock and weight, untreated with gonadotropic or oestrogenic hormones and killed during the course of acute experiments in this laboratory, was found to be 42 mg.

It thus appears that within the limits of the methods and the dosages here chosen, oestrone does not cause luteinization of the ovarian granulosa of the adult oestric rabbit. Oestrone also has little if any effect upon the pituitary weight of female rabbits which

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<sup>3</sup> Selye, H., Harlow, C., and McKeown, T., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1253.

<sup>4</sup> Hisaw, F. L., and Leonard, S. L., *Am. J. Physiol.*, 1930, **92**, 574.

possess no corpora lutea, or which exhibit corpora of less than 3 or 4 days' maturity. The administration of large doses of oestrone does not appear to interfere with post-coital ovulation and early corpus luteum development in the rabbit, though it may alter in marked manner the anatomic pattern of the coincidental progestational mucosa of the uterus. The undoubted stimulating effect of oestrone upon the anterior pituitary, as shown by the work of Wolfe,<sup>5</sup> Lane,<sup>6</sup> Hohlweg,<sup>1</sup> Selye, *et al.*,<sup>2</sup> and others,<sup>7-10</sup> might therefore be interpreted—as Selye has in fact already suggested—as the calling forth of pituitary factors which have functions other than that of initiating the luteinization of follicular granulosa.

## 8447 C

**Effects of Extract of Cattle Ant. Pituitary on Endochondral Ossification in Thyroidectomized Young Guinea Pigs.**

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In former investigations we<sup>1</sup> studied the influence of acid extracts of anterior pituitary gland of cattle on the growth of bone and cartilage in young guinea pigs. We analyzed the endochondral ossification in normal animals as well as the callus formation in guinea pigs in which a bone had been fractured; we could establish under these conditions a growth-promoting effect of the anterior pituitary extract. In about 20% of the cases the growth of both cartilage and bone was stimulated to the same extent, with consequent hypertrophy and hyperplasia of their various cells. However, in the majority of the animals the process of ossification predominated over the formation of cartilage, thus causing an earlier closure of the epiphyseal line than would have taken place otherwise.

<sup>5</sup> Wolfe, J. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 757, 1192.

<sup>6</sup> Lane, E. C., *Am. J. Physiol.*, 1935, **110**, 681.

<sup>7</sup> Burch, J. C., and Cunningham, R. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **27**, 331.

<sup>8</sup> Halpern, S. R., and d'Amour, F. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 108.

<sup>9</sup> Hisaw, F. L., *Am. J. Obs. and Gyn.*, 1935, **29**, 638.

<sup>10</sup> Nelson, W. O., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **32**, 452.

<sup>1</sup> Silberberg, M., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1423. Silberberg, M., and Silberberg, R., *Ibid.*, 1935, **33**, 177.