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Effect of Oestrin on Ovaries and Adrenals.

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The enlargement of the ovary observed during gestation in the rat is solely due to the corpora lutea attaining a much larger size than those of the normal cycle or those produced in non-pregnant animals by administration of gonadotropic substances; yet there has been little investigation of the factors regulating the size of the individual corpus luteum. We have shown¹ that the mere distension of the uterine cavity with paraffin leads to prolonged vaginal dioestrus and inhibits the involution of the corpora lutea of pregnancy even though the embryos and their placentae be removed; these corpora, however, showed histological signs of fatty degeneration and were unable to maintain the mammary glands in a fully developed condition. The hypophysis is not essential for the maintenance of the corpora lutea of gestation.^{2, 3} The administration of gonadotropic hormones produces larger corpora in the pregnant animal than in the non-pregnant.⁴ Since the gonadotropic hormones lead not only to formation of new follicles and corpora lutea but also to involution of corpora already present, we have repeated and modified these experiments. Six rats received 3 injections of 50 units of A.P.L. (anterior-pituitary-like hormone of pregnancy urine) on 3 successive days at about mid-pregnancy. The animals were sacrificed 7 days later; their ovaries were found to contain very large numbers of corpora lutea, all of which were as large as normal corpora of gestation. There is, therefore, some factor in the pregnant animal which transforms experimentally-produced corpora lutea into corpora lutea of gestation, and maintains them throughout the whole period of pregnancy.

There is evidence that oestrin is produced in large quantities in the pregnant rodent.⁵ Wolfe⁶ has shown that corpora lutea as

¹ Selye, H., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **31**, 488.

² Selye, H., Collip, J. B., and Thomson, D. L., *Anat. Rec.*, 1934, **58**, 139; *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 589.

³ Pencharz, R. I., and Long, J. A., *Am. J. Anat.*, 1933, **53**, 117.

⁴ Selye, H., Collip, J. B., and Thomson, D. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **32**, 530.

⁵ Selye, H., Harlow, C. M., and McKeown, T., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**.

⁶ Wolfe, J. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 757, 1192.

large as those of gestation are formed in non-pregnant animals treated with oestrin in large doses. We have confirmed these experiments, and find, in agreement with Scaglione⁷ and Hohlweg,⁸ that ovaries containing large numbers of large corpora lutea are produced by such treatment. Such observations suggest that oestrin may be the essential factor in the production of the ovarian structure typical of pregnancy; yet the ovaries obtained in these experiments differed from those of pregnancy in that several sets of corpora were present. Attempts were therefore made to study the effects of oestrin in conditions during which follicle maturation is known to be inhibited. In 8 late pregnant rats treated with 500 γ of oestrone* in oil daily, pregnancy was prolonged up to 24-26 days, at the end of which time most of the embryos died *in utero*, and only in 2 cases did delivery of the dead fetuses take place. In one case the corpora lutea were still fully developed and apparently functional, judged by their histological appearance and the condition of the endometrium on the 26th day of gestation. It seems that oestrin in these cases prolongs the life of the corpora lutea and thus interferes with parturition. In another experiment, 8 rats received 500 γ of oestrone daily in oil from the 4th to the 14th day of lactation. The vaginal smear remained dioestrous throughout; at autopsy on the 10th day of treatment the ovaries, pituitaries and adrenals were found to be greatly enlarged. The average weight of the ovaries was 93 mg., of the adrenals 71 mg., and of the pituitary 18.5 mg. The enlargement of the ovaries in this experiment was due solely to the increase in size of the corpora lutea; the ovary represented in Fig. 1 was the largest of the series, weighing 134 mg., yet the number of corpora lutea was not increased; the stroma of the ovary and those corpora lutea which apparently formed from subsequent ovulations were very poorly developed. The vaginal epithelium of these animals displayed a high degree of mucification, a condition regularly seen in pregnancy but not in lactation, although active corpora lutea are known to be present in both of these states.

We conclude from these experiments that the differences observed when the ovaries, endometrium, and vaginal epithelium of normal pregnant and normal lactating rodents are compared are attributable to the presence of oestrin during pregnancy and its absence during lactation, and that the characteristic picture of the ovary during

⁷ Scaglione, S., *Riv. Ital. Ginecol.*, 1930, **11**, 463.

⁸ Hohlweg, W., *Klin. Wchnschr.*, 1934, **13**, 92.

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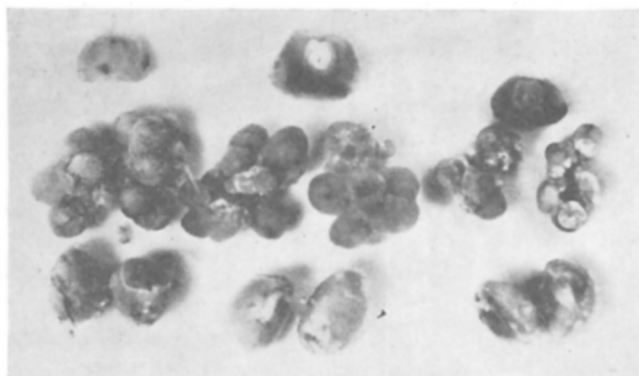


FIG. 1.

Showing that the pituitary, the corpora lutea and the adrenals of the oestrone-treated, lactating rat (in center) are not only larger than those of an untreated control at a corresponding stage of lactation (on the right) but exceed the size of those of a pregnant animal near term (on the left)—the number of the corpora lutea is not increased.

pregnancy is due to the action of oestrin in the presence of some factor inhibiting follicle maturation.

The fact that these observations are in apparent contradiction with those of other investigators^{9, 10} who found a decrease in ovarian size after oestrin administration may probably be explained by a difference in the dosage used. In 6 normal adult females treated with 20 γ of oestrin daily, the vaginal oestrus was almost continuous and the ovaries showed marked signs of atrophy at the end of 20 days; their average weight was 25 mg., and only in one case did they contain recent corpora. Hohlweg⁸ has stated that even large doses of oestrin do not lead to the appearance of corpora lutea in animals much below the age of spontaneous puberty; Lane,¹¹ however, found evidence of stimulation of follicular maturation in immature rats treated with small doses of oestrin. We have treated a series of 7 21-day-old female rats with 100 γ of oestrone daily, in oil; the animals were killed at intervals up to the 30th day of treatment; their ovaries ranged between 7 and 14 mg. and showed definite histological signs of atrophy; spontaneous puberty did not occur in any one of this series. The pituitaries and adrenals were also within the normal weight-range. It appears that oestrin does not lead to

⁹ Meyer, R. K., Leonard, S. L., Hisaw, F. L., and Martin, S. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **27**, 702.

¹⁰ Doisy, E. A., Curtis, J. M., and Collier, W. D., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **28**, 885.

¹¹ Lane, C. E., *Am. J. Physiol.*, 1935, **110**, 681.

enlargement of the pituitary or adrenals save in the presence of corpora lutea, nor is such enlargement obtainable in male animals.

It was of interest to ascertain whether the described actions of large doses of oestrin could be maintained over long periods. A series of 12 adult female rats received 500 γ of oestrone in oil daily, and were killed at intervals up to 4 weeks. After an initial appearance of vaginal oestrus, a prolonged dioestrus set in, during which the ovaries were seen to be greatly enlarged; after 3 weeks' treatment, however, the ovaries returned to approximately normal weights and vaginal oestrus reappeared. The size of the pituitaries and adrenals ran roughly parallel to the size of the ovaries.

In 12 lactating rats treated with 100 γ of oestrone daily the corpora lutea were not maintained after hypophysectomy performed on the 6th day of treatment, to judge by the sudden involution of the mammary glands and the histological appearance of the corpora; vaginal oestrus, however, did not reappear until 5-7 days after the removal of the pituitary. This, however, cannot be interpreted as a sign of maintained activity of the corpora lutea, on account of the following observation: 6 lactating rats receiving as much as 500 γ of oestrone daily were ovariectomized on the 6th day of treatment, but vaginal oestrus did not reappear until 5-6 more days had elapsed; no such inhibition was observed in 6 ovariectomized lactating control animals not subjected to pre-treatment with oestrin. The nature of this inhibition of vaginal oestrus is not easy to explain; it may possibly be due to remnants of corpus luteum hormone formed in large quantities under the stimulus of oestrin before ovariectomy not being rapidly excreted; it is also conceivable that organs which hypertrophied under the influence of corpus luteum hormone (like the mammary glands) may inhibit the vaginal effects of oestrin for some time after the ovaries are removed.

It is to be observed that in these experiments rapid involution of the mammary glands followed hypophysectomy, nor did the continued application of large doses of oestrin lead to repair of this involution; we conclude that the presence of the pituitary is requisite for the action of oestrin upon the mammary glands, although Ruinen¹² claims that oestrin causes development of the mammary glands of hypophysectomized male rats. Hypophysectomy also leads to involution of the adrenal cortex in spite of continued administration of large doses of oestrin; we conclude that the effect of oestrin in producing marked enlargement of the adrenals in the

¹² Ruinen, F. H., *Nederl. Tijdschr. Geneesk.*, 1933, 610.

presence of corpora lutea is an indirect action mediated by the anterior pituitary. Histological examination of the enlarged adrenals obtained by oestrin administration reveals that only small quantities of lipoids are present, whereas very large amounts of lipid material appear in the cortex within 48 hours of hypophysectomy in spite of continued oestrin administration; these observations are of interest, in that the relation between lipid content and functional activity of the adrenal cortex has been regarded by various authors from very divergent points of view.

It has been noted^{13, 14} that administration of oestrin enhances the luteinizing action of A.P.L. and its effect on ovarian weight; another type of synergism has been noted by Hisaw;¹⁵ others, however, have found the action of gonadotropic substances to be unaffected^{16, 17} or inhibited^{18, 19, 20} by oestrin. It is in fact easy to obtain synergistic or antagonistic effects at will. 40-day rats show only 30 mg. ovaries after 4 days' treatment with 100 units of A.P.L. if they have been pre-treated with 100 γ oestrone daily from the 21st day of life, while 60 mg. ovaries are found in controls not pre-treated with oestrin. On the other hand, 4 rats which received 100 γ of oestrone daily from the 21st day of life, and 50 units of A.P.L. daily from the 26th day, possessed ovaries weighing over 165 mg. at the 40th day of life, while the heaviest ovaries in 4 controls receiving the A.P.L. treatment only weighed 64 mg. In short, animals whose ovaries have been depressed by oestrin treatment do not respond normally to A.P.L. administration, while on the other hand corpora lutea produced by the action of A.P.L. are enlarged by concurrent and subsequent oestrin treatment.

¹³ Collip, J. B., *Internat. Clinics*, 1932, 4 (42), 65.

¹⁴ Magath, M. A., and Rosenfeld, R. M., *Arch. ges. Physiol.*, 1933, 233, 311.

¹⁵ Hisaw, F. L., Fevold, H. L., Foster, M., and Hellbaum, A. A., *Anat. Rec.*, 1934, 60, Proc. 52.

¹⁶ Spencer, J., D'Amour, F. E., and Gustavson, R. G., *Endocrinol.*, 1932, 16, 647.

¹⁷ Baum, O. S., and Pincus, G., *Am. J. Physiol.*, 1932, 102, 241.

¹⁸ Mahner, A., *Zentralbl. Gynäk.*, 1928, 52, 1754.

¹⁹ Dahlberg, G., *Klin. Wehnschr.*, 1930, 9, 1398.

²⁰ Aron, M., *Arch. d'Anat., d'Histol., d'Embryol.*, 1932, 15, 237.