filled with nitrogen 4 times and finally evacuated before heating. The dry heated powder when rubbed up with saline and injected into the breast and leg muscles in doses of 40 and 10 mg. respectively in each of 2 chicks produced 4 tumors, whereas the powder subjected to the same conditions of heating in the presence of water, when similarly injected in 2 birds gave negative results. In other words, when the protein was coagulated the activity was lost while when denaturing of the protein was limited by dry heat, activity was retained.

This preliminary work would seem to indicate that the active agent is water soluble and probably resides in the protein fraction.

9412 P

Effects of Androgenic Substances in the Female Rat.*

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Butenandt[1] and others (see Koch[2]) have reported that some of the unsaturated androgens, notably androstene-dione and dehydroandrosterone, will induce estrus in the normal infantile rat. Browman[3] reported the cessation of estrous cycles in adult normal rats injected with androsterone and testosterone. In spayed rats Nelson and Gallagher[4] found that androstane-diol and androstene-dione failed to produce vaginal cornification, but did induce uterine and mammary hypertrophy and prevented castration changes in the hypophysis.

The administration of testosterone† (0.5 and 1.0 mg. daily), androsterone (1.5 mg. daily), dehydroandrosterone (1.0 mg. daily) and androstane-dione (1.0 mg. daily) to groups of 2 to 4 spayed female rats for 30 days failed in every case to produce vaginal cornification (as determined by daily smears and histological sec-

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1 Butenandt, A., *Die Naturwiss.*, 1936, **24**, 15.

2 Koch, F. C., *Physiol. Rev.*, 1937, **17**, 153.

3 Browman, L., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 205.

4 Nelson, W. O., and Gallagher, T. F., *Science*, 1936, **84**, 230.

† The writers are very grateful to Dr. T. F. Gallagher and Dr. F. C. Koch for their kindness in preparing and furnishing us with the synthetic androgens used in this study.

tion). In all cases castration changes were completely or almost completely prevented. Testosterone and androstane-dione in all instances induced definite mammary proliferation and enlargement of the uterus. Androsterone and dehydroandrosterone failed to induce changes in the mammary glands or uterus.

The above androgens, and in addition, androstene-dione, cis-androstene-diol and trans-androstene-diol have been administered to groups of 2 to 5 adult normal female rats for 16 to 30 days. Daily vaginal smears were made, in each instance, for at least 2 weeks prior to the initiation of and throughout the period of treatment. Normal 4 to 5 day cycles were present in all animals prior to treatment.

Testosterone at the one mg. daily level completely suppressed cycles in one and partially suppressed them in 2 rats. At the 2.0 mg. daily level cycles were completely suppressed (3 animals). The mammary glands showed stimulation and the uteri were enlarged. At the 1.0 mg. level the ovaries appeared unaffected, but at 2.0 mg. the corpora lutea were enlarged. The hypophyses showed degranulation of the basophiles.

Androstane-dione at the 1.0 mg. daily level in 2 rats completely and in 2 partially suppressed the estrous cycles. At a 2 mg. level complete suppression occurred in 2 animals. The ovaries of the animals whose cycles were inhibited showed fairly large corpora lutea. The ovaries of the remaining animals were either unaffected or slightly subnormal in size. Mammary glands and uteri showed erratic responses in this series. Pituitaries showed basophilic degranulation.

Androstene-dione at a 1.0 mg. daily level only partially suppressed the cycles of 2 animals. At the 2.0 mg. level suppression was complete in these same animals. The ovaries were definitely subnormal in size. Mammary glands and uteri were definitely stimulated.

Trans-androstene-diol at a 1.0 mg. level in 2 animals failed to influence the cycles or to significantly affect the ovaries, mammary glands or uteri.

Cis-androstene-diol in 2 rats at the 1.0 mg. daily level induced markedly prolonged periods of estrus. Thus in one animal the vaginal smear during the 22-day experimental period was of the estrous type for 16 days and in the other for 17 days. The ovaries appeared normal, the mammary glands were not stimulated, and the uteri were of the estrous type.

The tendency shown by cis-androstene-dione to induce continued estrus was even more apparent for dehydroandrosterone. The latter when injected at the 1.0 mg. daily level in normal rats resulted

in estrous type smears for 16 of 18 days, 12 of 20 days, 19 of 22 days, and 21 of 22 days, respectively. The ovaries were small, the mammary glands were not stimulated, and the uteri were large.

The amazing effect of dehydroandrosterone in the adult normal female led us to administer this treatment to 4 hypophysectomized females. To our surprise, particularly in view of its failure to change the vaginal picture in the spayed rat, the hypophysectomized female reacted in a manner even more striking than the normal female. Estrous type smears were shown for 21 of 25 days, 22 of 22 days, 22 of 22 days, and 12 of 14 days, respectively. The ovaries were only slightly decreased, the mammary glands were not stimulated, and the uteri were enlarged.

All animals showed a marked enlargement of the preputial glands and in many instances a striking enlargement of the clitoris.

The marked tendency of cis-androstene-diol and dehydroandrosterone to produce periods of prolonged estrus in normal female rats may be due to their conversion by the ovary to an estrogenic substance. If this is true it seems apparent that ovarian function is not entirely suppressed by hypophysectomy since dehydroandrosterone shows the same effect in the hypophysectomized female rat.

9413 P

Maintenance of Spermatogenesis in Testis of the Hypophysectomized Rat with Sterol Derivatives.*

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Walsh, Cuyler and McCullagh[1] reported that the administration of urine concentrates containing male sex hormone would prevent for as long as 20 days the degenerative changes which occur in the testes of hypophysectomized rats. These observations were confirmed and extended by Nelson and Gallagher[2] and Nelson,[3] who were able to show that spermatogenesis could be maintained for at least 60 days after hypophysectomy, and that such males were able

* This study was aided by a grant from the Committee on Scientific Research of the American Medical Association.

1 Walsh, E. L., Cuyler, W. K., and McCullagh, D. R., *Am. J. Physiol.*, 1934, **107**, 508.

2 Nelson, W. O., and Gallagher, T. F., *Science*, 1936, **84**, 230.

3 Nelson, W. O., *Anat. Rec.*, 1936, **67**, 110 (suppl.).