ined 28 hours after the preparatory intradermal injections. In the third group, no reaction whatever was noticed both 24 hours and 28 hours after the intradermal injections. Microscopically, the sections of the skin removed from the first and second groups of control animals showed about the same changes as those of the negatively reacting test animals. In the sections of the skin of the third group of control animals, there were no abnormal findings except for a mild edema in the subcutaneous tissues.

Summary. Intradermal injection of rabbits with an extract of *Ascaris lumbricoides,* followed 24 hours later by intravenous administration of the same extract, produced hemorrhagic necrosis which grossly and microscopically conformed with that described by Shwartzman.

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Development of Female Characteristics in Adult Male Rabbits Following Prolonged Administration of Estrogenic Substance.

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Estrogenic substance was prepared by extracting acidulated human pregnancy urine with butyl alcohol in a continuous extractor as described by Veler, Thayer, and Doisy.[1] The alcohol was removed by distillation, using a vacuum pump, and the residue dissolved in ethyl ether. The ether extract was added to olive oil, and the ether evaporated. The resulting olive oil solution was then assayed for its estrogenic content by the technique of Coward and Burn.[2] The quantity required to produce estrus in 50% of 20 ovariectomized, sexually mature, albino rats constituted the rat unit used in the following experiment.

Twenty-four male albino rabbits of known parentage, 16-17 months of age, were employed. Eight of these were injected subcutaneously once a day, 6 days a week, with from 20-60 rat units of estrogenic substance in olive oil. Injections were continued in 6 animals for 250 days or more. Eight rabbits were injected in like

[1] Veler, C. D., Thayer, S., and Doisy, E. A., *J. Biol. Chem.*, 1930, **87**, 357.

[2] Coward, K. H., and Burn, J. H., *J. Physiol.*, 1927, **63**, 270.

manner with plain olive oil, and 8 were left untreated. All animals had a syphilitic infection given in connection with another experiment in which they were being used. Only those rabbits receiving injections of estrogenic substance showed the variations from the normal described below.

Mammary glands. Hypertrophy of nipples to the size of those of the lactating female rabbit developed after 80 days of treatment. Milk could be expressed from the nipples. In 6 animals, the secretion increased in amount and became thick and white, in 2 animals it remained thin and watery. After continuing for at least 90 days in all animals, lactation decreased, and the nipples became smaller. Subsequently, milk could be expressed only from a few nipples in most of the animals, and in one case it disappeared entirely after being present for 120 days. Four rabbits continued to secrete some milk for as long as 200 days. Lactation did not depend upon the stimulation of secretion by withdrawal of milk. Enlarged mammary glands and dilated superficial blood vessels were visible under the shaved skin. The abdominal skin looked thinner and smoother than normal.

The lactating males willingly fostered young rabbits, and in 2

FIG. 1.
The scrotal sacs of an untreated rabbit.

FIG. 2.
The scrotal sacs (covered with hair) of a rabbit treated with estrogenic substance.

instances suckled them. Three animals 8 days old survived under these conditions for 5-6 days, which was 1-2 days longer than 3 controls.

External genitalia. The testes became atrophic after 100 days of treatment. Decrease in size was subsequently progressive; in 6 animals treated for 250 days the testes were less than half their original size, and were held high in the scrotum at the inguinal ring. In this position some were fixed. With the atrophy and ascent of the testis, the scrotum shortened. The usual smooth surface of its dependent portion disappeared, leaving a shallow sac covered with hair.

FIG. 3.
The penis of an untreated rabbit.

FIG. 4.
The penis of a rabbit treated with estrogenic substance. The prepuce is everted showing a light longitudinal band, which is the atrophied cavernous bodies.

After 144 days of treatment, the glans penis softened and the prepuce became swollen. The urinary meatus elongated as dorsal cleavage and shortening of the glans penis rapidly progressed. Within a period of 3 weeks, the urethra had receded into the depths of the prepuce and the glans penis had entirely disappeared, leaving the atrophied cavernous portion of the penis which tapered distally to a narrow band on the inner ventral surface of the prepuce. At

this stage the prepuce, penis and urethra of 7 animals resembled the female pudenda with its clitoris and common urogenital vestibulum.

Skin and Hair. The coats of the feminized males were unusually heavy and clean except over the areas where the injections were given. After 200-235 days of treatment the skin under the chin became loose and redundant, encircling the anterior neck like a ruff. A similar formation of the skin may be found in normal female rabbits, especially after they have borne young.

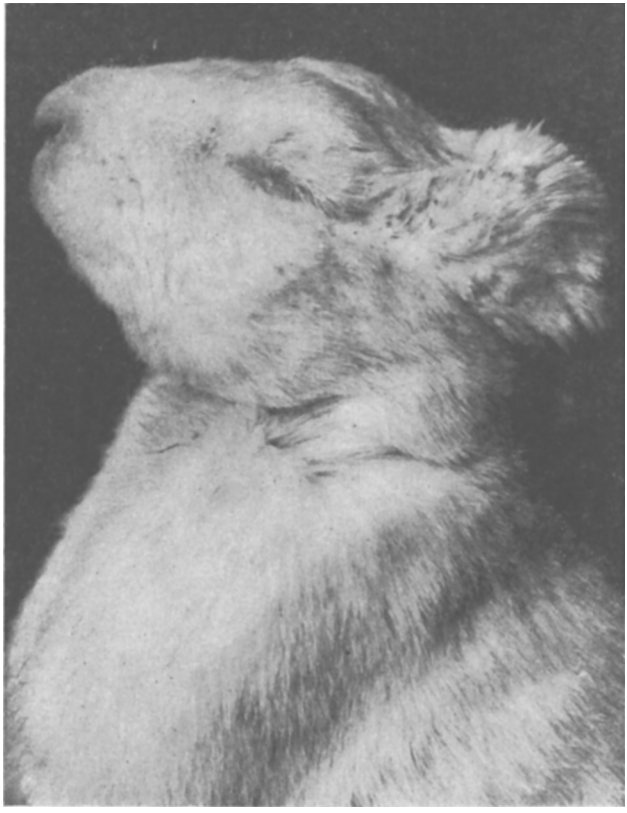

FIG. 5.
The neck of an untreated male rabbit.

Behavior. The feminized males were more passive and docile than the normal male rabbits except while fostering young ones, when they frequently resisted by biting. On repeated trials they were indifferent toward females, and evaded normal males who were persistent in attempting copulation. In one case copulatory actions

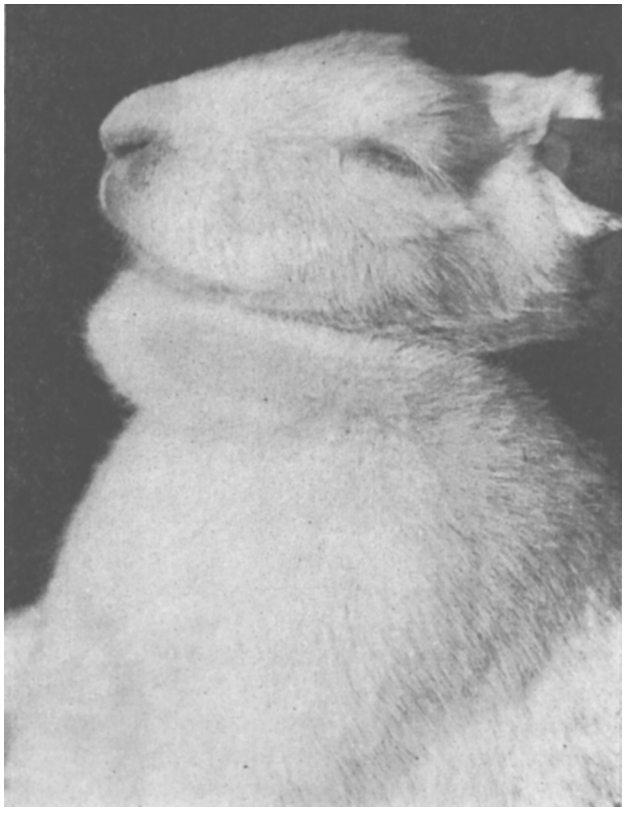

FIG. 6.
The fold of skin on the anterior cervical region of a male rabbit treated with estrogenic substance.

were terminated in ejaculation of semen by the normal male, but no spermatozoa could be found in the vestibulum-like structure of the receptive animal.

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Rate of Formation of Acetylcholine in Placenta In Vitro.

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We have demonstrated that placental acetylcholine originates in the syncitial layer of the villus, and that it exists in a free form.[1]

[1] Wen, I. C., Chang, H. C., and Wong, A., to be published.