The embryos were kept at a constant temperature of 38°, both during and between observations. They were not handled for one or 2 hours after being cultured, and then as little as possible. Recorded observations were made only after the culture had rested on the stage of the microscope for a few minutes. A few embryos have been observed in buffered salt solution (Tyrode) before removal from the uterine wall. These controls compared favorably with the observations made later in culture.

Summary. Rabbit embryos, removed during the ninth day of gestation, were cultured in hanging drops. Studies of the heart show that the 2 primitive lateral heart tubes beat regularly for some hours before they fuse to form one heart. There are marked differences between the 2 sides both morphologically and physiologically. The hearts develop considerably before fusing, as evidenced by the movement of cellular elements within the vessels. At early stages the contractions involve only the ventricles, but before fusion to form a single heart the impulses may be seen to originate in the respective atria.

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Andromimetic Effect of Estrogen upon the Clitoris of the Rat.*

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During the past 2 years, a number of female rats from an inbred strain of unrecorded origin have been found to possess spontaneous ovarian tumors which were invariably associated with defective external genitalia[1]. In order to interpret the physiology of these pathological ovaries, attempts were made to duplicate the abnormal external genitalia by the administration of sex hormones to normal females at different periods of postnatal life. It was observed that the genitalia, characteristic of the tumorous strain, could be experimentally produced by the injection of estrogen during the first week of postnatal life, but not if the treatments were begun thereafter. Since the studies on the ovarian tumor will be published subsequently, the present paper will be devoted to an interpretation of the effects of estrogen upon the external genitalia of the normal juvenile female rat.

It has been reported[2, 3] that the administration of estrogen to the

* The estrogen (Theelin) was supplied by Dr. Oliver Kamm, Parke, Davis and Company.

1 Turner, C. Donnell, *Anat. Rec.*, 1939, **73**, 75.

2 Hain, A. M., *Quart. J. Exp. Physiol.*, 1935, **25**, 131.

3 Greene, R. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 503.

pregnant rat, during the terminal stages of gestation, produced a characteristic modification of the clitorine prominence in the female offspring. Similar effects were observed when large amounts of estrogen were injected during the first few days of postnatal life.[3] Previous workers[2, 3] interpreted this abnormality as a hypotrophic effect of the estrogen which resulted in the absorption of the tissue which embryonically formed the anterior wall of the urogenital sinus. For this reason, the condition has been referred to as a "hypospadias." [3, 4]

TABLE I.
Postnatal Administration of Estrogen to Juvenile Rats.

Litter number	No. of treated animals	IU estrogen at each injection	No. of injections	Age in days at beginning of treatment	Age in days at autopsy
S1	1	100	60	2	120
S2	2	100	45	1	88
T1	4	100	17	20	118
S3	2	100	9	1	131
T2	2	50	10	1	65
T3	2	50	5	1	92
T4	3	100	20	10	146

Our observations are based upon 16 treated animals (7 litters) of the Wistar strain. The estrogenic treatments were begun at different ages and were continued for varying periods of time (Table I). The most pronounced effects were obtained when several large doses of estrogen were administered during the first few days after birth, the animal receiving no further treatment (litters S3, T2, T3). In these animals, the clitorine prominence is deeply cleft along the line of the raphe between the prominence and the vaginal orifice (Fig. 3). In the fissure, anterior to the urethral orifice, is a conspicuous teat-like elevation, the glans clitoridis. A slender frenulum extends caudally from the base of the glans. Small erectile bodies differentiate from the tissue between the glans and the urethral orifice (Fig. 3). When the estrogen is administered continuously from birth until postnatal life (litter S1), the grooved condition of the clitorine prominence is not so pronounced but slight hypertrophy of the glans clitoridis and the differentiation of small erectile bodies does occur (Fig. 2). At this writing, we are unable to explain why estrogen administered constantly from birth until postpuberal life has less influence upon the clitorine prominence than estrogen administered only for a short period subsequent to birth. The clitoris of the newborn rat seems to be particularly sensitive to estrogen since these treatments do not

[4] Greene, R. R., and Ivy, A. C., *Science*, 1937, **86**, 200.

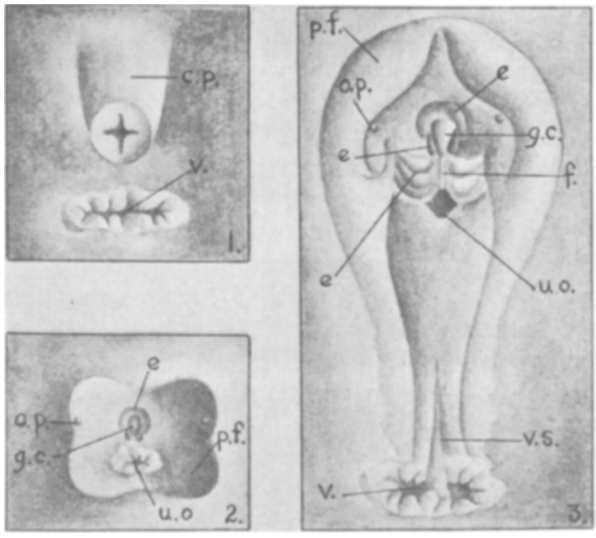

FIG. 1.

The external genitalia of a normal adult female rat. c.p., clitorine prominence; v., vaginal orifice.

FIG. 2.

An end view of the clitorine prominence of a rat (litter S1) which had received estrogen continuously from the second day of postnatal life until autopsy at 120 days of age. The preputial folds (p.f.) are separated in order to reveal the glans clitoridis (g.c.), erectile tissue (e.), urethral orifice (u.o.), and orifices of the preputial glands (o.p.).

FIG. 3.

The abnormal external genitalia of a rat (litter S3) which received estrogen only during the first eighteen days of postnatal life. The animal was autopsied at 131 days of age. Compare with Figs. 1 and 2. e., erectile bodies; f., frenulum; g.c., glans clitoridis; o.p., orifice of preputial gland; p.f., preputial fold; u.o., urethral orifice; v., vaginal orifice; v.s., vaginal septum.

produce grossly abnormal external genitalia when begun after the tenth day of postnatal life.

In the normal female rat, there is only very slight cleavage of the preputial fold (Fig. 1). The glans clitoridis of the normal adult is a very small protuberance situated anterior to the urethral orifice. The tissue around the base of the glans is heavily vascularized, and slight indication of erectile bodies may be observed occasionally. The glans clitoridis, erectile tissue and urethral meatus are surrounded by the invaginated preputial epithelium. The orifices of the preputial glands are located along the inner margin of the preputial fold and, in the adult smegma may be expressed from these pores.

Evidence of urinary incontinence was observed in only 2 of the 9 animals which received estrogen during the first week subsequent to birth. Previous investigators associated this functional impair-

ment with improper differentiation of the urinary sphincter[2, 3] and regarded it as "hypospadias" resulting from a hypotrophic effect of the hormone.[3] We believe that these abnormal external genitalia may be interpreted best on the basis of an andromimetic effect of the estrogen. The extensive fissure in the clitorine prominence apparently results from more extensive cleavage of the preputial fold than occurs normally. The estrogen also produces a slight enlargement of the clitoris and a feeble differentiation of erectile tissue. In affected animals, the urethral orifice is more posterior to the glans clitoridis than normally. This probably results from the separation of the preputial epithelium from the wall of the distal urethra and from the hypertrophy of the erectile tissue between the glans and the urethral meatus. If this is the correct explanation of the wide separation of the glans clitoridis and the urethral orifice in the treated animals, we question the justification of terming this defect a "hypospadias."

Summary. The administration of estrogen to female rats during the first week of postnatal life produces a defect in the external genital organs characterized by cleavage of the preputial fold, hypertrophy of the glans clitoridis, and a feeble differentiation of erectile tissues. Since these changes resemble, except for extent, those produced by postnatal androgen, it is concluded that estrogen exerts a mild andromimetic effect upon the clitoris of the rat.

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Distribution and Excretion of Sulfapyridine in the Guinea Pig.

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Since the guinea pig is almost exclusively used for experimental tuberculosis therapeutic studies on sulfonamide compounds, it is important to secure information on the distribution and excretion of the most potent anti-mycobacterial sulfapyridine compound in this animal[1]. Our early *in vitro* and *in vivo* studies of the bacteriostatic action of sulfanilamide and "prontosil soluble" on mammalian, avian, and paratubercular acid-fast bacteria[2], confirmed the findings of

1 Birkhaug, K. E., *Brit. Med. J.*, 1939, **2**, 54 (Review of literature).

2 Birkhaug, K. E., *Beretninger, Chr. Michelsen Institute*, 1939, **9**, 3.