duced by sulfanilamide differs from the macrocytic anemia just mentioned in that it develops between the third and sixth days of therapy and is rapidly progressive in degree. By virtue of its rapid onset after the use of therapeutic doses, it is reasonable to believe that this form of anemia is due to an idiosyncrasy or susceptibility to the drug, rather than to the administration of toxic doses.

The macrocytic anemia which commonly follows treatment with sulfanilamide can be ascribed either to the development of an acute hepatitis or the direct effect of the drug upon the bone marrow. Due to the relatively short interval between the onset of therapy and the macrocytosis, it is not likely that the anemia is induced by liver changes. On the contrary, evidence of the action of sulfanilamide on bone marrow is demonstrated by its normoblastic response.

Conclusion. Therapeutic doses of sulfanilamide commonly produce macrocytic anemia and a normoblastic bone-marrow reaction. In the acute hemolytic anemia the bone marrow shows a more marked normoblastic reaction with predominance of young forms.

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Estrogen-Induced Hypospadias in the Female Rat.*

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Normal development of the external genitalia of the female rat can be grossly modified by treatment with estrogens, either antepartum or immediately postpartum.[1-4]

The normal female rat has a clitorine urethra with the urinary meatus located at the tip of the clitoris (the word "clitoris" is used to indicate the female phallus and not just the glans clitoridis). The proximal portion of the clitorine urethra is anatomically a true

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1 Hain, A. M., *Edinburgh Med. J.*, 1935, **42**, 101.

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

3 Greene, R. R., and Ivy, A. C., *Science*, 1937, **86**, 200.

4 Turner, C. D., and Burkhardt, W. L., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 267.

urethra, while the distal portion of the functional urethra is formed by the preputium which surrounds and extends beyond the end of the glans clitoridis.

In the estrogen-modified animals no such clitorine urethra is present. The urethral meatus is located in an abnormal position, at the base of the phallus. The phallus itself is cleft on the caudal surface. Its component structures are therefore exposed.

A more extensive abnormality has been produced in an unreported group of 10 adult females. These animals received no direct treatment. However, their mothers received very large doses of estrogens during pregnancy. The mothers of 9 animals were given estradiol dipropionate in dosages varying from 3.0 to 50.0 mg between the 12th or 13th to the 17th or 19th days of pregnancy. The mother of the 10th animal received 5.5 mg of alpha estradiol between the 13th and 20th days of pregnancy. The clitorides of these animals presented the anomalous conditions already described. In addition, these animals had no discrete vaginal orifice. The small orifice located at the base of the cleft phallus, on investigation, proved to be a common opening for both urethra and vagina. These two structures joined to form a common canal 3 to 4 mm proximal to the external orifice.

Observations on normal development have been presented previously in conjunction with studies on the effects of androgens on sexual development.[5] In order to interpret the abnormality noted above it is necessary to review briefly the normal embryonic and postnatal development of the female. In the female rat the caudal portion of the vagina is formed during embryonic development by a longitudinal fission of the urogenital sinus into a ventral portion (urethra) and a dorsal portion (lower vagina). This fission, studied by consecutive wax reconstructions, proceeds caudalward, but even at birth is not quite complete. The most caudal portion of the vagina is still connected to the urethra by a bridge of epithelial cells and is not patent. The urinary meatus at birth is located at the caudal base of the phallus and represents the still unclosed primary urogenital ostium. Late in embryonic development the median urethral groove is formed on the caudal face of the phallus. At birth this median groove is continuous with the urinary meatus at the base of the phallus. Both are macroscopically visible. During normal postnatal development this urethral groove is roofed over by fusion of the 2 halves of the preputium which meet in the midline of the caudal surface of the phallus. As a result of this fusion the urethral groove

5 Greene, R. R., Burrill, M. W., and Ivy, A. C., *Am. J. Anat.*, 1939, **65**, 415.

is transformed into the clitorine urethra and the urogenital ostium is closed so that the urinary meatus is transferred from the base of the phallus to its tip. The male rat presents a more advanced stage of development at birth. With a few exceptions the penile urethra is already completely formed and the primary urogenital ostium is closed at birth.

There is an obvious similarity between the conditions of the external genitalia in the estrogen-modified females and the conditions in the embryonic states. In these modified adult females, as in the 21-day-old fetus, the more caudal portions of the vagina and urethra have not separated. Some development beyond the fetal stage has taken place in that the vagina is completely canalized, but the last 3 to 4 mm of both urethra and vagina are still represented by their normal precursor, the urogenital sinus. The urethral meatus in these animals is still situated as it is in the fetal and also the newborn state, in the position of the primary urogenital ostium, *i. e.,* at the base of the cleft phallus.

The phallus of these females remains in the cleft condition due to the fact that the preputial folds have failed to fuse and thus no clitorine urethra is formed. Subsequent growth of other parts of the phallus concomitant with growth of the animal to the adult state tends to exaggerate the defect. What appears as a groove in the fetus or newborn animal becomes a wide open cleft in the adult, exposing the glans clitoridis and also certain other homologues of the male cavernous structures which are normally present in newborn and adult female rats.

In the less modified animals previously reported by various workers, the degree of developmental arrest is less extensive and resembles more the conditions in the normal newborn female rather than the conditions in the fetus. Some degree of development occurs in that the vagina and urethra are no longer contiguous as they are normally at birth. The vagina therefore has an external orifice which is separate from the urethral orifice. The latter, however, is maintained in the location of the primary urogenital ostium at the base of the phallus.

The chief distinction between the two types of defects is that in the more highly modified animals the development of the caudal portion of the vagina is completely inhibited so that, in effect, the lower portion of the urogenital sinus is retained as in the fetal state, whereas in the less highly modified animals vaginal development continues to a point where the vagina has an external opening separate from that of the urethra. In both types, however, the urinary orifice is located in an abnormal position, at the base of the cleft phallus.

These genital defects, which are produced by estrogenic treatment, therefore, are due to inhibition of normal development.

The explanation of this defect provided by Turner and Burkhardt[4] obviously neglects consideration of embryonic and normal postnatal development. It is apparently based on gross observations of the abnormality in 6 out of 16 treated animals and on comparison with the normal fully developed female. These authors believe that the extensive fissure in the clitorine prominence apparently results from more extensive cleavage of the preputial fold than occurs normally. This is very unlikely inasmuch as no cleavage of the preputial fold normally occurs. The process involved is definitely an inhibition of the normal fusion of the two halves of the preputium. The "erectile" bodies noted by Turner and Burkhardt are merely the homologues of the male cavernous structures which are normally present in the female. The lack of development and fusion of the preputial folds has left these structures exposed.

The defect is not due to "resorption of a portion of tissue which developmentally forms the anterior wall of the urogenital sinus," as Hain has suggested[1] because there is no tissue covering the urethral groove to be resorbed.

Turner and Burkhardt have objected to the application of the term "hypospadias" to the genital abnormality under consideration. The normal female rat has a clitorine urethra while these modified animals do not. Instead, the urinary meatus is located at the base of the phallus in the position of the primary urogenital ostium. The term "hypospadias" therefore seems to be applicable, since it is anatomically descriptive of the defect. This same hypospadias has been produced in male rats by the administration of very high doses of estrogens to the pregnant mothers. In the males the defect is also due to inhibition of normal development.[6]

Summary. The administration of very large doses of estrogens to pregnant rats has caused a permanent hypospadias and lack of development of the most caudal portion of the vagina, in the female offspring. The abnormality represents an arrest of development so that conditions similar to those found in the 21-day fetus are retained.

[6] Greene, R. R., Burrill, M. W., and Ivy, A. C., unpublished data.