Differentiation of the Mammary Gland in Experimental Congenital Adrenal Hyperplasia Due to Inhibition of Δ^5,3β-Hydroxysteroid Dehydrogenase in Rats[1] (34187)

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(Introduced by T. N. Harris)

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It has been shown that the mammary glands of mouse fetuses whose gonads have been destroyed by X-irradiation undergo feminine organogenesis (1). In female fetuses of rats (2) and of mice (3) treated with androgens, nipple formation is suppressed. These findings led to the concept that the development of the male mammary primordi-

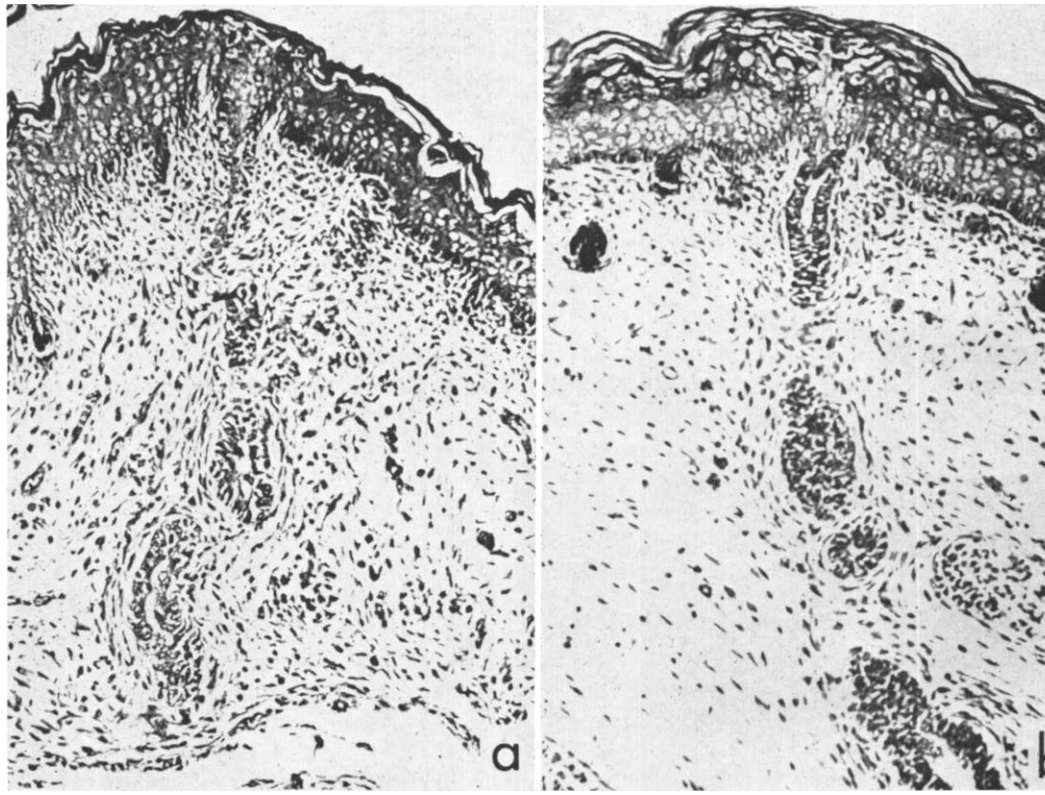

FIG. 1. Mammary anlagen of control male fetuses: (a) second inguinal, right; (b), first inguinal, left; ×200.

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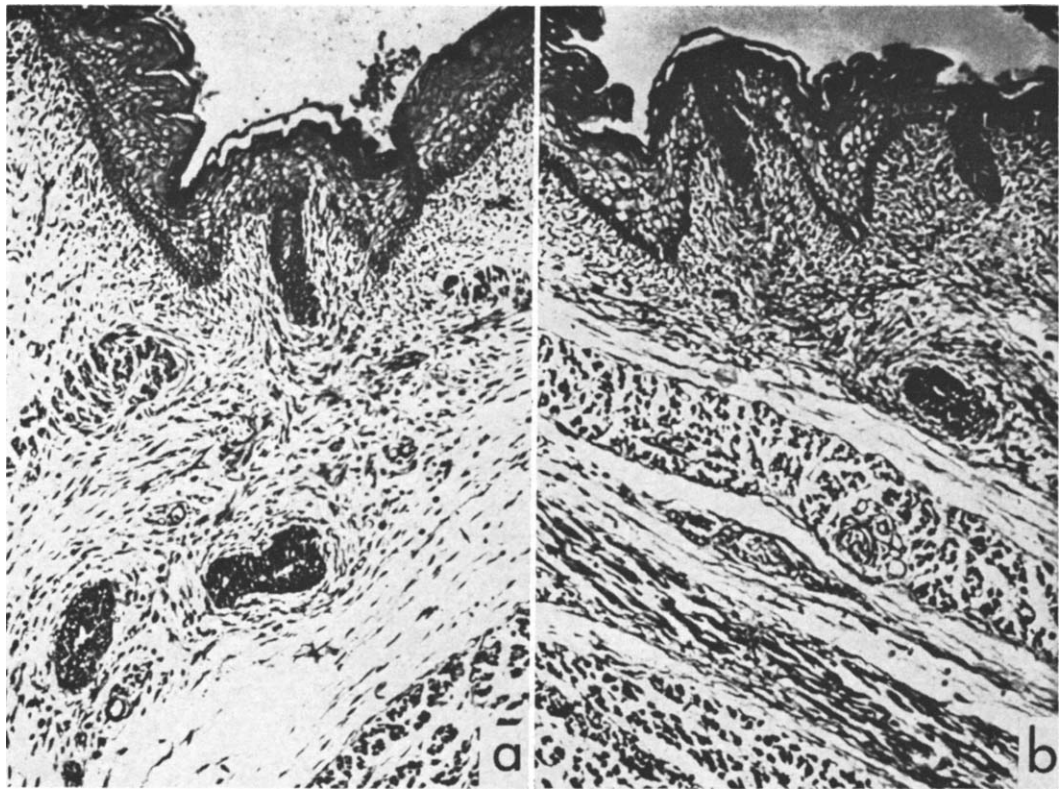

FIG. 2. Mammary anlagen of experimental males: (a), first inguinal, right; (b), second pectoral, right; ×200.

um is normally directed by hormones of the fetal testis. Much support for this hypothesis was given by the recent observation that male fetuses of rats treated with cyproterone-acetate (1,2α-methylene-6-chloro-Δ^4,6-pregnadiene-17α-ol-3-20-dione-17α-acetate) develop nipples (4). This steroid has no estrogenic or androgenic activity, and although it has some progestational action (5), its primary effect is considered to be a blockade of androgenic (testosterone) action in target organs (6–8).

A synthetic analog (2α-cyano-4,4,-17α-trimethyl-androst-5-en-17β-ol-3-one) of a C-19 substrate of 3β-hydroxysteroid dehydrogenase and Δ^5-Δ^4,3-ketosteroid isomerase injected into pregnant rats produces the anatomic and enzymatic defects characteristic of a rare form of human congenital adrenal hyperplasia due to a genetic deficiency of this enzyme system (9). These enzymes are essential for the conversion of Δ^5,3β-hydroxysteroids to the corresponding Δ^4,3-ketones in the early biosynthesis of nearly all biologically active steroid hormones. In the mature rat, the analog blocks adrenal corticoid biosynthesis, thereby stimulating endogenous ACTH secretion and adrenocortical enlargement (10–12). The analog depresses adrenal venous secretion of corticosterone and blocks the conversion of 3β-hydroxy-Δ^5-pregnene-20-one to progesterone by adrenal tissue (13). The enzymatic defects most likely can be attributed to the exceptionally tight, stoichiometric binding of the analog to the active site of the dehydrogenase and to the competitive binding of the analog to the active site of the isomerase (14–17). In the present study we investigated the effects of administration of the analog to pregnant rats on the development of mammary glands in their offspring.

Materials and Methods. Virgin Sprague-Dawley females of the Charles River cesarean-derived albino rat colonies 8–9 weeks of

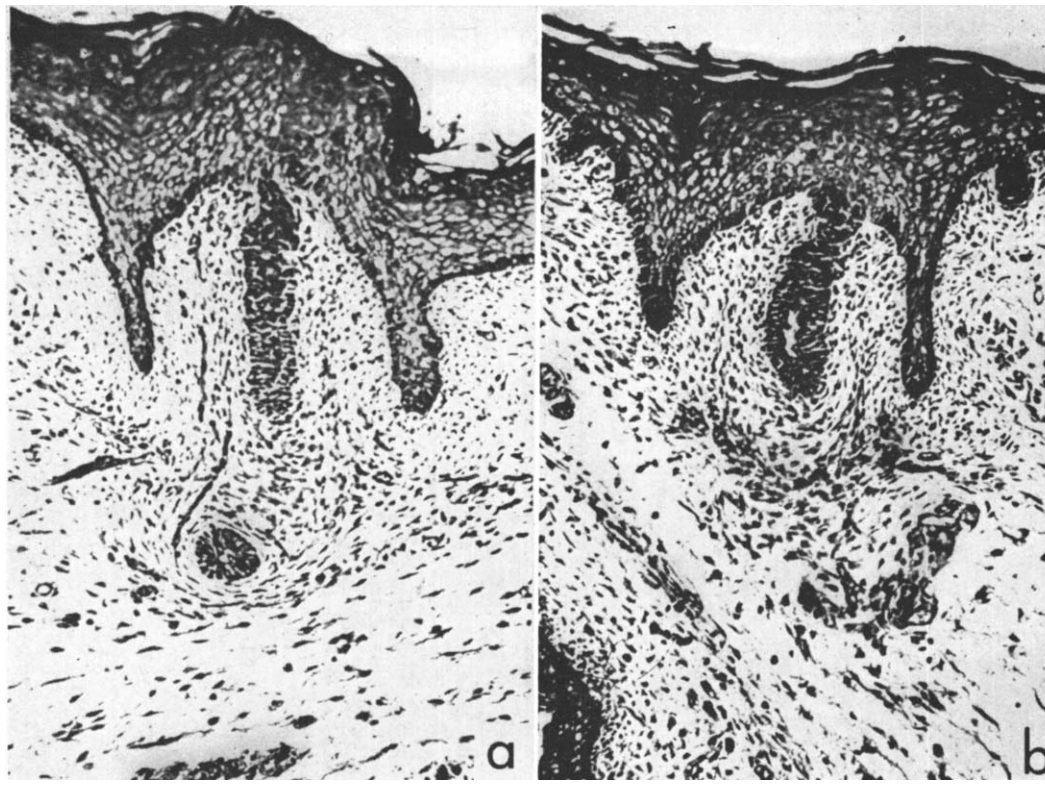

FIG. 3. Mammary anlagen of control female fetuses: (a), second inguinal, right; (b), first inguinal, left; ×200.

age weighing from 185 to 215 g were mated by the supplier (Charles River Breeding Laboratories, Wilmington, Mass.) according to the procedures described previously (18). Pregnant females were given a daily injection of either the analog dissolved in dimethylsulfoxide vehicle (DMSO) at 120 mg/kg, or DMSO alone on days 15–20. All fetuses were delivered on day 21 by cesarean section and fixed in formalin. Histologic serial sections of all nipple anlagen from 3 fetuses chosen at random from each group were prepared and stained with hematoxylin and eosin.

Results. Control males. The normal absence of male nipple development is shown in Fig. 1. The primary glandular bud is fully developed as in the normal female, but there is no epidermal thickening where the bud reaches the surface.

Experimental males. The nipple anlagen are evident in most nipple areas studied (Fig. 2). The circular invagination is not as deep as in normal females. Where nipple anlagen are present, the mesenchymal tissue shows the same pattern as in normal females. In those mammary rudiments where nipple anlagen are poorly developed, there is an epidermal thickening in the region where the primary glandular bud reaches the surface.

Control females. The normal mammary gland anlagen in the female fetuses are shown in Fig. 3. The circular epidermal invagination by which the nipple is formed reaches deep into the mesenchymal tissue. The epidermis is thickened throughout the nipple region. The primary glandular bud reaches the surface in the center of the nipple anlage. The mesenchymal tissue surrounding the epithelial invagination has a pattern typical of females.

Experimental females. All the nipple anlagen are smaller than those of control females (Fig. 4). The circular epidermal invagination is not as deep as in the controls, and

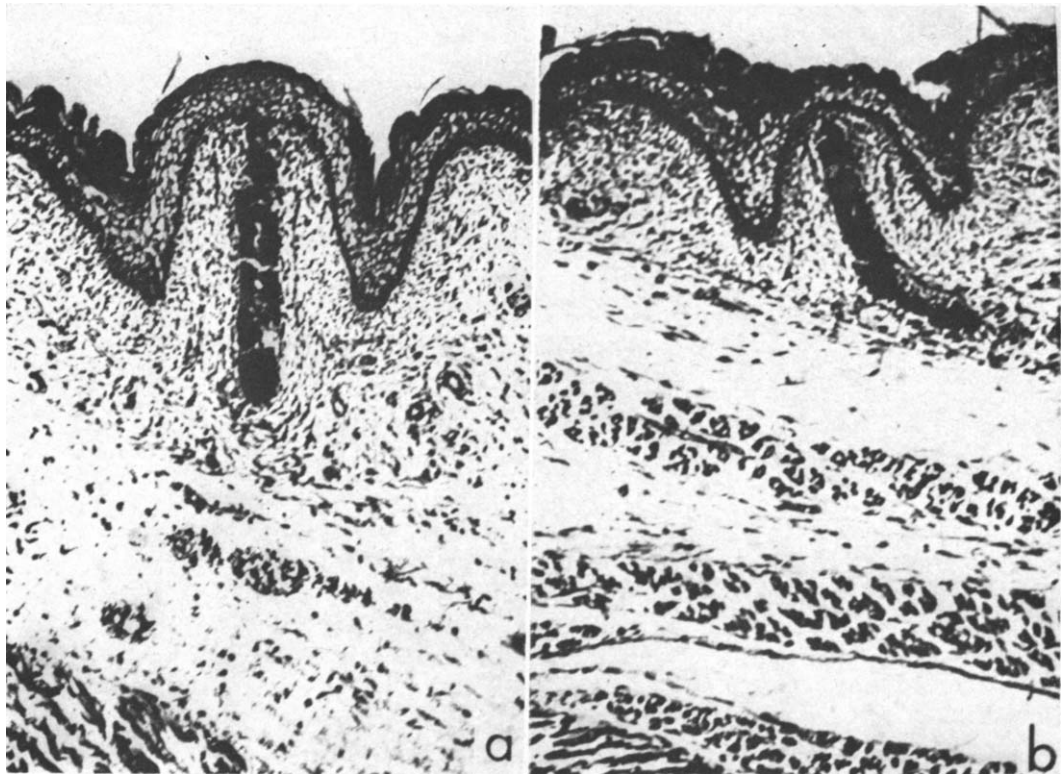

FIG. 4. Mammary anlagen of experimental females: (a), second pectoral, left; (b), third pectoral, left; ×200.

in some cases quite poorly developed. The glandular bud appears normal. However, the epidermal thickening at the juncture of bud and surface is less pronounced than in control females.

In summary, after administration of analog to pregnant rats, nipples occur in male fetuses, while nipple differentiation is inhibited in female fetuses.

Discussion. The present report has shown that after maternal injection of analog, nipple anlagen in male fetuses are insufficiently masculinized, while in female fetuses the anlagen are somewhat virilized, thus giving rise in both sexes to an intermediary state between normal female and male mammary development. These results are strikingly parallel to the effects of the analog on the development of the external genitalia (9). The analog produces incomplete masculinization of the penis in male fetuses resulting in hypospadias by inhibiting fetal testicular activity of the dehydrogenase, and virilization of the female external genitalia manifested by clitoral hypertrophy by inhibiting fetal adrenal activity of the dehydrogenase (19). The external genitalia of male and female fetuses of analog treated mothers resemble each other more than they do controls. The critical period for the production of hyposadias by the analog corresponds to the time of maximal activity of the dehydrogenase in the fetal testis, while that for the production of clitoral hypertrophy by the analog corresponds to the time of maximal activity of the dehydrogenease in the fetal adrenal. Testosterone prevents only the production of hypospadias by the analog, while corticosterone prevents only the production of clitoral and adrenal hypertrophy (20). Experimental embryologic evidence has suggested that an endocrinologically functioning testis is necessary for the normal development of a penis (21), and for the complete inhibition of feminine organogenesis of the nipple anlagen in male fetuses (1). In light of this evidence,

our present and previous work indicates that activity of the fetal testicular dehydrogenase system is essential for the production of Δ4,3-ketosteroidal androgens (testosterone) which induce the formation of a penis, and which prevent the formation of nipples in the male rat. This hypothesis is cogently supported by the observation that nipples develop in male fetuses of pregnant rats treated with cyproterone, an agent whose primary action is to compete with testosterone for sites in target organs.

The mild virilization of the nipple anlagen in the experimental female fetuses can be explained by the same mechanism presumed to account for the clitoral hypertrophy occurring in female fetuses of analog-treated mothers, i.e., corticotropin-induced excess adrenal production of 3β-hydroxysteroidal androgens, in response to inhibition of the fetal adrenal dehydrogenase system (22). Although less effective than testosterone, one such androgen, 3β-hydroxy-Δ^5-androsten-17-one, has a profound capacity to induce clitoral growth in the intact and castrated prepuberal rat (23) and mouse (24). The inhibition of the nipple anlagen in female fetuses by the analog suggests a virilizing effect of such androgens on nipple development.

Summary. The development of mammary glands has been studied in fetuses of pregnant rats treated with an analog (2α-cyano-4,4,17α-trimethyl-androst-5-en-17β-ol-3-one) of a C-19 substrate of 3β-hydroxysteroid dehydrogenase and Δ^{5-4}, 3-ketosteroid isomerase, which is a selective inhibitor of these enzymes. In male fetuses, the analog causes the development of nipples, which are normally suppressed by fetal testicular function. In light of the previous demonstration of the feminizing effect of the analog on the male external genitalia due to inhibition of the fetal testicular dehydrogenase, this observation suggests that inactivation of the testicular dehydrogenase system leads to insufficient endogenous Δ^4,3-ketoandrogen (testosterone) production, thus allowing the development of the nipple in the male. In female fetuses, the analog inhibits nipple development, probably because of adrenal overproduction of 3β-hydroxyandrogens in response to inhibition of the adrenal dehydrogenase system.

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