to hormone induction after a culture period of 3-4 days in its absence.

Present experiments suggest in addition that hydrocortisone stimulation of the specific activity of invertase is dependent on: 1) age of the initial tissue explant; 2) duration of exposure to the hormone; and 3) pretreatment of the tissue before hormone application. Maximum stimulation was observed after exposure to the hormone for 48 hours. In general, the extent of stimulation varied inversely with age of tissue beginning with the 14-day-old embryo. Without pretreatment, maximal responses were obtained with 14-day tissues and minimal responses occurred with 19-day tissue. If the tissues were first cultured for 3 to 4 days in the absence of the hormone and then placed in fresh medium and cultured for an additional 48 hours in its presence, however, this age dependent response was lost and even 19-day tissue cultures demonstrated a response similar to 14-day tissues. These preliminary experiments suggest the presence of an inhibitor which diffuses into the medium and is lost when the medium is changed. It is unlikely that these results could be explained by replenishment of essential nutrients alone since tissue *in vivo* does not attain these levels of activity (solid line in Fig. 4). These results may account for some observations reported by Moog(1) for HC induced alkaline phosphatase activity which increased when the cultures were transferred to balanced salt solution. The nature of this inhibitor is currently under investigation.

Summary. Embryonic duodenal tissue cultures respond to hydrocortisone with an increase in the specific activity of invertase. This response is dependent on: 1) the age of the initial tissue explant; 2) the duration of exposure to hormone; and 3) the pretreatment of the tissue before hormone application. These experiments suggest the presence of an inhibitor for the induction of invertase activity.

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The Role of Androgens in Differentiation of the Mammary Gland In Male Mouse Fetuses. (31564)

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Teats do not occur in normal male rats whereas they do develop in male rat fetuses under the influence of antiandrogens. Male mammary bud also showed greater glandularity under the influence of antiandrogens than is seen in normal male animals(1,2). Since the embryonic development of the mammary gland in the mouse is better known than that of any other animal species(3,4) we have extended our observations to the mouse. This is also appropriate because the glandular bud in the male mouse fetus is inhibited more markedly by fetal androgens than is the case in any other murine animal. Concentration of periglandular mesenchyma causes by 15th day of the embryonal development either the

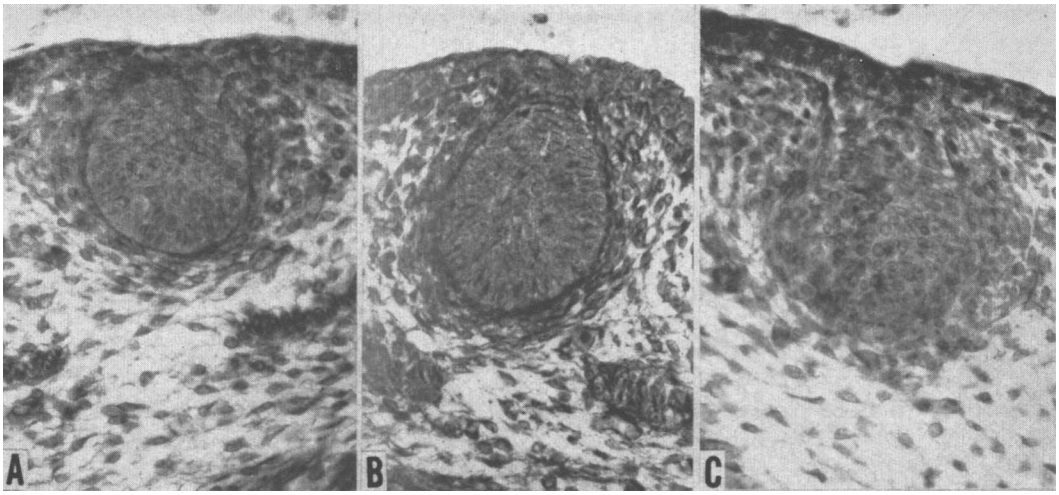

FIG. 1. Rudimentary mammary glands in the mouse on 14th day of embryonic development (Early phase of sexual differentiation). a) Female control. b) Male fetus. The mother received cyproterone acetate, 3 mg/day, from 12th day of pregnancy. c) Male control.

complete destruction of the glandular rudiments or their separation from the epidermis by the strangulation of the proximal sector (3).

Materials and methods. Female NMRI-mice weighing about 25 g each were placed with males during pro-estrus. If a vaginal plug was found on the following day, that day was considered as day 1 of embryonic development. The mothers were treated with cyproterone acetate (1,2α-methylene-6-chloro-$\Delta^{4,6}$-pregnadiene-17α-ol-3,20-dione-17α-acetate*) in doses of 3 mg/mouse/day intramuscularly in 0.1 ml of a 1:5 mixture of benzyl benzoate and castor oil from day 12 of pregnancy to the day of autopsy. We examined 4 fetuses on days 14 and 15 and 6 fetuses on day 18. After the fetuses had been removed from the uterus they were fixed in formalin (10%) and sectioned serially (vertical to the body axis) at 5 μ. Every 10th section was stained with hematoxylin-eosin. Male and female fetuses of the same age, obtained from untreated mother animals, served as controls.

Results. On day 14 of embryonic development, the rudiments (anlagen) of the mammary glands begin to show slight differences in controls of both sexes. The cells of the glandular rudiments are smaller in male fetuses than in the female controls and in the male fetuses of treated mothers. The cells are arranged irregularly and the outline of the surrounding mesenchyma is not clearly defined (Fig. 1). The mesenchyma which surrounds the glandular rudiments shows a more advanced development in male controls and in male fetuses from mothers which had been treated with cyproterone acetate. On the following day differences between normal male and normal female fetuses are even more pronounced. In the male fetuses from cyproterone acetate-treated mothers organogenesis follows the female pattern of development. The chromophilic mammary bud is well developed and distinct from surrounding mesenchyma. The primary sprout is connected with the epidermis through a sturdy chain of epidermal cells that stain poorly. The concentration of mesenchyma which in normal male fetuses leads to strangulation of the mammary bud, does not occur in male fetuses under the influence of cyproterone acetate (Fig. 2). On day 18 the primary sprout of male fetuses from treated mothers is more extensively developed than that in male controls. In several of the 6 male fetuses from treated mothers a circular indentation of the epidermis has formed around the insertion

* This compound was synthesized by Dr. R. Wiechert, Schering AG, Berlin.

site of the preserved mammary bud, representing a rudimentary teat (Fig. 3).

Discussion. As in the case in male rat fetuses, mammary bud and teats in male mouse fetuses as well as those in female fetuses will develop under the influence of an androgen antagonist. As far as can be ascertained, in those mice the primary sprout remains in contact with the epidermis and is always more extensively developed than in male controle, but not so fully developed as in female controls. The development of a mammilla is not always coupled with such anomalies.

The pronounced mesenchymal proliferation around the mammary bud that occurs in male fetuses after day 14 plays a leading role in destruction of the proximal mammary bud. The continuity of the primary sprout is preserved when the antiandrogen prevents the

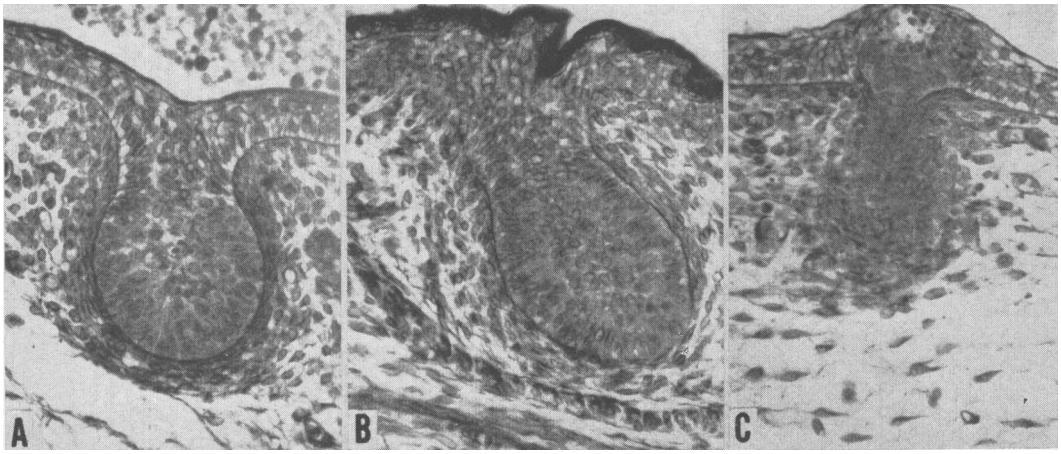

FIG. 2. Mammary gland rudiments of the mouse on 15th day of embryonic development (Sexual differentiation has progressed considerably over that shown in Fig. 1). a) Female control. b) Male fetus; the mother received cyproterone acetate, 3 mg/day, from 12th day of pregnancy. c) Male control.

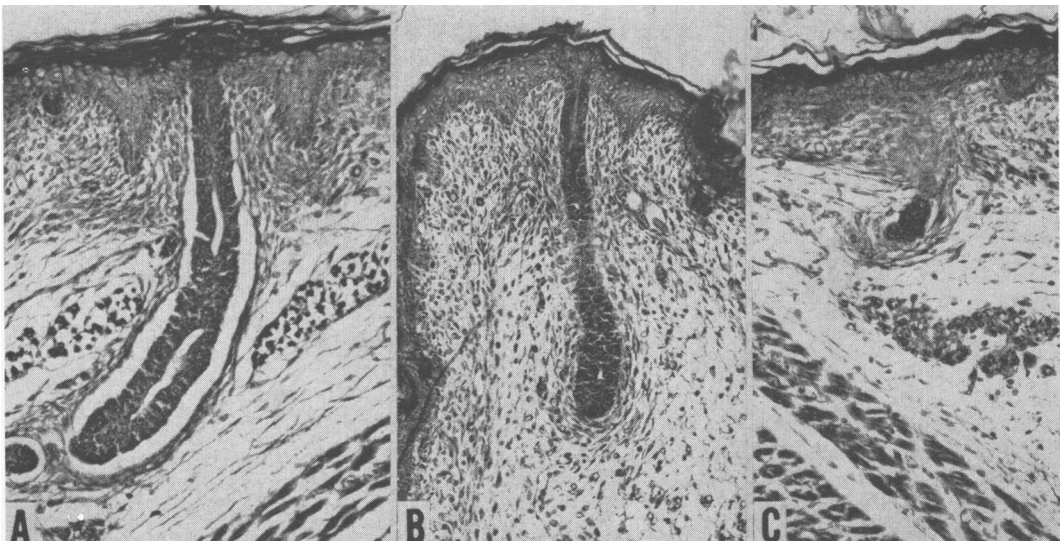

FIG. 3. Mammary gland rudiments of the mouse on 18th day of embryonal development (Formation of teats). a) Female control. b) Mammary gland rudiment in a male fetus. The mother received cyproterone acetate, 3 mg/day, from 12th day of pregnancy. c) Male control.

concentration of the mesenchyma.

Our findings emphasize the importance of androgens in differentiation of the mammary gland. Malformations in male fetuses, induced by antiandrogens, differ fundamentally from those that are induced by estrogens(3). In contrast to the estrogens, the antiandrogens induce a process which is already inherent in the biological "blueprint," the realization of which apparently is prevented only by the androgens of the male fetus. Our data suggest that the female pattern of development will be expressed up to puberty, irrespective of sex, in the absence of androgens.

Summary. In male fetuses of the mouse, mammary gland tissue is stimulated and frequently also the development of teats is observed under the influence of the androgen antagonist cyproterone acetate (1,2α-methylene-6-chloro-$\Delta^{4,6}$-pregnadiene-17α-ol-3,20-dione-17α-acetate). The continuity of the glandular process, normally lost in male fetuses because of a destruction of the epidermal sector, is maintained by cyproterone acetate. Inhibition of the endogenous androgens thus results in a female organogenesis of the mammary glands.

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Unequal Amino Acid Incorporation into Rabbit Serum Albumin Synthesized *in vivo*.* (31565)

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In recent studies on the mechanism of protein synthesis by *in vitro* preparations, it has been observed that short-term exposure of the synthesizing system to isotopic amino acid results in a pattern of amino acid incorporation compatible with the idea that the biosynthesis of the polypeptide chains proceeds from the amino terminal end. This confirmation of an hypothesis of Dagliesh(1) has come from work with hemoglobin(2), lysozyme(3), ribonuclease(4) and recently rat serum albumin(5).

The purpose of the present work is to describe unequal labeling of the moieties of an amino acid in a protein obtained with an *in vivo* preparation. Furthermore, persistence of the unequal labeling over relatively long periods has been observed. A preliminary report of this work has appeared(6).

Materials and methods. Biosynthesis of labeled albumin. 1.0 mc DL-1-C^{14} lysine (Orlando Research Chemicals, Inc.) dissolved in 0.1 ml saline was injected into a mesenteric vein of an anesthetized (nembutal), laparotomized male albino rabbit. The wound was covered with saline packs and anesthesia was continued. After 4 hr 10.0 ml blood samples were taken by heart puncture with heparinized syringes and the plasma was isolated by centrifugation. The plasma albumin was separated by starch block electrophoresis at pH 8.6, as described by Kunkel(7). The separated albumin was eluted with 0.5 N NaCl from starch segments cut from the central section of the albumin band, and the solution was dialyzed and lyophilized. The isolated albumin was homogeneous by the criteria of ultracentrifugation and paper electrophoresis.

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