caudal neurohemal areas, mostly within the tissue of the spinal cord. Electronmicroscope observations indicate reorganization of the normal relationship between caudal neurosecretory axons and capillaries. Accordingly, the possibility must be admitted that urophysial function is restored in *Tilapia* kept *for long periods* after urophysectomy, and the negative data obtained should be reviewed in this context. The unexpected regenerative ability of the caudal system is now under intensive study.

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Estrogens and Corticoids in Relation to Mammary Gland Growth in Male Rats. (30056)

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Development of the mammary glands of males differs widely among species. All possible variations seem to occur, from a few ducts hardly extending beyond the nipple area and fairly large glands consisting of ducts surrounded by dense clusters of alveoli lined with large, secretory epithelial cells. The growth of the mammary glands was believed to be elicited by androgenic hormones (1,4). Since androgenic hormones circulate in the blood of males of all species, the differences in degree and type of mammary development should be accounted for. Among possible causes are variations in the sensitivity of the effector tissues and influences exerted by other hormones. Interactions between androgenic and estrogenic hormones seem of special interest in this connection (2,7,8,9).

Few attempts have been made to investigate whether a rather extensive development of the mammary glands of males (*e.g.*, rats) may be due, at least partly to concomitant action of androgens and estrogens. The chief difficulties in such investigations are 1) to deprive the animal of estrogens, which may even originate from the metabolism of androgens and 2) to find what doses result in synergistic actions of the 2 hormones(2,7,8,9). For this reason, previous studies were performed on hypophysectomized rats under experimental conditions in which the mammary glands themselves either did or did not react upon estrogens. Results of these rather complicated experiments indicated that the development of alveolar lobules is dependent on delicately balanced actions of testosterone and estrogens(8,9).

Further inquiry appeared desirable, as, among other things, possible influences of the adrenal glands could not be excluded. The present work was performed on gonadectomized (gdx) adrenalectomized (adx) rats. Because of the metabolic deficiencies present in such animals it appeared likely that the response of the mammary glands to low, though not to higher, doses of testosterone would be markedly reduced(3,5,7). Possible effects produced in the mammary glands by additional treatment with cortisone, estrone, or both, could then be suitably studied.

Experimental. Male and a few female rats

TABLE I. Mammary Gland Responses to Testosterone Propionate (Tp), 0.125 mg Cortisone Acetate (Ca) and 0.05 μg Estrone (E) in Various Combinations in Adreno-Gonadectomized Rats After 25 Days of Treatment.

			Mammary glands rated†					
			I		II		III	
Group*	No. of rats	Treatment	No.	%	No.	%	No.	%
1: A	6	Tp	1	17	2	33	3	50
B	13	"	4	31	8	62	1	7
C	9	"	8	89	1	11	0	0
2: B	12	Tp Ca	5	42	6	50	1	8
C	10	" "	6	60	4	40	0	0
3: B	15	Tp E	6	40	7	47	2	13
C	10	" "	9	90	1	10	0	0
4: B	17	Tp E Ca	3	18	9	53	5	29
C	13	" " "	4	31	7	54	2	15

* A = $Tp_{0.5}$; B = $Tp_{0.2}$; C = $Tp_{0.1}$. For details see "Experimental".
† For system of rating see "Experimental".

from a closed colony bred at this Institute were used. Pelleted diets (Anticimex, 210 or 213) and tap water or 1% saline were freely available. At 3-5 (generally 3) weeks of age, the rats were gonadectomized. Adrenalectomy was performed when the rats were about 44 days old, the tap water then being exchanged for saline. At the end of experiments, tap water was replaced instead of saline. Rats lacking adrenal cortex tissue did not survive for more than 10 days when saline was absent. This was confirmed at autopsy by inspection of the operation field at a magnification of 2.5 and by microscopic examination of doubtful tissues. All rats surviving the tap water regimen longer than 10 days have been excluded from the present material. Injections were given from one week after adrenalectomy onwards.

Testosterone propionate,* 0.5 mg ($Tp_{0.5}$) and 0.2 mg ($Tp_{0.2}$) every other day as well as cortisone acetate,* 0.125 mg (Ca) and estrone,* 0.05 μg (E) daily, were given intramuscularly. During the present study the dosage of testosterone was of utmost importance. The lowest dose, 0.1 mg every other day ($Tp_{0.1}$), was then given in 0.05 ml oil subcutaneously.

Apart from the groups represented in Table I, non- or sham-adrenalectomized controls as well as untreated adx rats with or without testes and gdx and adx rats injected with 1) E and Ca singly or together and 2) higher doses of the 3 hormones in various combinations were studied. Observations on this material will be referred to only briefly.

Thoracic mammary glands were removed 1) either at adrenalectomy or, one week later, prior to injections and 2) after 14, 25 or 40 days of treatment. Variations of the period of treatment did not exceed 1 or 2 days. Whole mount preparations and microscopic sections of mammary glands were prepared and examined as in previous work(6). Development of the mammary glands was rated as follows: I: Poor duct tree with few side buds or moderately branched duct system with or without thickened ducts, ductal lobules or end buds; II: like I, but clear alveolar lobules in varying though limited amounts present; III: More or less large or compact gland showing not many alveolar lobules, which, however, did always cover the whole duct system (Fig. 1-4).

Results. All glands removed before start of injections were poorly developed though some end buds and/or a few lobules were sometimes found. The glands of untreated gdx adx rats observed for 25 or 40 days were atrophic. The effect of treatment with E and Ca, singly or combined was negligible. The observations made on 9 groups of gdx adx rats after 25 days of treatment are summarized in Table I and illustrated in Fig. 1-4. Modifications in the pattern of mammary growth were not clear at 14 days and did

* Kindly supplied by Ciba, Basle, Leo, Hälsingborg (Estrone).

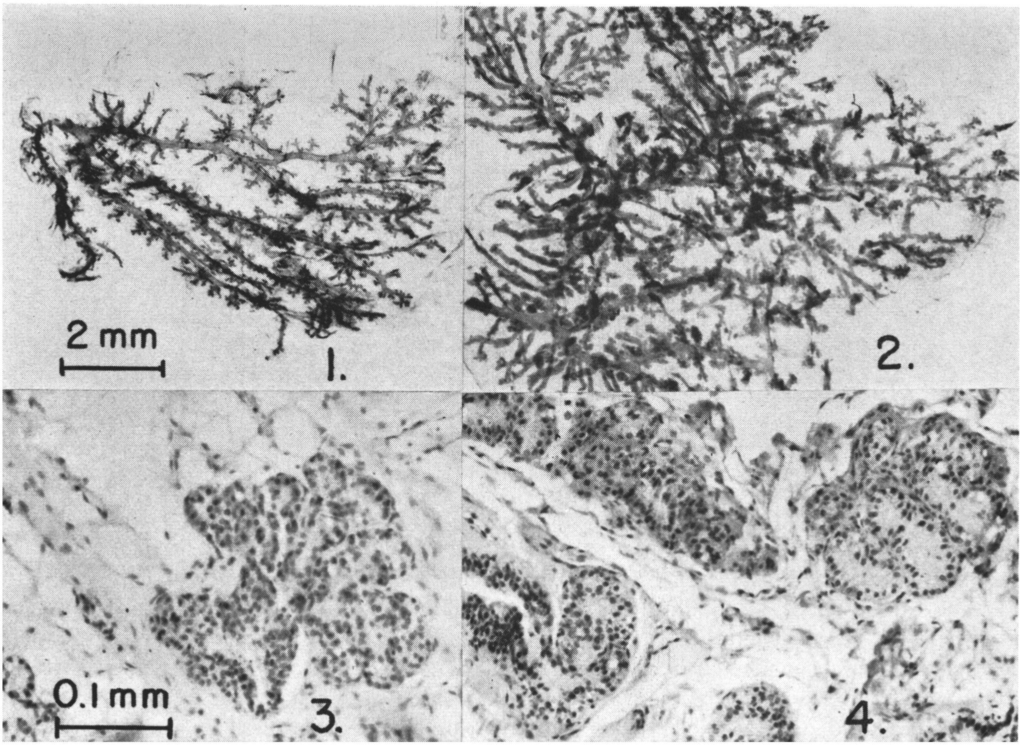

FIG. 1-4. Whole mount preparations (1, 2) and microscopic sections (3, 4) of mammary glands of gdx adx rats treated for 25 days. Magnification, see scale on Fig. 1 and 3, respectively.

Fig. 1. $Tp_{0.1}$. Rating 'I'. No alveoli. Fig. 2. $Tp_{0.1}$, E, Ca. Rating 'II'. Many alveoli and thick ducts. Fig. 3. $Tp_{0.1}$, Ca. Rated on whole mount as 'II'. Alveolar lobule. Fig. 4. Same as in Fig. 2. Alveolar lobules.

not markedly differ between glands examined after 25 or 40 days of injections.

Mammary glands of gdx controls injected with $Tp_{0.5}$, $_{0.2}$ or $_{0.1}$ presented and mostly exceeded the development required for rating 'III.' After adrenalectomy the number of glands developing alveoli decreased with the dose of testosterone given (Table I, Group 1). Thus, in Group 1A given $Tp_{0.5}$ the majority of glands had alveoli. The opposite was true for Group 1C injected with $Tp_{0.1}$. Addition of Ca to $Tp_{0.2}$ resulted in glands covering a larger area, but alveoli did not appear as often as without Ca. With $Tp_{0.1}$, on the other hand, Ca increased the development of alveoli. Injections of E improved the mammary responses neither to $Tp_{0.2}$ nor $Tp_{0.1}$ (Groups 1B, 3B and 1C, 3C). The effect of a combined treatment with Ca, E and $Tp_{0.2}$ or $Tp_{0.1}$ (Group 4) was different from that found in Groups 2 and 3. Distinct alveoli varied, often considerable amounts were present in a greater majority of the glands than in any of the other groups except, perhaps, for Group 1A given $Tp_{0.5}$. It should be recalled that glands showing ductal lobules were rated as 'I' and that end buds were generally absent in glands rated 'II' or 'III.'

Discussion. The results obtained from Group 1 (Table I) confirmed the assumption that the deficiencies prevailing in adx rats would markedly impair the growth reaction of the mammary glands to low and, less so, to higher doses of Tp. Preliminary experiments on adx gdx rats treated with estrone and/or cortisone in various doses showed that modifications of the response to $Tp_{0.5}$ could not be demonstrated with any certainty. From other trials it was apparent also that estrone and cortisone in higher doses induced effects of their own, whereas Ca and E singly or together did not produce any or only limited growth of mammary tissues of adx gdx rats. The growth reaction or the development of alveoli induced by Tp was enhanced by Ca. Injections of E did not enhance the responses of the mammary glands to $Tp_{0.2}$ or $Tp_{0.1}$. These findings agree with those after local application of testosterone and injections of either cortisone or estrone into castrated adrenalectomized rats(5). The result obtained from Group 4 (Table I) treated with $Tp_{0.2}$ or $_{0.1}$, Ca and E showed an 'androgen' response (alveolar lobules) in 82 or 69% of the mammary glands of 17 and 13 rats studied, respectively. A complete restoration to normal was not achieved. In view of the difficulty of finding properly balanced doses of the 3 hormones, this does not seem surprising. Of importance is that Ca or E given singly enhanced the effects of $Tp_{0.2}$ or $_{0.1}$ slightly (Ca) or not at all (E), but that Ca and E together permitted the stimulation of alveolar development in the mammary glands of a considerable number of gdx adx rats given $Tp_{0.2}$ or $Tp_{0.1}$. The result supports the view(8,9) that the actions of testosterone on the mammary glands of male rats are superimposed on those of estrogens. In agreement with others(5) the present findings show furthermore that adrenal cortex hormones facilitate mammary reactions.

Summary. The reaction of the mammary glands to testosterone propionate (Tp) was studied in gonadectomized (gdx), adrenalectomized (adx) rats given estrone (E) and cortisone acetate (Ca) singly or together. Compared with controls with intact adrenals the mammary responses to Tp were reduced after adrenalectomy. With few exceptions alveolar lobules failed to develop in gdx adx rats injected with 0.1 mg Tp every other day. When daily injections of 0.125 mg Ca were added to the treatment with 0.1 mg Tp the number of glands presenting alveoli increased slightly. E, 0.05 μg daily, failed to modify the mammary response to 0.1 mg Tp. Combined treatment with Ca, 0.125 mg, and E, 0.05 μg, daily and Tp, 0.1 or 0.2 mg, every other day resulted in development of alveolar lobules in a great majority of mammary glands of gdx adx rats. The findings support the view that the mammary response to androgens is dependent on concomitant actions of estrogens and adrenal cortex steroids.

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Growth and Glycolysis in the Human Diploid Cell Strain WI-38.* (30057)

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The serial cultivation of human diploid cell strains provides a unique system for biochemical studies since these cells, in contrast to the commonly used heteroploid cell lines, retain many of the characteristics of normal cells *in situ, e.g.*, a stable diploid karyotype, a limited life span, and non-malignant growth *in vivo*(1,2).

Extensive studies have been carried out

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