During this interval cooling of the skin in the hyperemic area did not result in vasoconstriction in the gastrointestinal tract, nor did further application of a warm pack result in increased vasodilatation in the viscus. This is in full accord with the observation reported by Ruhmann[4] that local warming of the skin does not elicit reflex responses of the gastrointestinal musculature until dilatation of the cutaneous vessels in the stimulated area has taken place.

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Response of Gonads and Gonaducts of Ambystoma Larvae to Treatment with Sex Hormones.*

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For many years Ambystoma has served as an experimental animal in studies of sex differentiation and sex development. Such studies have been made principally by means of parabiotic union of larvae or by gonad transplants. Since synthesized crystalline sex hormones are now available it seems of interest to determine the effects of these compounds upon the sexual development of the same species of salamanders and to compare the results of such experiments with those obtained by the above mentioned methods.

First reports on effects of crystalline sex hormones on sex differentiation in Ambystoma were made by Burns,[1, 2] who concluded that injection of testosterone propionate into *Ambystoma punctatum* larvae causes genetic females to differentiate in a male direction, while estrone causes differentiation of genetic males in a female direction. Ackart and Leavy[3] obtained results similar to those of Burns with injections of estrone into *Ambystoma tigrinum* larvae.

The animals used in our study were larvae of *Ambystoma maculatum* of two races, a differentiated race from Georgia, and a semi-differentiated race from Arkansas, and a small lot of *Ambystoma tigrinum* larvae from Iowa. The sex hormones used were estrone (Theelin: Parke-Davis Co.), estradiol dipropionate (Diovocylin:

4 Ruhmann, W., *München. med. Wchschr.*, 1933, **80**, 17.

* Aided by grants from the National Research Council, Committee for Research in Problems of Sex.

1 Burns, R. K., *Anat. Rec.*, 1938, **71**, 447.

2 Burns, R. K., *Anat. Rec.*, 1939, **73**, 73.

3 Ackart, R. J., and Leavy, S., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 720.

Ciba), and testosterone propionate (Perandren: Ciba).† These hormones, dissolved in absolute ethyl alcohol, were added daily to aquarium water in concentrations of 500 gamma per liter. The entire lot of animals was divided into three groups, one to receive testosterone treatment, a second to be treated with estrogens, and a third to serve as controls.

The larvae of *Ambystoma maculatum* (Georgia) of differentiated race received hormonal treatment between the ages of 70 and 142 days. *Ambystoma maculatum* (Arkansas) larvae of semi-differentiated race were treated when between 57 and 110 days old. Hormonal treatment was stopped when these animals metamorphosed. The tiger salamanders received treatment from the 50th day to the 221st day, the treatment being continued 70 to 100 days after metamorphosis. At the time of first administration of hormone there were 50 control larvae, 70 animals to be treated with testosterone propionate, and 70 animals to receive estrogens. Animals which died before the end of the experiment are included in data given in Table I.

TABLE I.

Treatment	Females	Hermaphrodites	Males with cortex	Males
Controls	27	8	6	12
Testosterone	34	8	19	7
Estrogens	53	13	2	0

Some of the control males retain a vestige of cortex but otherwise possess typical testicular structure. Some gonads of the larvae in the Arkansas group are of distinctly hermaphroditic character but must still be considered as genetic males (Witschi[4]). The gonoducts in control animals show no evidence of stimulation (Fig. 1). The Wolffian ducts are developed to their full length in all larvae. In *Ambystoma maculatum* only the anterior portion of the Mullerian ducts is present at the time of metamorphosis, but in the older *A. tigrinum* animals they reach the cloaca. These ducts are of the neutral juvenal type as described by Rodgers and Risley.[5] In no cases were the cloacal glands stimulated.

The sex ratio in the group of larvae receiving testosterone propionate treatment remains unaltered (34 females and 34 males). This seems to indicate that the male hormone has little, if any, effect upon sex differentiation. The ovaries seem to have been affected by the treatment in that there is no indication of the presence of an

† We wish to express our thanks to Ciba Pharmaceutical Products, Inc., and to Parke-Davis Co. for the generous supply of sex hormones.

4 Witschi, Emil, *J. Exp. Zool.*, 1933, **65**, 215.

5 Rodgers, L. T., and Risley, P. L., *J. Morph.*, 1938, **63**, 119.

ovarial cavity (Fig. 2). In male sex glands one observes paradoxical feminization effects which will be discussed in a forthcoming paper. In all animals of this group, regardless of genetic sex, there is an extreme stimulation of the Wolffian duct (Fig. 2) and the cloacal glands. The Wolffian and pronephric ducts are much convoluted, with crypts and pouches protruding from their walls, and in most cases the ducts are deeply pigmented and extend the full length of the body cavity. The Mullerian ducts cannot be seen in the testosterone-treated animals. This picture offered by the ducts is unequivocal proof that the testosterone is actually taken up from the water and produces characteristic effects even at very early stages of larval development.

In estrogen-treated animals the sex ratio is definitely shifted in the female direction, with 53 females and 15 males. These 15 "males", none of which possessed typical testes, are all found among the larvae which died relatively early. They have hermaphroditic gonads with testicular and ovarial features of various proportions. The ovaries of the 53 females resemble those of controls (Fig. 3). It is statistically evident that these "females" are of two genetical types, true females and sex reversed males, though morphologically they cannot be separated. While in metamorphosed animals of control groups the oviducts extend through the full length of the body cavity down to the cloaca, in estrogen-treated animals of same age they often end blindly before reaching the cloaca. This inhibition in longitudinal growth is in contrast to the considerable inflation and consequent enlargement in diameter of these oviducts (Fig. 3). At the larval stage only the upper parts of the oviducts, growing down from the pronephric region, are present and exposed to hormonic influences. These cranial parts are moderately enlarged in most cases. The Wolffian ducts and cloacal glands retain a condition as in animals receiving no hormonal treatment. The appearance of the oviducts is sufficient proof that, like the testosterone, the estrogenic hormones are also taken up by the larvae.

Conclusions. The presented data indicate that estrogens produce sex reversal in male salamanders, while testosterone propionate exerts no corresponding influence upon genetical females. As in adult animals, the secondary sex characters respond also in the larval salamanders to the administration of sex hormones. The female hormone stimulates slightly the larval Mullerian ducts, and the male hormone causes an extensive and very precocious stimulation of the larval Wolffian ducts and cloacal glands. It is remarkable that the estrogens affect most profoundly the male gonads and the testosterone the male secondary sex characters. The hormones do not induce

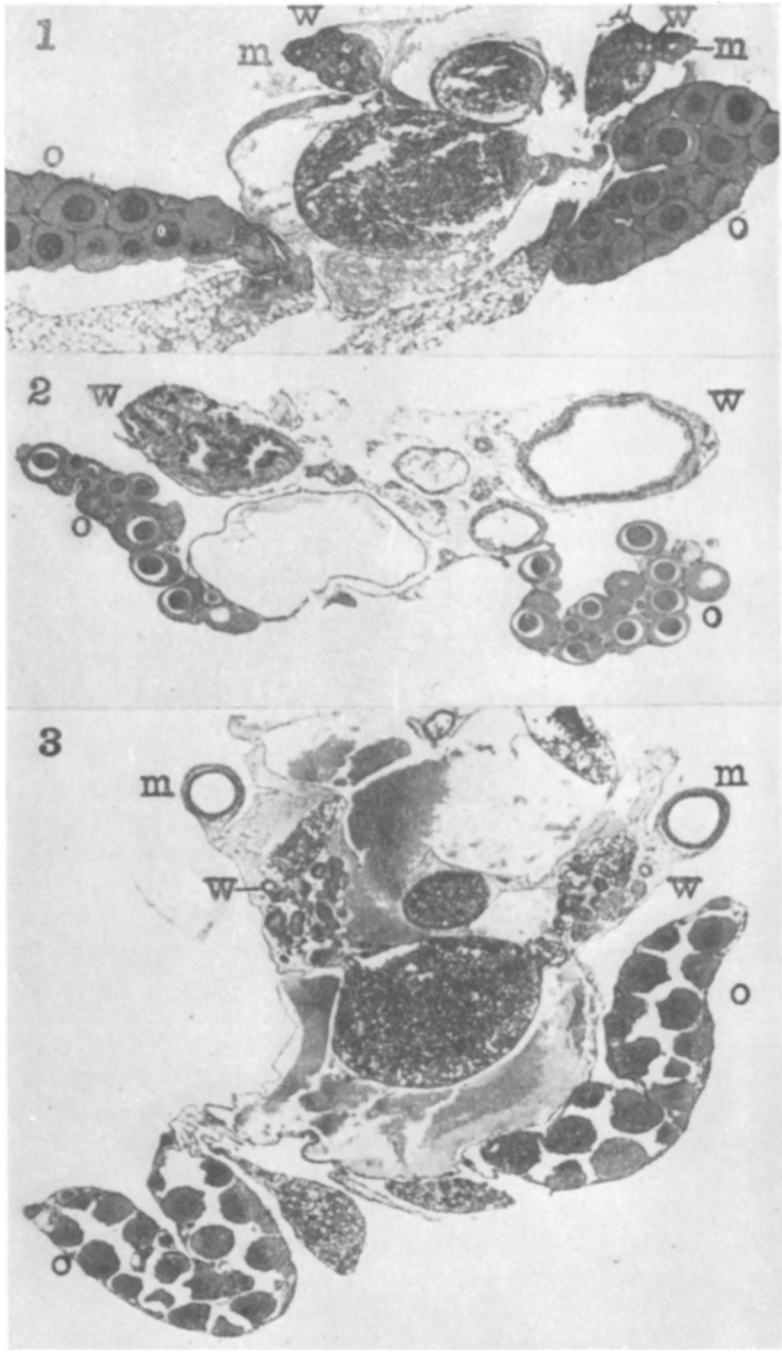
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FIG. 1.
Cross section through the gonads and gonoducts of *A. tigrinum* control female. Note unstimulated Mullerian ducts (m) and Wolffian ducts (w).

FIG. 2.
Cross section through the gonads and gonoducts of *A. tigrinum* female treated with testosterone propionate for 171 days. Note absence of Mullerian ducts and stimulated Wolffian ducts (w) and close resemblance of the right duct (left in the picture) to the frog seminal vesicle.

FIG. 3.
Cross section through the gonads and gonoducts of *A. tigrinum* female treated with estradiol dipropionate and theelin for 171 days. Note stimulated Mullerian ducts (m) and unstimulated Wolffian ducts (w). All figures ×25.

original formation of gonoducts; they stimulate only the secondary (functional) enlargement of parts of ducts already present. It has been shown by means of parabiosis that the testes of males release some inductive substance which inhibits the development of the ovaries of female cotwins and indirectly may cause some genetic females to continue development in a male direction.[6] On the other hand, in the male-female parabiotic combinations there is no precocious stimulation of either gonoducts or cloacal glands. These fundamental differences in the observed reactions prove that the crystalline sex hormones used in this experiment cannot be identical with the substances which normally act as inductors of sex differentiation.

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Effect of Foods on Serum Esterase of Rats.

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In a previous publication[1] it was reported that the tributyrin splitting property (here called esterase concentration) of the serum of rats is markedly increased after poisoning with carbon tetrachloride and after feeding a high fat diet. On the other hand, substances such as xanthine, which exert a protective action on the liver against carbon tetrachloride, will, when subcutaneously injected into rats, definitely lower the serum esterase concentration. Before proceeding further with the investigation into the possible significance of the serum esterase change in the animal's resistance to carbon tetra-

[6] Witschi, Emil, *Allen's Sex and Internal Secretions*, Baltimore, 1939, 2nd ed., 145.

[1] Forbes, J. C., and Outhouse, E. L., *J. Pharm. and Exp. Therap.*, 1940, **68**, 185.