jecting of a given volume only a small portion actually stays in the epidermis, the remainder going into surrounding tissues which are not susceptible to this virus. Hence, if more virus is injected than can stay in the skin it has no effect. Up to a certain point as shown in Table I the increase in the volume of injected solution does increase slightly the chance that a susceptible cell will become infected. It should be emphasized that this does not negate the theory that 1 particle causes infection. It does, however, show that an erroneous figure may be obtained in calculating the number of particles in a virus suspension if the volume of injection used is too large.

Although the above explanation at present seems the most likely, further experimentation must be done as there is the possibility that this phenomenon represents a much more fundamental character of virus infection. We are continuing the study of this subject.

Summary. Experiments are reported in which it is shown that under usual experimental conditions the concentration and not the volume of a vaccinia virus dilution is important in determining the number of infectious units contained in a given virus suspension.

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Experimental Reversal of Sex in Salamanders by the Injection of Estrone.*

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Experimental evidence derived from the work of Burns, Humphrey, Witschi and others[1] has established the fact that differentiation of sex in salamanders may be modified or even completely reversed through the action of sex-differentiating principles produced in the gonads of a parabiotic partner of opposite sex, or by a gonad of different sex type resident as a graft. This report presents briefly the results of experimental sex reversal in the salamander *Amblystoma tigrinum* (Green), during the period of sex differentiation,

* The crystalline substance used was very kindly supplied by the Schering Corporation.

1 Allen, E. (Editor), 1939, *Sex and Internal Secretions*, 2nd Ed., Chapters III and IV. Baltimore, Williams & Wilkins Co.

induced by treatment with a female sex hormone of *mammalian origin*—crystalline estrone. It seemed of interest to determine whether such hormones are specific in their actions throughout vertebrates in general, and if so whether their effects are exerted through the differentiating mechanisms already demonstrated for amphibians. The latter point might be determined by close comparison of the histological process in the two cases.

Thirty-six larvae of *A. tigrinum* were selected, closely approximating 30 mm in length, at which stage sex differentiation is beginning in this species. Fourteen were reserved as controls, subject to the same environmental conditions as the experimental group. The 22 experimental subjects each received 2 injections weekly of .01 cc of a solution of estrone in sesame oil over a period of 6 weeks. Each injection contained 25 γ of the crystalline substance, the total treatment representing approximately 350 γ. Injections were made into the body cavity by means of a modified microinjection apparatus.

During the experiment individuals were reared in isolation, at a dietary level permitting a relatively slow rate of development, thereby prolonging the period of sex differentiation. At the end of treatment the subjects were still larvae and approximately 65 mm in length.

During the experiment 7 animals died as a result of injury or from unknown causes. Most of these deaths came early in the experiment while the subjects were quite small. From the data on experimental sex reversal in salamanders generally, it can be safely concluded that this mortality was incidental and without significance for the problem at hand. A few specimens subsequently showed a mild form of ascites, due probably to infection admitted to the body cavity while injecting. Some deaths may have been due to this cause.

After preservation, examination by gross dissection at once showed entire absence of typical males in the experimental group. Controls, on the other hand, proved to be distributed equally as to sex—7 males and 7 females. At this stage of development, ovaries are large, irregular and convoluted, or folded along their free borders. Numerous large, growing ovocytes in their follicles give the surface a coarsely granular character. Testes, on the contrary, are much smaller, smooth and spindle-shaped, frequently lightly sprinkled with fine pigment. The two normal types can scarcely be confused at this stage of development. Of the 15 injected specimens, 9 resembled control females so closely, both in form and size of the gonads, as to lead to the conclusion that these individuals were doubtless genetically female, and that experimental treatment had not significantly modified the normal course of ovarian development. The remaining 6

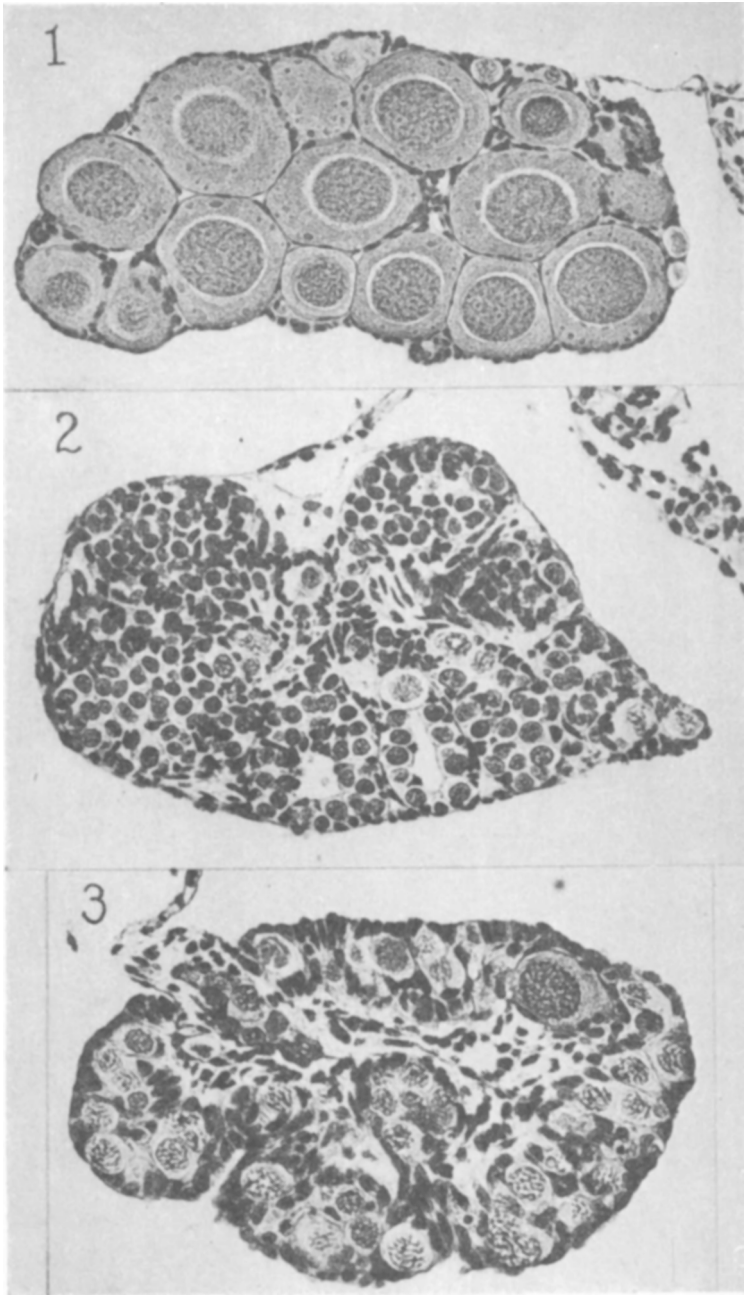

FIG. 1.
Normal ovary of a control specimen, × 150. Large growing ovocytes in their follicular envelopes virtually obliterate the ovarial cavity.

FIG. 2.

Normal testis of a control male, × 150. The germinal elements are in spermatogonial and spermatocyte stages. The medullary substance is compact, with no internal sac.

FIG. 3.

Typical section of an experimentally produced ovo-testis, × 150. The cortical elements have the cytological characteristics of ovocytes. The medullary area is degenerate and shows evidence of cavity formation in the region corresponding to the ovarial sac.

cases varied greatly in size, but none approached the proportions of normal ovaries. All were irregular in form, more closely resembling immature ovaries than testes at any stage of development. Histological study was necessary to determine their real character.

Microscopic examination of these atypical gonads revealed ovo-testes in every case. The rudimentary cortex of the larval testis, under the influence of the hormone, had developed to a condition resembling that of retarded ovaries. Varying amounts of medullary tissue remained, however (Fig. 3). In the cortex of the ovo-testes, the germinal elements had the cytological characteristics of ovocytes, while in the medulla regressive changes, such as degeneration and vacuolization, were usually apparent. In some cases the medullary region had been practically obliterated and the gonads were rudimentary ovaries. In others it remained a definite structure, but cortical development always greatly exceeded that normal for developing testes. The principal difference between the ovo-testes and young normal ovaries consisted in the large amount of medullary stroma in the former, and the more advanced condition of the cortex in the latter. (Compare Figs. 1, 2 and 3.) The type of reversal here described is very similar to that reported by Burns[2] in *A. punctatum,* following estrone injections.

No definite modification of the gonoducts was noted in these animals, due doubtless to the generally immature condition of the subject at the conclusion of treatment, since in somewhat older *A. punctatum* (at metamorphosis) Burns[2] had found a moderate but unmistakable stimulation of the oviducts following estrone administration.

The histological changes produced by injection of estrone are quite similar in detail to the results of parabiotic twinning, and the reversals obtained by Humphrey through gonad grafting. However, Padoa,[3] by adding "folliculin" to the water in which the tadpoles *(Rana esculenta)* were developing, obtained a "paradoxical effect"; namely, the differentiation of males only. Witschi and Crown,[4] using the

2 Burns, Robert K., Jr., *Anat. Record,* 1938, **71**, 447.

3 Padoa, Emanuele, *Arch. Ital. Anat. et Embriol.,* 1938, **40**, 122.

4 Witschi, E., and Crown, E. N., *Anat. Rec.,* 1937, **70**, 121.

same method of administration, failed to observe this paradoxical effect in *Rana pipiens* larvae, but obtained female or hermaphroditic differentiation using the female hormone, dihydrotheelin, and male differentiation after adding testosterone propionate. Except for the above-mentioned result of Padoa, it appears that mammalian sex hormones have a sex-specific effect on amphibian differentiation, at least in the period of larval development. Recently Foote and Witschi,[5] using sexually differentiated larvae of *Rana clamitans,* have caused a secondary transformation of ovaries into testes by injecting testosterone propionate, though they found estrogenic hormones to have little effect on the structure of the testes of these animals. Puckett,[6] however, obtained a precocious sexual differentiation in *Rana catesbiana* tadpoles after injecting pituitary extracts in addition to sex hormones, a result not obtained by injection of sex hormones alone. In his experiment, theelin plus pituitary preparation produced a 100% differentiation of ovaries, while testosterone propionate plus pituitary preparation produced a differentiation of testes in 100% of the animals receiving this combination.

Summary. 1. Twenty-two larvae of *A. tigrinum,* at the beginning of sexual differentiation, were each injected with 350 γ of estrone, distributed over a period of 7 weeks. Seven cases died. 2. Macroscopically, the gonads of 9 treated animals closely resembled those of control females, while the gonads of 6 were atypical. The latter were of smaller size, smoother in contour, and in other respects suggestive of abnormal testes rather than ovaries. 3. Histological examination showed these atypical gonads to be ovo-testes so modified by estrone as to resemble retarded ovaries with irregular medullary remains.

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5 Foote, C. L., and Witschi, Emil, *Anat. Rec.,* 1939, **75**, 75.

6 Puckett, W. O., *Anat. Rec.,* 1938, **75** (Suppl.), 89.