in chambers of the latter (Fig. 5) yet the sections in Fig. 7 show a complete degeneration of follicles and ova 60 days after grafting.

Conclusions. Homografts of the rat ovary do not depend on vascularization to maintain the production of estrogenic substances up to 60 days. Semipermeable millipore filter membranes were used to form a chamber in which to embed ovarian transplants in previously castrated recipients. These chambers excluded leucocytes and prohibit vascularization, yet permitted passage of gonadotropic hormones. Vaginal smears and uterine weights demonstrated the production of estrogens from these grafts two months later. The functional survival of similar preparations in the monkey is now under investigation.

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Hypophysectomy and the Tail Darkening Reaction in *Xenopus.** (23016)

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It has long been known that if the hypophyseal placode is removed from amphibian embryos, the resulting larvae will be very pale in appearance and vary in color in different species from white or silver, to golden yellow(1,2). Pallor generally results from persistent contraction of epidermal melanophores and expansion of both dermal and epidermal guanophores(2). It is therefore surprising to observe that in hypophysioprivic *Xenopus* larvae, melanophores of the tail are capable of considerable expansion or contraction under appropriate conditions. The experiments reported here were designed to study the influence of the hypophysis on the behavior of some of the chromatophores of *Xenopus* larvae, and to gain some insight into the peculiar activity of melanophores in the tail.

Methods. All tadpoles were raised from naturally spawned eggs of *Xenopus laevis* Daudin. At an early tailbud stage, the hypophyseal placode was removed from 378 embryos. Judging from the number of pale larvae resulting, removal of the hypophysis was accomplished in about 85% of the operated animals. Only about 25% of these larvae survived after 2 weeks, because of severe damage to their mouth parts.

Melanophore counts were made in the ventral fin of both normal and hypophysioprivic larvae of the same egg batch. An area about one-third the distance from the anterior margin of the pigmented region of the tail and midway between fin and somites was selected for the counts. These were made using an ocular micrometer on 6 larvae from each group. Under magnification of 45X, melanophores enclosed in a square, one-sixteenth of a square cm in size, were counted for 25 squares and the average number per square was then determined for each tadpole.

Results. In hypophysioprivic larvae a very great reduction in number of melanophores was noted in all regions except the tail, and those that were present were maximally contracted. This was especially true of melanophores present on thymus, nerves, and blood vessels (Fig. 1, 2). In the tail, melanophore number was not greatly influenced by hypophysectomy (Fig. 3, 4, 5). A reduction of about 30% occurred in the number of tail melanophores in hypophysioprivic tadpoles. This is a remarkably small percentage compared with the great reduction observed in other parts of the tadpole.

In normal *Xenopus* larvae, guanophores are

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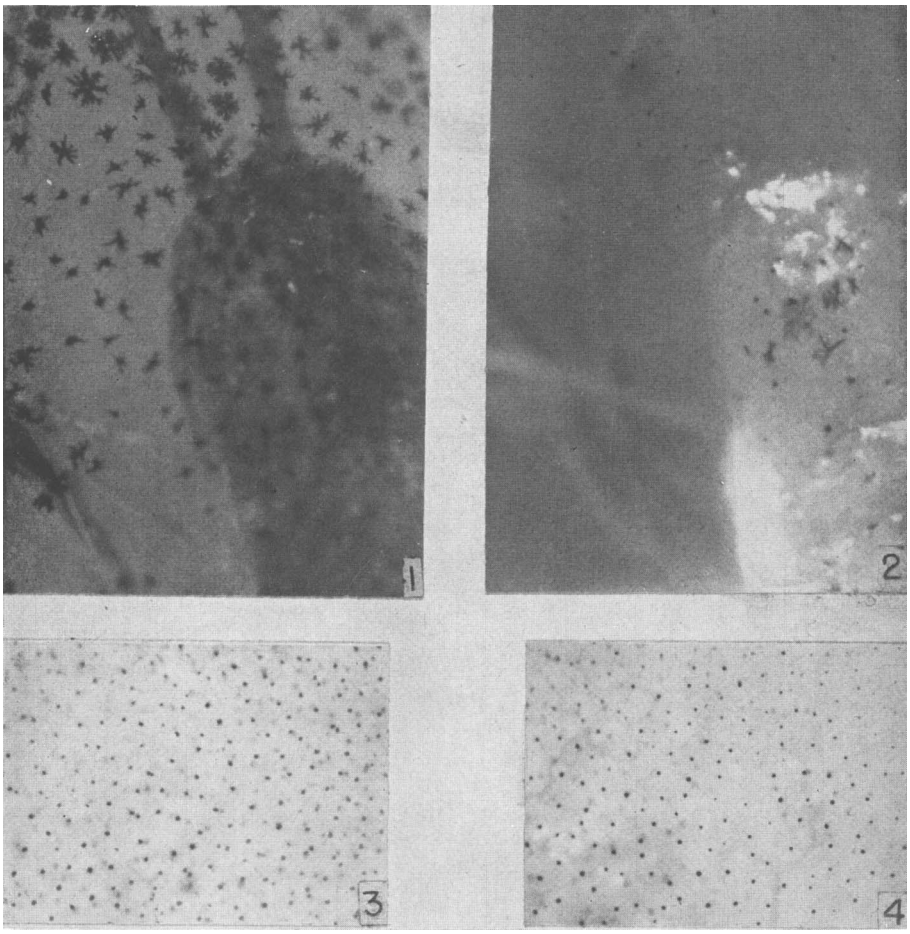

FIG. 1. Anterior head region of normal *Xenopus* larva showing distribution of melanophores over brain, in skin, and on deep nerves and blood vessels. X45.

FIG. 2. Anterior head region of a hypophysioprivic *Xenopus* larva showing reduction in number of melanophores over brain, in skin, and on deep nerves and blood vessels, and the presence of guanophores. X45.

FIG. 3. Contracted melanophores in ventral fin of normal *Xenopus* larva. X60.

FIG. 4. Contracted melanophores in ventral fin of hypophysioprivic *Xenopus* larva showing only slight reduction in number of melanophores. X60.

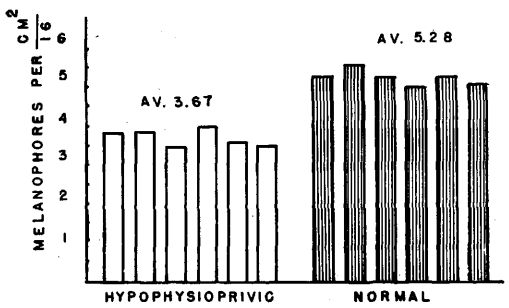

FIG. 5. Diagram showing relative number of melanophores in ventral fin of 6 hypophysioprivic and 6 normal *Xenopus* larvae.

restricted to eyes and abdomen(3). In hypophysioprivic larvae, however, they were present as large glistening bodies in the integument of head and tail (Fig. 2, 6, 7).

When either normal or hypophysioprivic tadpoles were placed in the dark at room temperature for about 30 minutes, tail melanophores expanded greatly, causing the tail to appear very black. When tadpoles were returned to the light, these melanophores contracted and within 5 or 6 minutes, the tails regained their former light appearance. Be-

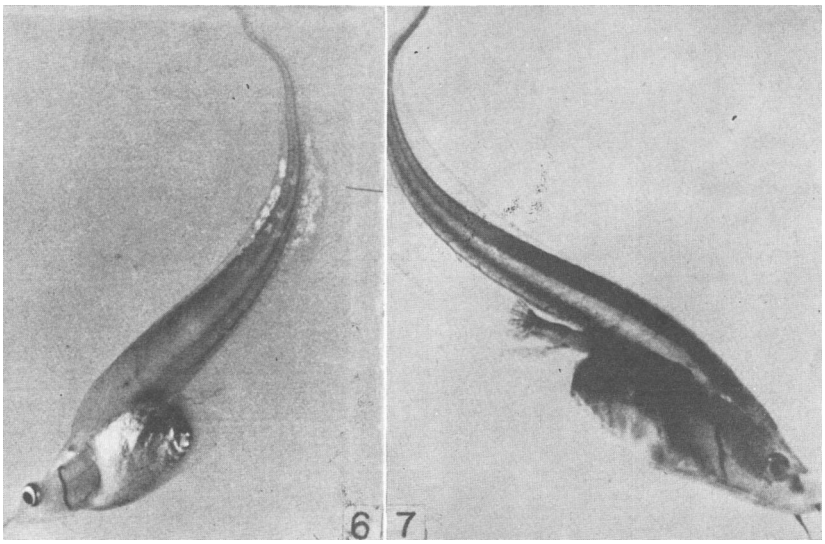

FIG. 6. Hypophysioprivic *Xenopus* larva showing typical paleness and presence of guanophores in the tail. X1.5.

FIG. 7. Normal *Xenopus* larva. Note darkness, and lack of guanophores in tail. X1.5.

cause tail darkening reaction obviously involves reception of light, the eyes were removed from 5 hypophysioprivic and 10 normal larvae. During the next few days, these larvae were tested repeatedly and in all cases displayed the same positive tail darkening reaction. To determine whether direct nervous mediation of tail melanophores might be a factor in the tail darkening reaction, the spinal cord and lateral line nerves of the tail were severed in 4 normal larvae. A disappearance of the sinusoidal wave pattern of tail movement occurred, but ability of tadpoles to carry out the tail darkening reaction was in no way impaired by the operation.

With the eyes and direct ennervation eliminated as important elements in the tail darkening reaction, it seemed possible that tail melanophores might be directly stimulated by light. To explore this possibility, tails were cut from at least 50 normal larvae and placed in Petri dishes containing tap water. Some dishes were placed in the dark, while others serving as controls were left illuminated. After 30 minutes, the tails were brought back into the light and were very dark due to expansion of their melanophores. Just as in intact tadpoles, 5 or 6 minutes after exposure to light, the tails became pale and appeared no different from the controls. This reaction could be consecutively repeated at least 3 times.

In a similar experiment, a microscope ocular was replaced with a small light source so that a narrow beam of light could be passed through the microscope and focused on the stage. By using this microscope in a dark room, it was possible to illuminate only a small region of tail mounted on the stage. In such experiments, melanophores in the illuminated region remained contracted, while those in the dark area displayed the typical tail darkening reaction (Fig. 8).

Discussion. Just as in most amphibians, removal of the hypophysis of *Xenopus* results in marked reduction in number of melanophores over the entire tadpole. The fact that this reduction is relatively small (30%) in the tail, is probably related to the tail darkening effect, which seems to operate independently of the hypophysis. This does not imply that tail melanophores are not stimulated by the hypophysis, in fact, Thing(4) uses expansion of tail melanophores in *Xenopus* as an index in the assay of the chromatophorotrophic hormone.

Appearance of guanophores in such regions as the tail and head of hypophysioprivic *Xen-*

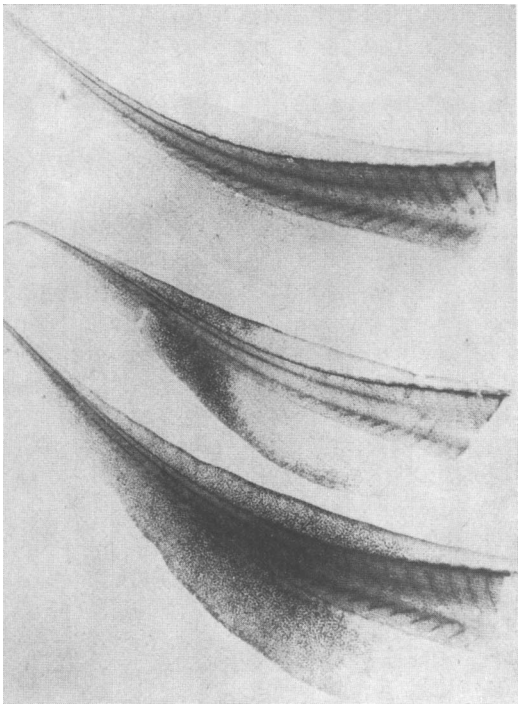

FIG. 8. Tails of normal *Xenopus* larva; upper, under lighted conditions; middle, tail darkening reaction except for center part which was left illuminated; bottom, at height of tail darkening reaction. X3.

opus larvae possibly reflects a general characteristic of anurans wherein the chromatophorotrophic hormone of the hypophysis elicits a guanophore inhibition. Hence, guanophores are contracted in normal larvae of *Rana,* but are expanded in hypophysioprivic tadpoles. This inhibition is perhaps of even greater potency in *Xenopus* and could account for the lack of differentiation of guanoblasts in the trunk and tail, as reported by Stevens(3).

The apparent direct stimulation of tail melanophores by light suggests that these cells possess a photochemical system. Additional evidence for this hypothesis can be derived from the fact that contraction of melanophores in the dark reacted tail occurs within 6 minutes after removal from the dark. The 30 minute period required for tail darkening could correspond with the synthesis of a photochemical substance, while the 5 or 6 minute periods might relate to its rapid inactivation or destruction by light. Support for this hypothesis is gained from some experiments now in progress which indicate that these melanophores can dark adapt.

Hypophysectomy has a profound effect on distribution of chromatophores in *Xenopus* larvae. A great reduction in number of melanophores occurs over the entire tadpole except in the tail where guanophores now appear. The tail melanophores can behave independently of the hypophysis and respond directly to stimulation by light.

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