

organism making the blip, careful calibration may even make it possible to arrive at a rapid but fairly accurate estimate of changes in velocity by comparing the lengths of blips under different conditions. This, however, may have limitations, owing to the relative direction of the movement of the scanning spot and the organism.

The system is particularly well suited to the recording of pathways at low power (that is, with a 1-inch objective) in organisms with a wide range of size variation around the general order of magnitude of 100 μ . With little doubt, however, the apparatus could be used at higher magnifications and high velocities of travel. An advantage could be obtained when high velocities are encountered by the use of higher scanning rates and a long-persistence display tube which would enable one to photograph the "stored" track image.

A final advantage is that, since with the system illumination of the field is low and intermittent, heating is minimal. In our studies the miracidia were simply placed in an open drop on a well-slide without undue drying occurring during a long series of exposures. This makes easy the addition of materials to the drop, the effects of which one wishes to study.

The value of this apparatus as a tool for the analysis of linear and angular velocity under different conditions of many sorts of organisms (protozoa, trochophores, microplankters—indeed, motile cells of all sorts) can, we feel, be immediately grasped from the accompanying photographs (5).

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5. We wish to express our appreciation to Rank Cintel Ltd. for making possible the use of the flying-spot particle resolver and to Donald Claughner for technical assistance.

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Response of the Neotenic Salamander *Haideotriton wallacei* to a Metamorphic Agent

Abstract. The neotenic, cave-dwelling plethodontid salamander *Haideotriton wallacei* undergoes only a few minor integumentary changes and loses only a single bone, the coronoid, when treated with sodium laevo thyroxine. Since neither the animal's thyroid nor the supplementary thyroxine is sufficient to effect substantial metamorphosis, genetic factors probably restrict the animal's ability to transform.

The systematic relationships of neotenic animals are best understood when the morphology of experimentally metamorphosed individuals can be compared with that of fully transformed types. The induction of metamorphosis also assists in ascertaining whether the neotenic features are due to environmental, physiological, or genetic factors.

The odd cavernicolous neotenic salamander, *Haideotriton wallacei*, was assigned by Archie Carr, who described it, to the lungless family Plethodontidae on the basis of its general conformation to the anatomy of larval plethodontids. I have shown in previous experiments (1) that the neotenic plethodontid salamander, *Eurycea tynerensis*, is clearly assignable to the genus *Eurycea* (it undergoes transformation upon administration of thyroxine) but that *Typhlomolge rathbuni*, another cavernicolous plethodontid, can undergo only a partial metamorphosis upon administration of thyroxine and therefore appears to have genetic resistance to the production of many of the skeletal and integumentary features of transformed plethodontids. It comes as no surprise, then, to find that *Haideotriton* is a second supposed plethodontid neotene that displays strong resistance to metamorphic agents.

For experimentation several subadult *Haideotriton* from Florida and Georgia were utilized (2). The animals were treated with various concentrations of sodium laevo thyroxine, a drug that normally causes almost complete transformation in neotenes such as *Eurycea tynerensis*, *E. neotenes*, and *E. nana*, and in various salamander larvae, within 3 weeks at laboratory temperatures and in the concentrations used for the experiment reported here (3).

The experimental animals were maintained in finger bowls containing 100 ml of the thyroxine solution made up with native water kept in a B.O.D. incubator in complete darkness at $19.5 \pm 0.5^\circ\text{C}$ (the temperature of the natural cave habitat). The thyroxine was

first dissolved in a drop of 0.1N NH_4OH and then diluted to the desired concentrations. Three controls maintained under the same conditions exhibited no changes in the course of the experiment, and they showed no changes during the succeeding 2 months in which they were maintained.

The results of treatment with thyroxine are shown in Table 1. In no case did any treated animal give complete external evidence of loss of larval characteristics; gills atrophied but were never completely lost or resorbed. That the animals perhaps cannot withstand complete transformation is suggested by the failure of any to survive more than 25 days of treatment, whereas all the control animals were in excellent health for some time after the death of the last treated animal. The alterations that occurred were essentially only the most simple integumentary changes, such as loss or reduction of fins, gills, and labial folds. The only skeletal change observed was the loss of the coronoid (a bone which was not reported by Carr in his original description but which is definitely present in untreated animals). The only peculiarity in the response pattern is that a preparation from a large untreated adult male, on staining, shows ossification of the posterior basibranchium to form an os thyroideum, whereas none of the treated animals give any indication of such ossification. This throws some doubt on the response observed in this species, for earlier stages of amphibian larvae tend to lack the potential to develop all adult structures when forced to metamorphose precociously (4).

All the treated animals were immature or female, but their response should be no different from that of males. What would happen if larger, older animals were treated with sodium *l*-thyroxine remains questionable at this time. Adult animals are exceedingly rare and difficult to capture, and further experimentation must await better collecting opportunities.

In addition to the responses shown in Table 1, the nonoccurrence of a number of other changes normally exhibited during metamorphosis was noted. None of the following occurred: development of nasolabial groove and nasolacrimal canal; development of eyelids; transformation to adult skin structure; development of a new integumentary pattern; atrophy of pterygoid; development of maxillary, prefrontal, nasal, and septomaxillary bones; devel-

Table 1. Results of experimental transformation of *Haideotriton wallacei*: ++, complete transformation; +, partial transformation; —, no change.

Concentration of thyroxin (μg/lit.)	Time treated (days)	Body length (mm)	Sex or stage	Loss of fins	Loss of labial folds	Gill atrophy	Narial shift to adult position	Development of gular fold	Loss of lateral line organs	Atrophy of coronoid
10,000	15	27	F	+	++	+	+	—	+	++
2,000	18	22	F	++	++	+	+	+	++	++
2,000	25	25.5	F	+	++	+	+	+	++	++
500	17	16	Immature	++	++	+	++	++	++	+
500	18	18	Immature	++	++	+	+	+	++	++

opment of adult vomerine tooth pattern and of choanal notches in the vomers; ossification of parasphenoid bone; and development of parasphenoid dentition.

Haideotriton, though exhibiting limited response to thyroxin, possesses distinct thyroid follicles containing colloid. A histological preparation made from the throat region of an untreated female approximately 24 mm from snout to vent contains a series of small follicles on either side, spread over an antero-posterior distance of 520 μ. The follicles, which do not exceed 30 μ in diameter, are lined with squamous-to-low-cuboidal epithelium. No attempt was made to identify thyroid hormone in the colloid, but as the experiment shows, the mere existence of an ample supply of thyroxin does not insure that metamorphosis will occur. Thus, *Haideotriton*, like *Necturus* and *Typhlomolge*, appears to be genetically resistant to the thyroid secretions, even though the animals' own thyroids produce and presumably release thyroid hormone or hormones.

Haideotriton proves to be the most resistant to thyroxin of all the plethodontids investigated. The results of the experiment described indicate that *Haideotriton* should be recognized as a plethodontid that has deviated markedly from other members of its family in that it has lost many of the usual adult bony structures and integumentary features, probably through genetic change. In many cases a classification of family is based upon the lack of certain bones found in more generalized families, but we see here a case where relationships are based only on larval similarities.

A moot point, then, is where to draw the line in declaring a higher category, for in a sense *Haideotriton*, and for that matter *Typhlomolge*, each of which lacks certain bones, is not comparable to transformed plethodontids and could represent independently lungless salamanders of a closely related but different family. If *Haideotriton* and *Typhlomolge* are retained as plethodontids

we must realize that the characteristics of animals in which neoteny occurs should be defined on the basis of both larval and adult features, so that the full identity is known. Thus, the characteristics of a salamander family are not solely those given for the transformed adult, and the necessity for concise descriptions of larvae is strongly indicated (5).

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1. H. A. Dundee, *Copeia* 1957, No. 1, 52 (1957).
2. The experimental animals were collected with the assistance of Richard Warren of Gainesville, Fla.
3. The sodium L-thyroxin pentahydrate (lot O.N. 23267) was kindly supplied by the manufacturer, Smith Kline & French Laboratories.
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5. This study was supported by a grant from the National Institutes of Health.

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New Genetically Homogeneous Background for Dystrophic Mice and Their Normal Counterparts

Abstract. A new type of genetically homogeneous dystrophic and comparable normal mice, from an F₁ hybrid cross between 129/Re-dy and C57BL/6J-dy, is now available as a result of a special breeding program. The clinical manifestations in these F₁ hybrid dystrophics correspond closely with those observed in 129/Re-dy mice, and they give completely comparable results on a variety of research tests. They are, however, considerably healthier than previously available dystrophics, with a growth rate closer to normal and a greatly increased life-span.

A research development at the Roscoe B. Jackson Memorial Laboratory which may be of interest to investigators studying muscular dystrophy in other institutions is the recent production of genetically uniform F₁ hybrid dystrophics (*dydy*) and comparable normal (*DyDy* and *Dydy*) counterparts. These animals come from a cross between the 129/Re-dy and C57BL/6J-dy inbred

strains, made possible by the introduction of the *dy* gene onto the C57BL/6J background by repeated backcrossing, now at backcross 10. At this backcrossing generation, approximately 98 percent of genes not closely linked to *dy* are expected to be identical with those of C57BL/6J (1).

As in the case of regular maintenance of *dy* in its strain of origin, 129/Re (2), this breeding program has involved transplantation of *dydy* ovaries into histocompatible normal host females (3). In each successive backcross generation, host females were F₁ hybrids between inbred strains 129/J and C57BL/6-a'a'. These females accept tissues from all backcross generations, since they carry one dose of all histocompatibility alleles carried by both of the original parental strains (4), and have the added advantage of providing genetic markers (*A^w*, *a'*) for identification of possible offspring from regenerated host ovaries. These host females, bearing ovaries transplanted from black dystrophic (*aa B- C- dydy*) females (offspring of *Dydy* carriers from the previous backcross generation), were mated to C57BL/6J (*aa BB CC DyDy*) males. The resulting *Dydy* offspring were intercrossed, and their dystrophic female offspring used in the next backcross generation.

There is little to choose in health or survival time between dystrophic individuals in the two inbred strains, 129/Re-dy and C57BL/6J-dy. When a cross is made between carrier individuals from these two inbred strains, clearly identifiable *dydy* individuals, showing the characteristic dystrophic syndrome (5) appear in the expected proportion. These are, however, considerably healthier, show less difference from normal in weight, and survive much longer than the dystrophics in either parental strain. Although 17 littermate comparisons (Table 1) of the body weight of *dydy* dystrophics and normals confirmed previous observations (in 129/Re) of significant size difference between genotypes, the weight differential was less than is usually observed in 129/Re-dy and all individuals appeared to thrive.

The contrast in life-span of inbred and uniform F₁ hybrid dystrophic animals is very striking. The mean life-span of 129/Re-dy dystrophics on a good laboratory diet is approximately 180 days (6), that of C57BL/6-dy dystrophics 106 days (7). It is not yet possible to estimate the mean life-span of 129B6-dy F₁ dystrophics, but 47/50