

THE EFFECTS OF PROLACTIN AND THYROXINE ON TAIL
FIN HEIGHT, HABITAT CHOICE, AND FORELIMB
REGENERATION IN THE ADULT NEWT
(*NOTOPHTHALMUS VIRIDESCENS*)¹

ROY A. TASSAVA, Department of Zoology
CARL KUENZLI, Developmental Biology Program, Ohio State University, Columbus, OH 43210

Abstract. Near maximum tail height of freshly collected adult male newts was maintained in the laboratory at 21.5°C by intraperitoneal injections of prolactin alone or by a combination of prolactin and thyroxine. Tail height of male newts at 5°C was also maintained with or without thyroxine treatment. Tail height decreased by over 40% when male newts at 21.5°C were given saline or thyroxine. Both male and female newts, after 1 week at 21.5°C laboratory conditions, begin to leave the water. This land habitat choice is enhanced somewhat by thyroxine but completely abolished by prolactin with or without thyroxine. In addition to effects on tail fin height and land-water choice, prolactin also enhanced limb regeneration rate of adult newts.

OHIO J. SCI. 79(1): 32, 1979

The spotted newt (*Notophthalmus viridescens*) has a life cycle consisting of three distinct phases: in water as a larva, on land as a red eft, and again in water as an adult (Wald 1958, Grant 1961). Treatment of spotted newts with prolactin and/or thyroxine causes physiological changes which are shown in behavioral and morphological differences (Wald 1958, Grant 1961, Grant and Cooper 1965).

Grant and Cooper (1965) demonstrated that prolactin treatment inhibited larvae from undergoing primary metamorphosis, whereas thyroxine administration accelerated primary metamorphosis. Furthermore, it has been shown that the active principle involved in inducing the water drive (second metamorphosis) of efts is prolactin (Grant and Grant 1958). Adult aquatic newts, however, will again assume a land habitat upon treatment with thyroxine (Grant and Cooper 1965).

Tail fin keeling, which is characteristic of adult males in breeding condition (Singhas and Dent 1975), is also observed in efts which are induced to migrate to water by prolactin administration. Vel-

lano *et al* (1970) determined that prolactin injections increased the tail height of adult crested newts (*Triturus cristatus carnifex*), whereas thyroid stimulating hormone treatments did not.

Prolactin and thyroxine appear to be essential for survival and for limb regeneration of adult aquatic newts. The rate of forelimb regeneration in intact adult newts is enhanced by prolactin administration (Waterman 1965), whereas exogenous thyroxine acts to inhibit cell proliferation in regenerating limbs (Hay 1956). In hypophysectomized newts, a combination of prolactin plus thyroxine is effective in promoting survival and limb regeneration (Connelly *et al* 1968, Tassava 1969).

The experiments described here are a further investigation of the seemingly antagonistic effects of prolactin and thyroxine in water-land drive and tail fin keeling of adult newts, and the synergistic effects of prolactin and thyroxine in regeneration of intact adult newts.

MATERIALS AND METHODS

Adult male aquatic newts (*Notophthalmus viridescens*) were collected in the spring in southern Ohio. All of the newts were in breeding condition and were kept in this condition by storage in a refrigerator kept at 5°C. When

¹Manuscript received 28 October 1977 and in revised form 26 July 1978 (#77-84).

experiments were in progress, newts were kept at $21.5^{\circ}\pm 0.5^{\circ}\text{C}$ with either normal room lighting or complete darkness in an incubator.

NIH ovine prolactin was dissolved in 0.9% saline. A dose of 0.15 units/0.1 ml was administered 3 times/week to each newt by intraperitoneal injection. Thyroxine (L-thyroxine sodium salt, Calbiochem) was added to aerated water to give the desired concentration of 1×10^{-6} or 1×10^{-7} (1 part thyroxine/million or 10 million parts H_2O). Thyroxine solutions were changed 3 times/week. Control newts received saline injections of 0.1 ml of 0.9% NaCl 3 times/week.

In the tail fin height experiment (Series I), newts were kept in round plastic containers holding 500 ml of water. Measurements of the highest part of the tail were made 3 times/week through the eyepiece of a dissecting microscope which contained a micrometer scale (0.7 units equal 1 cm). Measurements were made at low power from a constant height. A total of 8 newts were treated with prolactin, 10 with thyroxine (1×10^{-7} ppm) plus saline, 10 with saline and 12 with prolactin plus thyroxine (1×10^{-7} ppm). All of the above were kept at $21.5^{\circ}\pm 0.5^{\circ}\text{C}$. An additional group of 4 newts was compared with a large group of controls routinely stored at 5°C .

The Series II regeneration experiment included both male and female newts picked at random, with 8 newts in each of the 3 experimental groups. Both forelimbs of each newt

were amputated distal to the elbow two weeks prior to hormone treatment. The treatments were similar to those administered in the tail height experiment, except that the thyroxine concentration used was 1×10^{-6} ppm for the first 12 days of treatment, and then 1×10^{-7} ppm for the duration of the experiment in the thyroxine plus saline group. A concentration of 1×10^{-6} ppm thyroxine was used throughout in the thyroxine plus prolactin group.

In Series III, experiments were designed to test the effects of prolactin and thyroxine on the behavior of aquatic adult newts with regard to choice of land or water habitat. Both male and female newts, randomly mixed, were divided into groups of 8 to 10 and placed into plastic crispers which were tilted so that both water and sand were available in equal areas in each crisper. All newts were in breeding condition and were taken from a 5°C refrigerator and placed immediately into the test containers which were at $21.5^{\circ}\pm 1^{\circ}\text{C}$. At 5:00 P.M. on each day, the numbers of newts in the aquatic vs. the land areas of each container were recorded. In the occasional cases where newts were found partly submerged, the body area of each newt which was out of water was estimated. Newts with over 50% of their body area out of water were recorded as on land, under 50% as in water. Series III newts were given one of the following, 3 times per week: 0.1 ml saline (Group A); 1×10^{-7} ppm thyroxine and 0.1 ml saline (Group B); prolactin, 0.15 units/newt (Group C); or 0.15 units

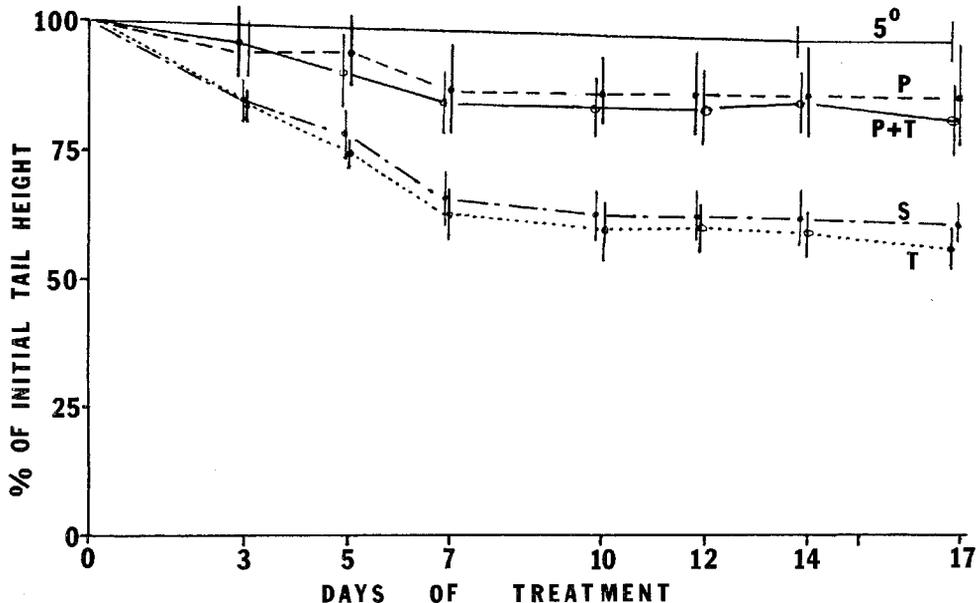


FIGURE 1. A comparison of the percent tail height remaining of untreated adult male newts at 5°C and of adult male newts at 21.5°C which were treated with either prolactin (P), prolactin plus thyroxine (P+T), saline (S), or thyroxine (T). An additional 4 male newts were maintained at 5°C and treated with thyroxine. The tail fin height of the latter group did not differ from the 5°C group which was not treated (top line). Each group contained at least 8 newts. Vertical bars represent \pm S.E. of the mean.

prolactin/newt plus 1×10^{-7} ppm thyroxine (Group D).

RESULTS

The results of the tail fin height experiment are shown in figure 1. The only male newts which did not show a significant reduction in tail height were newts kept at the 5°C storage temperature. These newts, with or without thyroxine treatment, showed a reduction of less than 5% of the tail height recorded at the beginning of the experiment. Nuptial pads and claspers of male newts in these two groups retained their maximum breeding condition.

All 4 groups of newts maintained at room temperature ($21.5 \pm 0.5^\circ\text{C}$) during the experiment exhibited a reduction in tail fin height. Newts treated with prolactin alone or with prolactin plus thyroxine lost about 15% of the tail height during the first week at room temperature (fig. 1). During the subsequent two weeks, only a small additional decrease occurred, so that by 21 days after treatment was begun, prolactin-treated newts retained 85% of the original tail height and prolactin plus thyroxine-treated newts retained 80% of the original tail height. Nuptial pads and claspers of these newts lost some of their black coloration but were still in good condition in 3 weeks.

Newts which were treated with thyroxine alone or control (saline) newts all lost significant portions of the tail fin, the major reduction occurring during the first week of treatment. Newts in these two groups retained only 60% (saline) and 55% (thyroxine) of the original tail height. Nuptial pads and claspers also were slowly lost, so that these typical male breeding characteristics were no longer present after 3 weeks of treatment.

In the Series II regeneration experiment, it was clear that prolactin treatment enhanced the regeneration rate of adult newts. When newt regenerates were staged according to Tank *et al* (1976) at 3 weeks after amputation and again after 4 weeks, it was clear that the most advanced stages of regeneration were reached by prolactin-treated newts, followed by prolactin-thyroxine-treated newts and saline controls, which regenerated at an equal rate, and finally thyroxine-treated newts. A complicating

factor was the finding that newts treated with thyroxine alone began to die after 3 weeks and were obviously in poor health. Of 6 newts treated with thyroxine alone, none reached palette stages or beyond and only 3 newts survived to 4 weeks after amputation. Newts treated with prolactin alone were approximately 1 stage of regeneration more advanced. Thus when prolactin-treated newts were at the palette stage of regeneration (3 weeks), controls and prolactin plus thyroxine-treated newts were at the late bud stage. This one stage difference was maintained through 4 weeks.

The results of Series III experiments are shown in figure 2. Initially, after 1 day, newts in all 4 groups remained in the water. By two days and especially at 1 week, however, saline-treated control newts began assuming a land habitat as did newts treated with thyroxine. By day 11, all newts of the thyroxine-treated group preferred to be on land as did nearly all of the saline controls. Usually, in the latter group, only 1 or 2 newts remained submerged or partly submerged after day 10 (fig. 2).

In contrast, none of the prolactin-treated newts were ever observed on land and only occasionally were newts of the prolactin plus thyroxine-treated group observed on land. It is clear that the land choice of control newts and thyroxine-treated newts is reversed by prolactin.

DISCUSSION

The present results, which suggest that prolactin promotes and maintains tail fin height in male spotted newts (*Notophthalmus*), are in agreement with earlier reports on effects of prolactin on tail fin height of male spotted newts (Singhas and Dent 1975) and of European newts (*Triturus cristatus*) (Vellano *et al* 1970). In both species, decreases in tail fin height (fin regression), which occur when male newts are maintained at laboratory temperature, can be prevented by prolactin administration, as was observed in the present study. Interestingly, testosterone is important to nuptial pad formation, along with prolactin, but does not affect tail fin height (Singhas and Dent 1975).

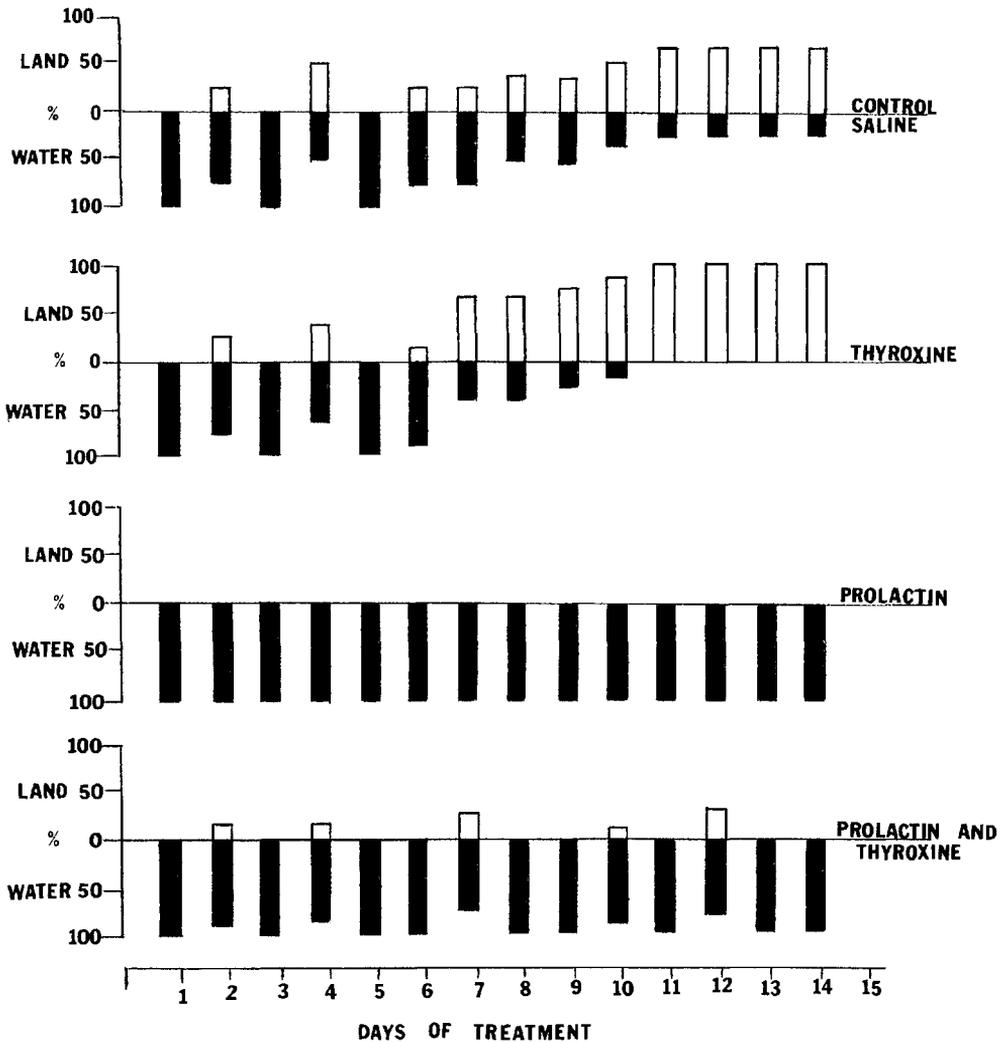


FIGURE 2. A comparison of the daily percent distribution of adult newts in aquatic or terrestrial habitat following treatment with saline, thyroxine, prolactin, or prolactin plus thyroxine. Each aquarium and each treatment group contained 8-10 newts.

In both of the above species, tail fin height of male newts exhibits periodic changes, correlated with the temperature and season. Tail height is maximum in the late fall, winter, and early spring and is reduced during the warmer months (Vellano *et al* 1970, Singhas and Dent 1975, Tassava, unpublished observations). Singhas and Dent (1975) suggest that seasonal variations in tail fin height relate to prolactin secretion, i.e., prolactin secretion is highest during cold

months. Presumably prolactin secretion is also high when newts are maintained in the laboratory at cold temperatures, as in the present study using 5°C (fig. 1), and as reported by Singhas and Dent (1975).

Singhas and Dent (1975) postulated a role for thyroxine in the formation and regression of nuptial pads and in the restoration of tail height in male newts. Although the role of thyroxine in tail fin regression is apparently minimal (fig. 1), Singhas and Dent (1975) obtained maxi-

imum tail fin height restoration in newts with prolactin and testosterone only when combined with thyroxine. The mechanism by which thyroxine interacts with prolactin and testosterone to stimulate tail fin height restoration is not known. Singhas and Dent (1975) suggest only a passive role for thyroxine (i.e., molt stimulation) in nuptial pad loss and restoration.

Prolactin enhanced limb regeneration of both male and female newts in the present study. Others have reported similar positive effects of prolactin in both intact newts (Waterman 1965) and hypophysectomized newts (Connelly *et al* 1968, Tassava 1969). Seasonal variations in rates of limb regeneration, interestingly, do not correlate exactly with tail fin height maxima and minima (Schauble 1972). While regeneration rate is high in the fall and spring and low in the summer, rates are also low in the winter when tail fin height is maximum and prolactin secretion is presumably high. Singhas and Dent (1975) suggest that this discrepancy may be because of other hormones operative in winter (see also Vethamany-Globus and Liversage 1973).

It has long been known that red eft (the land stage of the newt) will migrate to water when exogenous prolactin is given (reviewed by Grant 1961). This migratory phenomenon has been called "water drive" or "second metamorphosis" (Chadwick 1940, Wald 1958). These behavioral effects of prolactin may somehow involve sodium and water balance, possibly via integumentary changes (Dent 1975). One might predict, therefore, that adult aquatic newts would resume a land habitat under conditions of low prolactin secretion. In fact, the results of the present study show this to be true. When newts are maintained at room temperature in aquaria with both land and water habitats available, not only does male tail height decrease but both male and female newts move to a land habitat (fig. 2). It is of interest that thyroxine enhances land habitat choice whereas newts given prolactin alone or a combination of prolactin and thyroxine remain in the water (fig. 2). Grant and Cooper (1965) examined hab-

itat choice of newts but did not use the combination prolactin-thyroxine treatment. Also, their control newts, which were maintained in Ringer's solution, remained in the water whereas control newts in the present study, which used aerated tap water, preferred a land habitat after the first week. The reasons for this difference are not known. Little quantitative data are available on thyroxine secretion by newts in relation to seasons or temperature (Tassava and Lorscheider 1972), and thus, whether thyroxine or prolactin is the influential hormone on newt behavior is not clear. For example, newts which resume a land habitat may do so because prolactin secretion decreases thus reducing the peripheral inhibition of thyroxine action (fig. 2). Likewise, thyroxine, when given with prolactin (fig. 2), may have little effect because of peripheral inhibition of prolactin on thyroxine action (Etkin 1968).

It is clear from the present results and from earlier findings, that prolactin affects tail fin height, regeneration and behavior of spotted newts (Singhas and Dent 1975, Tassava 1969, Grant and Cooper 1965). The mechanisms by which prolactin, and possibly also thyroxine, affect these phenomena, remain to be elucidated.

Acknowledgment. This work was supported, in part, by grant PCM 76-11807 from the National Science Foundation.

LITERATURE CITED

- Chadwick, C. S. 1940 Identity of prolactin with water drive factor in *Triturus viridescens*. Proc. Soc. Exp. Biol. Med. 45: 335-337.
- Connelly, T. G., R. Tassava and C. S. Thornton 1968 Survival and limb regeneration of prolactin treated hypophysectomized newts. J. Morphol. 126: 365-371.
- Dent, J. N. 1975 Integumentary effects of prolactin in the lower vertebrates. Amer. Zool. 15: 923-935.
- Etkin, W. 1968 Hormonal control of amphibian metamorphosis. p. 313-348. In: W. Etkin and L. I. Gilbert (eds.) Metamorphosis. Appleton-Century Crofts, New York.
- Grant, W. C. 1961 Special aspects of the metamorphic process: second metamorphosis. Amer. Zool. 1: 163-171.
- and G. Cooper 1965 Behavioral and integumentary changes associated with induced metamorphosis in *Diemictylus*. Biol. Bull. 129: 510-522.
- and J. A. Grant 1958 Water drive studies on hypophysectomized efts of *Diemictylus viridescens*. Part I. The role of the lactogenic hormone. Biol. Bull. 114-1-9.

- Hay, E. D. 1956 Effects of thyroxine on limb regeneration in the newt *Triturus viridescens*. Bull. Johns Hopkins Hosp. 99: 262-285.
- Schauble, M. K. 1972 Seasonal variation of newt forelimb regeneration under controlled environmental conditions. J. Exp. Zool. 181: 281-286.
- Singhas, C. A. and J. N. Dent 1975 Hormonal control of the tail fin and of the nuptial pads in the male red-spotted newt. Gen. Comp. Endocrinol. 26: 382-393.
- Tank, P. W., B. M. Carlson and T. G. Connelly 1976 A staging system for forelimb regeneration in the axolotl, *Ambystoma mexicanum*. J. Morphol. 150: 117-128.
- Tassava, R. A. 1969 Hormonal and nutritional requirements for limb regeneration and survival of adult newts. J. Exp. Zool. 170: 33-54.
- and F. L. Lorscheider 1972 ^{131}I incorporation into thyroids of newts. Amer. Zool. 12: 671.
- Vellano, C., V. Mazzi and M. Sacrdote 1970 Tailheight, a prolactin-dependent ambisexual character in the newt (*Triturus cristatus carnifex* Laur.). Gen. Comp. Endocrinol. 14: 535-541.
- Vethamany-Globus, S. and R. Liversage 1973 *In vitro* studies of the influence of hormones on tail regeneration in adult *Diemictylus viridescens*. J. Embryol. Exp. Morphol. 30: 397-413.
- Wald, G. 1958 The significance of vertebrate metamorphosis. Science 128: 1481-1490.
- Waterman, A. J. 1965 Prolactin and regeneration of the forelimbs of the newt. Amer. Zool. 5: 237.
-