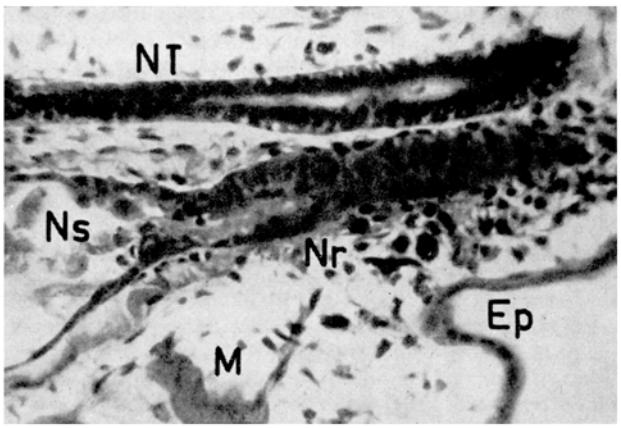

Fig. 2. Sagittal section of the proximal end of an isolated tail tip (7 mm), with beginning regeneration, 5 days after amputation. Ep = epidermis, Nr = regenerated notochord, Ns = notochord of the stump. For legends see Figure 1.

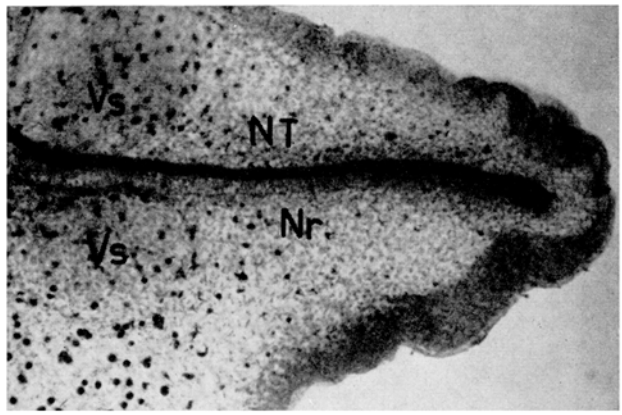

Fig. 3. Heteropolar regenerate of a tail tip (7 mm), 15 days after amputation. For legends see Figure 2.

grow out into the regenerate. So far no differentiating myoblasts have been observed in regenerates of isolated tails.

Isolated tails with abolished circulation form small heteropolar regenerates, whereas the amputated tail stumps connected with the normal stump vessels develop into rather large homopolar tail regenerates. The problem as to whether the difference in polarity actually accounts for the restricted capacity of regeneration in isolated tails needs further investigation. The completion of the culture medium by nutrient components might be decisive in assessing the maximum capacity of heteropolar regeneration in isolated tails. Since our improved method enables us to cultivate whole isolated tails, distal regeneration of tips may possibly also be obtained and compared with regeneration of vascularized stumps.

Zusammenfassung. Isolierte Schwänze von *Xenopus*-larven lassen sich in Holtfreterlösung + 0.05% Sulfothiazol mindestens einen Monat am Leben erhalten. Sie bilden trotz Abwesenheit des Blutkreislaufes ein kleines, heteropolares Regenerat, in welchem Chorda und Neuralrohr, nicht aber die Muskulatur, typische Differenzierungsleistungen zeigen.

R. HAUSER and F. E. LEHMANN

Zoologisches Institut der Universität Bern (Switzerland), November 28, 1961.

Induced Metamorphosis in Isolated Tails of *Xenopus* Larvae

In *Xenopus* larvae the involution of tail tissue during metamorphosis coincides with characteristic changes in the activity of a number of enzymes[1–4]. Since anuran metamorphosis sets in as a response to the thyroid hormone by competent larval tissues[5], the problem arises whether or not thyroxine has a direct effect on enzyme levels in responding larval tissues. In approaching this problem it was thought essential to realize first a simple larval system in which the metamorphic response could be obtained at will and with a minimum of interfering factors. By following an experimental design of SHAFFER[6], isolated tail tips of *Xenopus* larvae were found to represent a very suitable material, for they survive well in culture[7] and in addition promptly respond to thyroxine treatment. Considering the wide possibilities of this method in the experimental study of anuran metamorphosis, a short description of the procedure, as well as of the results on metamorphosis *in vitro* appears to be justified.

From *Xenopus* larvae of stage 53/54[8], tail tips, measuring 7–20 mm in length, were amputated and transferred into Holtfreter-solution, containing 0.05% sulfothiazol. The tail tips were kept individually in 5 ml of this solution, using Petri-dishes with a diameter of 3 cm. At 18°C, three days were required for wound-healing at the site of amputation, and then the healthy specimens were selected for thyroxine treatment. Metamorphosis was induced by adding thyroxine «Roche» to the culture medium at final concentrations of 1:1 million and 1:5 millions respectively. Every second day the solutions were changed.

Under these conditions, treated tail tips shrunk to half or one-third of the original length within 3–6 days of treatment, depending upon the concentration of thyroxine (Figure 1). In all cases the involution of the tail fin, beginning from the very tip, was the first detectable sign of metamorphosis. This quick response shown by isolated tails to thyroxine treatment is of particular interest, since in our experience, tails on tadpoles, exposed to similar concentrations of thyroxine, e.g. 1:5 millions, exhibit a comparable degree of reaction only after about 10 days of treatment.

As seen from sections (Figure 2) the shrinkage of treated tail tips coincides with marked histological changes in

1 R. WEBER, Exper. *13*, 153 (1957).
2 R. WEBER, Rev. suisse Zool. *64*, 326 (1957).
3 R. WEBER und B. NIEHUS, Helv. physiol. Acta *19*, 103 (1961).
4 B. NIEHUS und R. WEBER, Helv. physiol. Acta *19*, 344 (1961).
5 W. ETKIN, in B. H. WILLIER, P. A. WEISS, and V. HAMBURGER, *Analysis of Development* eds. (W. B. Saunders Co., Philadelphia 1955).
6 B. M. SHAFFER, Demonstration at the IXth Internat. Congr. for Cell Biology, St. Andrews (1957).
7 R. HAUSER and F. E. LEHMANN, Exper. *18*, 83 (1962).
8 P. D. NIEUWKOOP and J. FABER, Normal Table of *Xenopus laevis*. (North Holland Publishing Co., Amsterdam 1956).

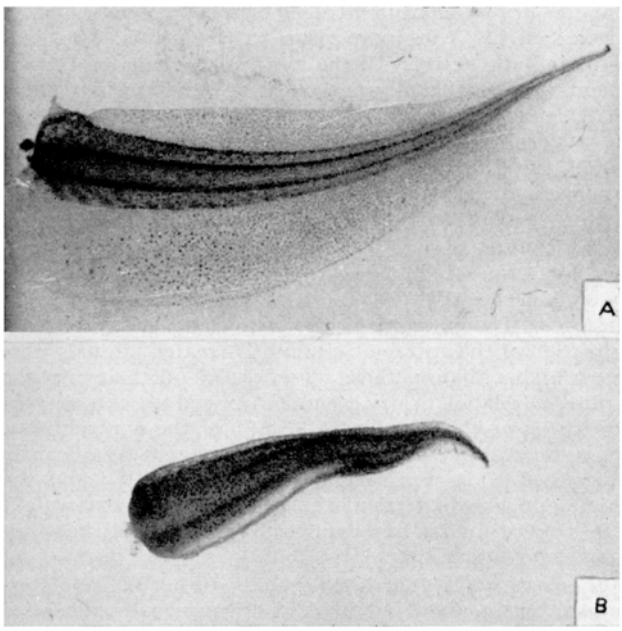

Fig. 1. Comparison of living tail tips on the 6th day of the metamorphosis experiment. A = untreated control, B = typical example of metamorphic response upon addition of thyroxine (1:5 millions). 6× actual size.

macrophages. In both cases, however, the histological pattern of the metamorphosis reaction is very similar.

These preliminary observations show that in isolated tails of *Xenopus* larvae a typical metamorphic pattern of tissue regression may be elicited by thyroxine. According to our present experience, this response is highly reproducible, and takes less time in isolated tails than in intact ones of similarly treated tadpoles. The problem as to what accounts for this delay in metamorphic reaction needs further investigation. Meanwhile the method of inducing metamorphosis in isolated larval tissues might prove useful in studies on the biochemical changes preceding the dramatic involution of larval structures during metamorphosis[9].

Zusammenfassung. Isolierte Schwanzspitzen von Xenopuslarven können durch Thyroxinbehandlung (1:1–1:5 M) zur Metamorphose veranlasst werden. Die *in vitro* auftretenden Abbauvorgänge im Schwanzgewebe sind denjenigen der normalen Metamorphose, mit Ausnahme der weniger stark entwickelten Gefässkapillaren und der fehlenden Blutzirkulation, durchaus vergleichbar. Schwanzisolate sprechen auf Thyroxin rascher an als die Schwänze von gleich behandelten intakten Larven. Isolierte Schwänze dürften sich in besonderem Masse eignen zu Untersuchungen über den Einfluss von Thyroxin auf biochemische Dedifferenzierungsvorgänge in larvalen Geweben.

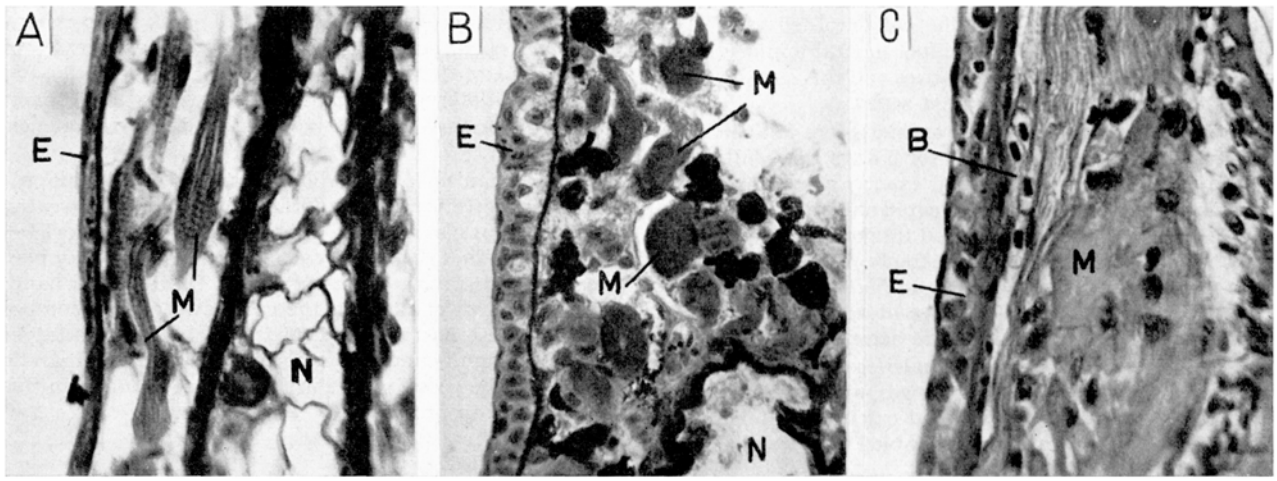

Fig. 2: Histological changes in tail structures at metamorphosis. Frontal sections of tail tips, 310×. A = Control with well developed muscle cells (M), vacuolated cells of notochord (N), thin epidermis (E) with underlying black pigment cells. B = Induced metamorphosis after 6 days of thyroxine traetment (1:5 millions). Obvious changes: thickening of epidermis (E), involution of muscle cells (M) and notochord (N), accumulation of black pigment. C = Tail undergoing metamorphosis on tadpole, early stage of tissue regression. Blood capillary (B), visible between epidermis (E) and degenerating muscle cells (M).

various tail structures, namely thickening of epidermis, migration of pigment cells, and most conspicuously the involution of the notochord, the neural tube and muscle cells. In sections of untreated controls, no such changes are noticed. Compared to tails undergoing metamorphosis on tadpoles, isolated ones are lacking a well developed system of blood capillaries and apparently contain fewer

R. Weber

Zoologisches Institut der Universität Bern (Switzerland), November 10, 1961.

[9] This work was supported by funds from the 'Swiss National Foundation for the Promotion of Scientific Research'. The author is also greatly indebted to Mr. T. Wyler for valuable technical assistance.

Tissue Lipolytic Activity in Calciferol Intoxicated Rats

In a previous paper[1] the *in vitro* inhibitory effect of high calcium and magnesium concentrations on tissue lipolytic (lipoproteinolytic) activity[2–5] was shown. The above results stimulated further work, where the effect

[1] D. Grafnetter and T. Zemplényi, Cor et Vasa *3*, 63 (1961).
[2] T. Zemplényi and D. Grafnetter, Gerontologia *3*, 55 (1959).
[3] D. Grafnetter and T. Zemplényi, Hoppe Seylers Z. *316*, 218 (1959).
[4] T. Zemplényi and D. Grafnetter, Brit. J. exp. Pathol. *40*, 312 (1959).
[5] T. Zemplényi, Z. Lojda, and D. Grafnetter, Circul. Res. *7*, 286 (1959).