disturbance lasts for the first two weeks. Riboflavin, too, at higher concentrations exerts deleterious effects on growth (Table III). The exact dosage at which riboflavin becomes toxic could not be determined because the casein used in the diet was not made free from this vitamin[2].

The present investigations reveal that some of the B vitamins at higher concentrations become toxic to *Corcyra* larva – a fact observable in many higher animals[3]. (A larger quantity of biotin also has a toxic effect on *Tribolium confusum*[4]). The phenomenon of the mode of action of these vitamins is being investigated.

Résumé. La biotine et la riboflavine, fortement concentrées, administrés à la larve de la phalène du riz, *Corcyra cephalonica*, sont toxiques. Les hautes concentrations de l'acide nicotinique n'ont des effets défavorables que sur la croissance des plus jeunes larves de l'insecte.

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Regeneration in Isolated Tails of *Xenopus* Larvae[1]

Normally regenerating tails of *Xenopus* larvae possess a good blood supply. In connection with TSCHUMI's[2] experiment which revealed a correlation between blood supply and limb development, it was of interest to investigate whether or not a similar relationship may be found in tail regeneration. The culture of isolated tail tips with abolished blood circulation proved to be the method of choice. Similar attempts to culture isolated tails of *Xenopus* larvae have already been made by SHAFFER[3] for other purposes. In the present paper, a short account of our preliminary results will be given.

From a variety of nutrient-free culture media, the best results were obtained with Holtfreter solution[4]. In order to avoid bacterial infections we later added 0.05% sulfothiazol (Geigy). Prior to amputation the larvae were thoroughly rinsed with glass distilled water, and then transferred into aqueous solution of 0.05% sulfothiazol for 24 h. The isolated tail tips were kept individually in small Petri dishes, containing 5 ml of culture medium at constant temperature (18°C). Since they were lying close to the surface of the culture medium, sufficient supply of oxygen was provided. Under these conditions it was possible to supress mortality almost completely and to keep even whole tails alive. Due to the absence of blood circulation, the supply of nutrient material is of course reduced, thus allowing isolated tails to survive only for a limited period of time. In a first experiment in which 7 mm of the tail tips of *Xenopus* larvae, measuring 30 mm in length, were amputated, the following times of survival were observed in the absence of sulfothiazol. From a total number of 12 tail tips, 3 died until the 6th day after amputation, the remaining 9 specimens survived, but showed some distortions, and the muscle tissue gradually became transparent. Between the 30th and the 44th day after amputation these tail tips died. It is likely that tissues are partially absorbed. According to our present experience, however, also whole tails, measuring 20 mm in length, survived in good condition for at least one month.

The vitality of isolated tails is best illustrated by (1) the persistence of muscular contraction, even resulting in displacement of the tails, and (2) in the formation of a *regenerate* at the (proximal) site of amputation. In isolated tail tips, measuring 7 mm in length, the maximum of regeneration is obtained on the average 15 days after amputation. The regenerate is then about 1 mm long (Figure 1A). In contrast, the tails of the tadpoles from which these tips were amputated, and which have a normal blood supply, produced within 15 days regenerates of 4.0 to 4.5 mm in length (Figure 1B). The histological analysis reveals a limited capacity of tissue regeneration in isolated tails. Thus the *epidermis* regenerates rather well, but shows irregular proliferations and vesicles at the site of amputation. In the regenerate, *notochord* as well as *neural tube* differentiate almost normally, although they are considerably reduced in size (Figure 2 and 3). In addition, apparently new *blood capillaries* may be found in the regenerate, even containing blood cells, whereas in the original tail tip these same elements are subject to degeneration. The behaviour of the *muscle* cells is still obscure, since we have no evidence as yet that myoblasts

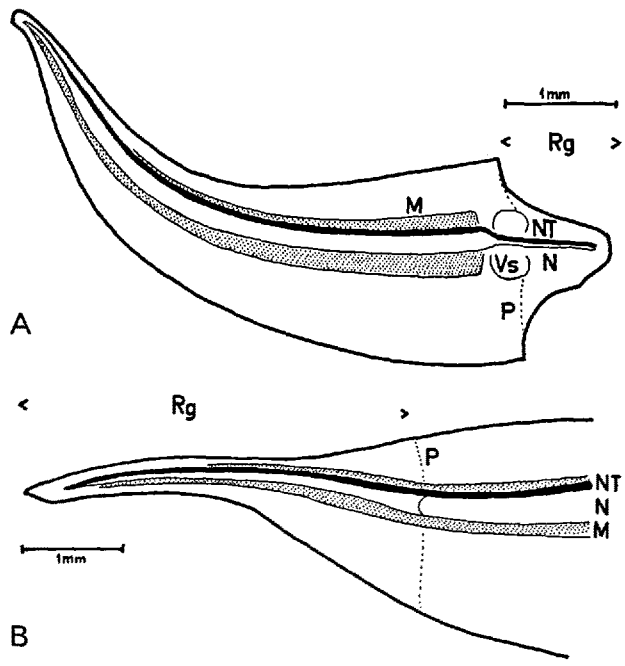

Fig. 1. (A): Isolated tail tip (7 mm), 15 days after amputation, with small heteropolar regenerate.
(B): Normal homopolar tail regeneration of a *Xenopus* larvae, 15 days after amputation (removed 7 mm).
M = muscle, N = notochord, NT = neural tube, P = pigment boundary, Rg = regenerate, Vs = vesicles.

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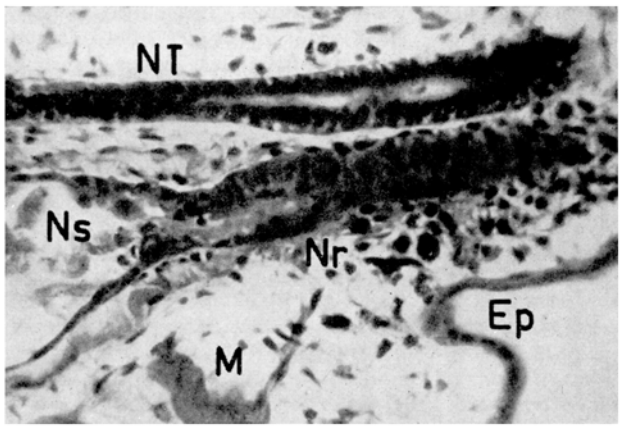

Fig. 2. Sagittal section of the proximal end of an isolated tail tip (7 mm), with beginning regeneration, 5 days after amputation. Ep = epidermis, Nr = regenerated notochord, Ns = notochord of the stump. For legends see Figure 1.

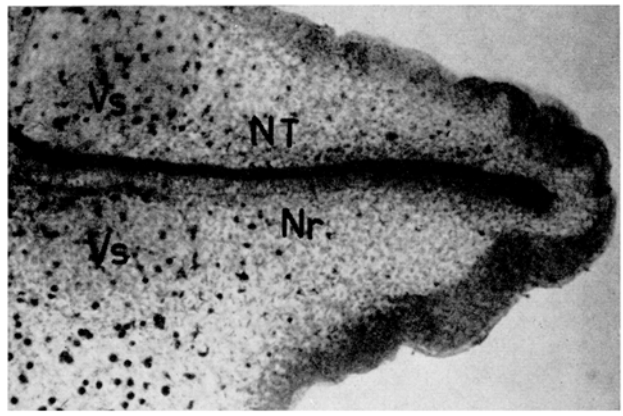

Fig. 3. Heteropolar regenerate of a tail tip (7 mm), 15 days after amputation. For legends see Figure 2.

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.

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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

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