glycolipids. These glycolipids were purified further by TLC with chloroform-methanol-water, 60:35:8. The purified lipids were then examined by continuous development for 3 h with chloroform-methanol-water (80:35:7) on TLC plates. The first spot (M) further separated into 5 spots (I–V, Figure 2) in this solvent and these lipids were isolated in amounts adequate for analysis. Analytical data on the glycolipids, as presented in the Table, suggest that all the 7 lipids were mannophosphoinositides containing varying amounts of mannose (1–6) and fatty acids (2–4)/g-atom of phosphorus. Monomannoside was the only phospholipid among the isolated lipids with a fatty acid-phosphorus ratio of 2. The mannose-containing lipids accounted for 30% of the total phospholipids of *M. 607* of which the monomannoside was the major glycolipid.

Mannophosphoinositides of *M. 607*

Phospho-lipid	No. of moles/g-atom P			Inference
	Mannose	Carboxyl ester	Inositol	
I	5.7–6.2 (4)	4.0–4.5 (3)	1.0	Hexamannoside
II	4.9–5.3 (4)	4.0–4.2 (3)	1.0	Pentamannoside
III	3.4–3.5 (4)	3.1–3.3 (3)	1.1	Tetramannoside
IV	2.8–3.0 (3)	3.5–3.9 (2)	1.2	Trimannoside
V	1.6–1.8 (2)	3.5–3.7 (2)	0.8	Dimannoside
VI	1.1–1.3 (4)	2.3 (4)	1.3	Monomannoside
VII	2.2–2.4 (4)	4.2–4.4 (4)	1.0	Dimannoside

No. of determinations are given in parentheses.

The identification of only 2 dimannosides and 1 pentamannoside in *M. 607* is in contrast with the higher number, some of which with different fatty acid-phosphorus ratios, found in BCG and *M. tuberculosis* by PANGBORN[4] and in *M. phlei* by BALLOU[7]. The dimannosides in *M. 607* differed in their mobility on TLC plates probably due to differences in the position of the fatty acids on the molecules. The extent of variation in the glycolipids due to differences between strains, and to age and conditions of growth within a particular strain, is unknown and such a study might throw some light on this aspect[14].

Zusammenfassung. Aus *Mycobacterium 607* wurden 7 Mannophosphoinositiden isoliert und durch präparative Dünnschichtchromatographie getrennt. Diese Lipide machen ca. 30% der gesamten Phosphatide des Stammes aus und setzen sich aus einem Mono-, zwei Di- und je einem Tri-, Tetra-, Penta- und Hexamannosiden zusammen. Mit Ausnahme der Monomannoside (2) haben sämtliche Mannoside mehr als 3 Fettsäuren pro Phosphor-g-Atom.

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Inhibitory and Modifying Influence of Excess of Vitamin A on Tail Regeneration in *Bufo* Tadpoles

Excess of vitamin A is known to exert a profound influence on morphogenesis and differentiation on embryonic and adult tissues both in vivo and in vitro[1–5]. This communication reports the results of a study on the influence of excessive vitamin A in the medium on tail regeneration in tadpoles of *Bufo andersonii* Bouleng.

Appropriate quantities of Arrovit drops (Vitamin A palmitate, Roche, India), dissolved in a little ethanol, were added to tap water to prepare vitamin A solutions of 1, 2.5, 5, 10, 15, 20 and 30 I.U./ml concentration. The experimental tadpoles were immersed in these solutions immediately after amputation of the tail across the middle. They were maintained in these solutions either for the entire duration of the experiment or for 1 day only after which they were transferred to conditions similar to those of controls. A small quantity of ethanol was added to the water in which controls were kept. The animals were fed boiled spinach regularly and their medium was changed every 2 days. The experiments were made at room temperature during July–September.

Apart from greater mortality and retardation in general body growth in vitamin A treated tadpoles their regenerated tails differed from those in controls in several respects (Figure a–d). The length of the tail regenerated in experimental tadpoles was much less than in controls; it decreased progressively with rise in vitamin A concentration in the medium. Thus, while the regenerated tail in controls attained a length of 2.95–4.7 mm in 9 days the animals immersed in 1 I.U./ml vitamin solution for all this period regenerated tail lengths of 1.8–2.95 mm only. In solutions of 20 and 30 I.U./ml strength, no regenerate attained a length of more than 1 mm even in 12 days. Generally, the tadpoles treated with vitamin A solutions for 1 day only showed greater regeneration than their fellows kept in these solutions for the entire period of the experiment. The difference was, however, small in concentrations below 5 and above 10 I.U./ml. The small regenerates in tadpoles kept in high vitamin A concentrations also appeared to be deficient in pigmentation.

Regeneration of the axial tissues was affected particularly severely and none of the experimental tadpoles regenerated them as well as the controls. There was some regeneration of these tissues in animals treated with 1, 2.5 and 5 I.U./ml vitamin A solutions, those maintained in the last mentioned concentration showing the least amount of growth. The tadpoles kept in these concentrations of vitamin A for only 1 day showed relatively better

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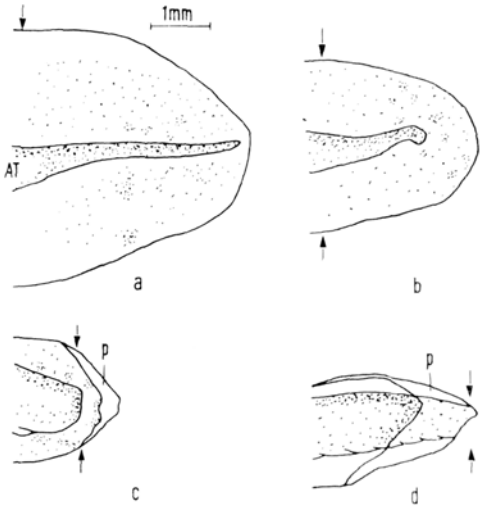
growth of the axial tissues than their fellows kept in vitamin solutions for the entire duration of the experiment. In concentrations of 10 I.U./ml and above, however, regeneration of the axial tissues was almost completely suppressed irrespective of the duration of treatment (Figure b and d). When these tissues did regenerate to some extent they frequently grew in a postero-dorsal or postero-ventral direction in the regenerating tail.

The caudal margin of the regenerating tail-fin in experimental tadpoles often became folded to the right or left side early after amputation. The folding spread anteriorly along the dorsal and ventral margins of the tail-fin ultimately forming a distinct pocket of fin-tissue at the regenerating end (Figure c and d). In tadpoles kept in vitamin A solutions for the entire duration of the experiment frequency of such a fin-pocket formation was nearly 0% in 1 I.U./ml solution, over 30% in 2.5 and almost 100% in concentrations of 5 or more I.U./ml. In animals given this treatment for only the first day after amputation the frequency of fin-pocket formation rose from nearly 0% in 1 and 2.5 I.U./ml solutions to over 70% in 20 and 30 I.U./ml vitamin A concentrations.



Camera lucida drawings of tail regenerates of *Bufo* tadpoles. (a) 9-day-old control; (b) 9-day-old experimental, maintained in 2.5 I.U./ml vitamin A solution for all 9 days; (c) 5-day-old experimental, kept in 5 I.U./ml vitamin A solution for only the first day after amputation; (d) 6-day-old experimental, kept in 30 I.U./ml vitamin A solution for only the first day after amputation. AT, axial tissues; P, fin-pocket formed at the regenerating end. Arrows indicate level of amputation.

The results clearly indicate that excess of vitamin A in the medium exerts a strong inhibitory influence on tail regeneration in *Bufo* tadpoles. The degree of inhibition depends upon concentration of the vitamin in the medium and duration of treatment. Since, subjection to even low concentrations for only 1 day after amputation produced visible retardation and in high concentrations even such a short treatment completely inhibited regeneration of at least some tissues the influence of the vitamin must have operated on the early stages of regenerative development including the phases of demolition and dedifferentiation. The suggestion that vitamin A probably acts by liberating cathepsin-like proteases from intracellular lysosomes[2,5] may be relevant in explaining our results for enhanced activation of cathepsins is said to retard or inhibit regeneration[6]. An adverse effect on cell division[2,3,7] may have also been involved. It must, however, be noted that in the experimental animals regeneration was not merely retarded or inhibited but there was also a marked change in morphogenesis of the tail-fin which formed a peculiar fin-pocket with a remarkably high frequency.

In mammalian embryos hypervitaminosis A is reported to cause serious alterations in presomitic mesoderm leading to various somitic malformations[4]. Preliminary histological observations made during the present study also indicate particularly defective muscle regeneration with regards to the quantity, distribution and segmentation of this tissue in tail regenerates of vitamin A treated tadpoles. Perhaps some tissues are affected more than others by vitamin A.

Zusammenfassung. Bei Zugabe von Vitamin A zum Zuchtwasser von Krötenlarven wird die Schwanzregeneration unterbunden. Ausserdem kommt es zu morphologischen Veränderungen (Taschenbildungen).

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Reproduction in Urodeles II. Observations on the Spermatheca[1]

Previous reports on the reproductive habits of the urodeles have noted that the sperm are transferred via a spermatophore and are then stored in a spermatheca[2]. Observations on the spermatophore of *Triturus viridescens* show that the sperm are in an inactive state, probably in preparation for storage[3]. The spermatheca of the female newt, *Triturus*, is seen to contain sperm at all times of the year, however, the greatest accumulations are seen during the spring in the true mating season and in the autumn during the false breeding season[4,5]. Females maintained in the laboratory over the winter with no contact with males can be induced to lay viable eggs by pituitary or chorionic gonadotropin injection. Embryos developing from such eggs give ample evidence that the stored sperm are indeed capable of fertilization.

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