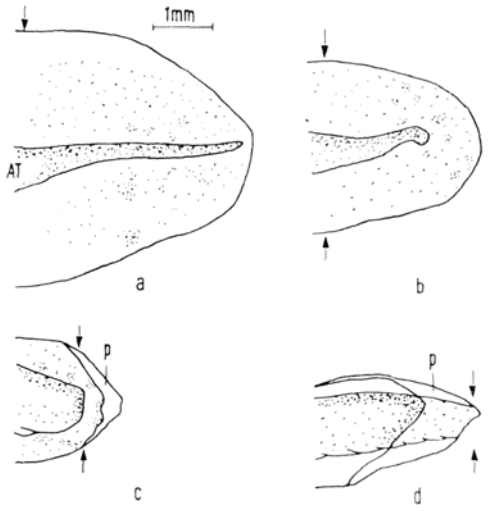
growth of the axial tissues than their fellows kept in vitamin solutions for the entire duration of the experiment. In concentrations of 10 I.U./ml and above, however, regeneration of the axial tissues was almost completely suppressed irrespective of the duration of treatment (Figure b and d). When these 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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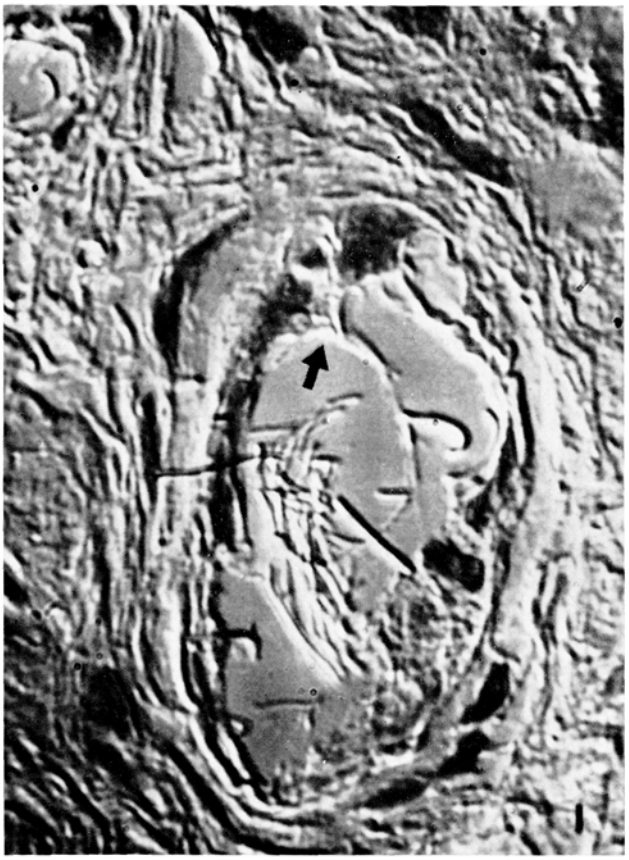
It is the intent of this report to present an observation that may shed light on one possible mechanism involved in sperm storage.

Methods. Female newts were killed by decapitation following anesthetization with MS-222 (Sandoz) and their cloacal regions were fixed in either 10% neutral buffered formalin or Bouin's fluid. These tissues were processed in the usual manner to obtain paraffin sections and the sections were stained with azure A (0.1% in 30% ethanol) or in a Feulgen-Naphthol yellow sequence.



Section through a spermathecal tubule containing several spermatozoa. The arrow indicates the site of sperm-epithelial cell contact. × 500.

Observations. It was seen that the sperm were accumulated in the proximal ends of the spermathecal tubules and were, for the most part, highly coiled. In order to study the positioning of the sperm in the tubules it was necessary to use animals which had only a small amount of sperm since it was rather difficult to trace the sperm from section to section when the concentration was high.

Within the spermathecal tubules the sperm heads appeared to be in contact with the epithelium (Figure) tubules. This condition was seen in animals killed at all seasons of the year. With the light microscope, the contact appeared to be in the region of the acrosome, however, a definitive statement on the sperm-epithelial cell contact must await further investigation with the electron microscope.

Discussion. An observation of the type reported is very suggestive of a nutritive or maintanance role on the part of the spermathecal epithelium. Although the sperm appear inhibited in the spermatophore it is doubtful that the polysaccharide material thought to be responsible for this inhibition could keep them in an inactive yet viable condition for a period of several months[6]. Histochemical studies on the spermatheca reveal that none of the polysaccharides of the spermatophore persist. Therefore, it is not unreasonable to suggest that the sperm of the urodele are maintained by the tubule epithelium in a relationship similar to that which exists between the Sertoli cells and sperm of the mammalian testis.

Zusammenfassung. Die Spermatheca des Weibchens zeigt bei den untersuchten Urodelen eine innige Beziehung zu den Spermien, so dass eine Ernährungsfunktion der Spermatheca wahrscheinlich gemacht werden kann. Es wird auf die Sertoli-Zellen der Mammalia verwiesen.

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The Cytochemical Demonstration of Dehydrogenases and Oxidases in the Cells of Fungi

In human pathology, methods for the intracellular detection of dehydrogenases and oxidases by means of more or less specific staining procedures have been developed and successfully used in the study of many organs. Extended investigations have shown that some of these methods are useful for the demonstration of enzymes in the fungi *Neurospora crassa, Oospora lactis* and *Saccharomyces cerevisiae.*

(1) *Dehydrogenases.* Dehydrogenases can be demonstrated with tetrazolium salts as indicators that are reduced to more or less insoluble, intensively coloured formazan granula[1]. Out of a great number of available tetrazolium salts, whose usefulness for fungi has been studied, Nitro-BT proved to be the best indicator[2]: after an incubation period of 30 min, optimal for nearly all investigated enzymes, the cells contained blue-black formazan deposits that were equally distributed in the nearly colourless cytoplasm. MTT, frequently used in medical histochemistry, was unsuitable for the detection of several dehydrogenases because the solution of $CoCl_2$, necessary for the stabilization of the formazan, proved to be toxic for many fungal enzymes.

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