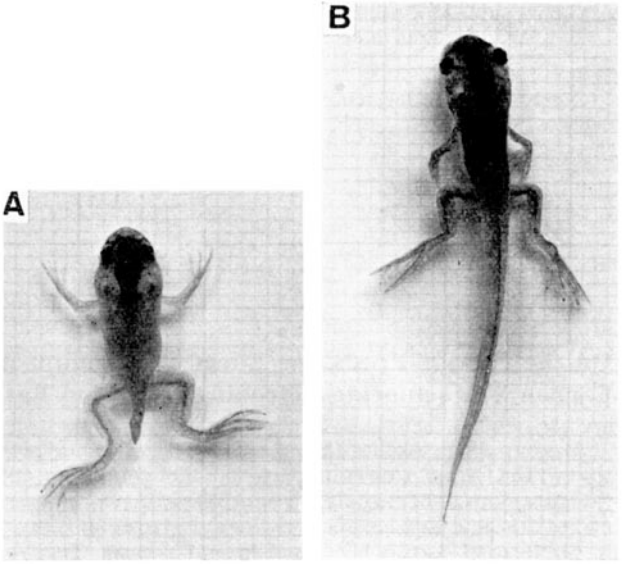
Inhibitory Effect of Actinomycin D on Tail Atrophy in *Xenopus* Larvae at Metamorphosis[1]

The involution of the tail in metamorphosing tadpoles represents a striking example of physiological tissue regression which like the other events of metamorphosis is known to be under the control of thyroid hormones. At the biochemical level, tail atrophy has been found to coincide with a marked increase in the activities (expressed per tail) of cathepsins[2,3], acid phosphatase[4] and other acid hydrolases[5], which are apparently involved in tissue destruction. Histochemical methods revealed a progressive accumulation of acid hydrolases by macrophages, probably in response to the initiation of phagocytosis in the regressing tail tissue[6,7]. From these and other findings it was concluded that the increase in activity of acid hydrolases in the regressing tail of the tadpole might reflect the *synthesis* of such enzymes by activated macrophages[8], rather than their *release* from preformed cytoplasmic constituents – a mechanism of tissue involution originally proposed by the 'lysosome' theory[9].

In order to elucidate further the mechanism of tissue involution in relation to metamorphic stimuli, the application of *actinomycin*[10] appeared of particular interest. This compound, which has been reported to inhibit specifically DNA-dependent synthesis of RNA should, therefore, interfere with enzyme synthesis by macrophages, but not with the release of preformed enzymes in the regressing tissue. Preliminary observations on the effect of Actinomycin D on metamorphosing *Xenopus* larvae, especially on the activity of cathepsins in the tail tissue, will be reported below.

Actinomycin D (AMD) was administered to *Xenopus* larvae at the onset of the 'metamorphic climax', as indicated by the eruption of the forelimbs. As shown in Table I, the immersion method proved to be much less effective than intraperitoneal injection of AMD. With doses of 0.1 μg per animal the survival time was sufficiently long so that the inhibition of metamorphosis was clearly discernible. According to our present experience, AMD-treated larvae exhibit a considerable delay in the transformation of the head (gill region) and the intestine, as well as in the involution of the tail (Figure). At higher doses, however, the survival time is considerably reduced, the most conspicuous effect of AMD being characteristic malformations in fore- and hindlimbs. It may be mentioned that the teratogenic action of actinomycins has been reported repeatedly since the original observation by TUCHMANN-DUPLESSIS and MERCIER-PAROT[11] on rat embryos.

The obvious inhibition of tail atrophy by moderate doses of AMD has made it possible to assay acid hydrolases under the condition of delayed histolysis. Since earlier work has shown cathepsins to increase considerably in regressing tail tissue[2,3], this enzyme system appeared most promising for exploring the effects of AMD on acid hydrolases. Table II contains the results of these experiments in which catheptic activity was determined on tail homogenates by our standard procedure[12]. It is noted that in tails (reduced to approximately half the original length) of normally metamorphosing tadpoles (controls) there is a much higher activity of cathepsins per unit total nitrogen (TN) than in tails of AMD-treated larvae. Since in all experiments larvae of equal size were used, the total activities (per tail) may be regarded as an equally meaningful criterion. In fact, these data unequivocally reveal greatly reduced levels of cathepsins in the tail tissue of



Effect of Actinomycin D on metamorphosis. A: Control, 7 days after the eruption of the forelimbs. B: Tadpole, same age as A, but injected with 0.1 μg Actinomycin D. Inhibition of metamorphic transformation indicated by larval appearance of head region and persistance of tail. Besides slight teratogenic effects are noticed in hind- and forelimbs.

Table I. Effect of Actinomycin D on survival and progress of metamorphosis in *Xenopus* larvae

Mode of administration	AMD	N	Mean survival time (days)	Inhibition of metamorphosis
Immersion	5 μg/ml[a]	3	10.3	0
	10 μg/ml[a]	4	5.6	+
Injection	0.1 μg[b]	10	ca. 7	++
	0.2 μg[b]	10	ca. 5	+, teratogenic effects
	0.5 μg[b]	2	4	+, teratogenic effects

[a] Solutions made up in tap water at this concentration. [b] Dose per animal, injected in 5 μl Holtfreter solution. N = Number of animals.

[1] This work was supported by a grant from the Schweiz. Nationalfonds zur Förderung der wissenschaftlichen Forschung (Project 2546).

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AMD-treated tadpoles. From the close agreement of the values obtained, the inhibitory effect of AMD on the accumulation of cathepsins in the tails of prometamorphic tadpoles appears to be highly reproducible.

On the basis of this evidence it is concluded that cathepsins are, indeed, functionally related to histolysis and that the accumulation of these enzymes in the regressing tail must be due to neosynthesis. Further work on the mechanism of action of AMD, particularly in relation to macrophages, is now in progress[13,14].

Table II. Effect of Actinomycin D on catheptic activity in tail tissue

Experiment		Days after AMD injection	AMD µg/animal	Specific activity µg casein/h/µg TN	Ratio C/T	Total activity µg casein/h/tail	Ratio C/T
1	C	5	–	30.77	13.6	2981.6	3.82
	T	5	0.2	2.26		781.1	
2	C	7	–	45.38	12.5	2913.8	3.82
	T	7	0.1	3.63		763.4	
3	C	7	–	32.88	13.5	3224.2	3.48
	T	7	0.1	2.44		924.4	
			Mean ratio C/T		13.2		3.71

C = Controls, undergoing normal metamorphosis. T = AMD-treated tadpoles.

Zusammenfassung. *Xenopus*larven, denen zu Beginn der «Metamorphoseklimax» 0,1 γ Actinomycin D intraperitoneal injiziert wurden, zeigten eine erhebliche Verzögerung der Metamorphose. Biochemisch ist die Hemmung der Schwanzresorption bei behandelten Larven durch die geringe Aktivität der Gewebekathepsine gekennzeichnet.

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[13] The author is greatly indebted to the Merck Sharp & Dohme Research Laboratories, Rahway (N.J. USA) for the generous supply of Actinomycin D. The able technical assistance of Mr. T. WYLER is also gratefully acknowledged.

[14] Note added in proof: Further experiments have shown that the inhibitory effect of AMD is not abolished by the exposure of the injected tadpoles to L-thyroxine (1:50 million). This indicates that AMD exerts its action by suppressing the responding capacity of the larval tissues.

On the Hepato-Protective Effect of Selenium in Carbon Tetrachloride Poisoning in Albino Rats

A group of researches (SCHWARTZ et al.[1,2]) established that in the liver of rats submitted to a diet containing sufficient amounts of proteins, vitamins, salts and calories, necrotic lesions appear in cases where the protein is given in the form of certain species of brewer's yeast.

According to the findings of SCHWARTZ, in certain yeasts this hepato-protective factor is present, while in others it is lacking. In the yeast extracts showing a hepato-protective effect, it was possible to demonstrate the presence of a selenium compound.

The lack of selenium causes various morphological modifications, varying from one species of animal to another. In rats the effect assumes the form of hepatic necroses. The hepato-cellular modifications show a great variety, from hydropic aspects or those of fatty degeneration up to symptoms suggesting the presence of hepato-cellular necroses. Several authors[3–9] have studied the effect produced by the lack of selenium. The object of this paper is to investigate the effect produced by the administration of small doses of selenium upon the course of acute carbon tetrachloride poisoning.

Our experiments were made on albino rats of both sexes, weighing between 120–150 g. The animals were divided into 4 groups, each consisting of 30 rats. The animals were kept on a semi-synthetic diet, poor in selenium, while the controls were given the customary non-treated diet. The selenium was given per os in the form of sodium selenite dissolved in distilled water.

Group I. During the first 10 days of our experiments, each animal was given intraperitoneally 0.1 ml of a mixture of carbon tetrachloride and sunflower oil in the proportion of 1:1. Following the carbon tetrachloride poisoning, the rats received per os a daily dose of 1 γ selenium/100 g body weight, in the form of sodium selenite, for 21 days.

Group II. The animals were submitted to intoxication during 10 days with a method similar to that used in the case of group I with carbon tetrachloride, but simultaneously we also initiated the administration of a daily dose of 1 γ selenium/100 g body weight. The administration of selenium lasted 31 days, i.e. another 21 days following carbon tetrachloride poisoning.

Group III. During the first 21 days, the animals received a daily dose of 1 γ selenium/100 g body weight. Then, starting with the 21st day, in addition to selenium, the animals were also given carbon tetrachloride, using the same method as the one applied in group I.

Group IV. The animals received the customary diet to serve as controls. After completion of the experiments, the animals were sacrificed.

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