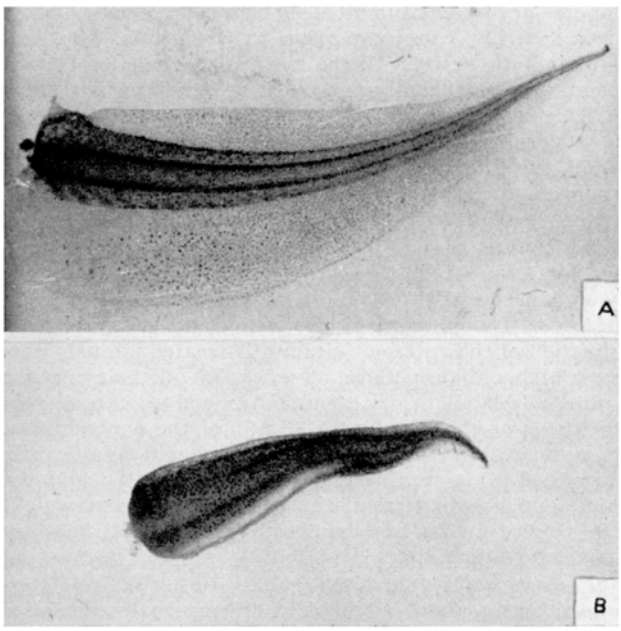

Fig. 1. Comparison of living tail tips on the 6th day of the metamorphosis experiment. A = untreated control, B = typical example of metamorphic response upon addition of thyroxine (1:5 millions). 6× actual size.

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.

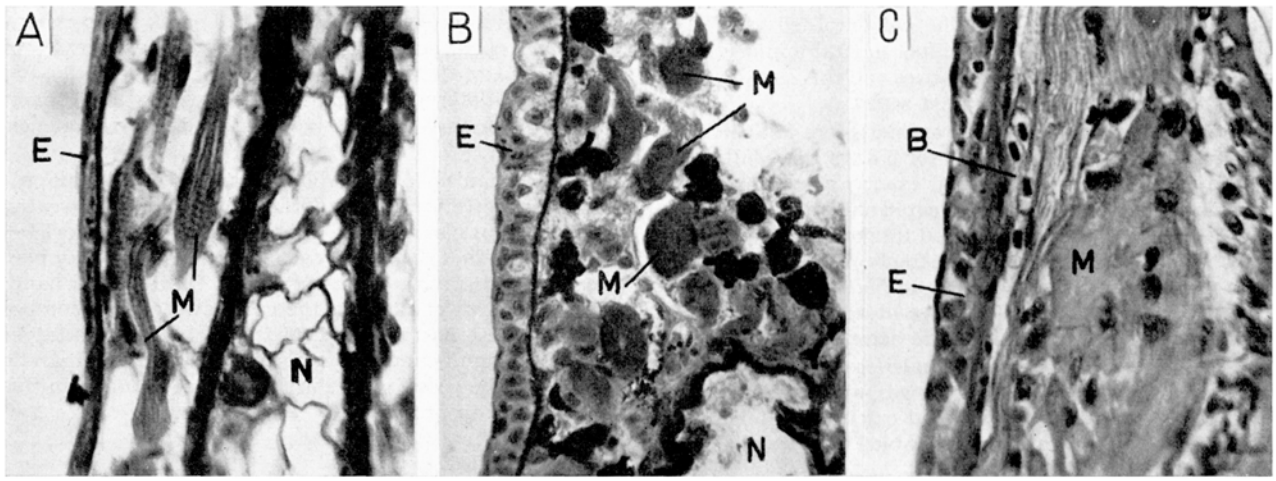

Fig. 2: Histological changes in tail structures at metamorphosis. Frontal sections of tail tips, 310×. A = Control with well developed muscle cells (M), vacuolated cells of notochord (N), thin epidermis (E) with underlying black pigment cells. B = Induced metamorphosis after 6 days of thyroxine traetment (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

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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).
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[3] D. Grafnetter and T. Zemplényi, Hoppe Seylers Z. *316*, 218 (1959).
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of mineralization, following calciferol feeding, on the lipolytic activity was studied.

Materials and Methods. Adult white male rats, maintained on normal laboratory diet, were used. Vitamin D in oil (Calciferol Spofa) was fed to unanaesthetized rats (by means of a syringe with a short polyethylene tubing attached). Aortae, carefully freed of periaortal tissue, were dissected longitudinally and incubated at 37°C with a lipaemic substrate for 90 min at pH 7.6. In some experiments, heart breis were incubated under the same conditions. Lipolytic activity was assayed by titration[6] of fatty acids released from the substrate, i. e. from human lipaemic serum diluted 1:1 with NH_4Cl-NH_3 buffer. All vessels were shaken constantly during the incubation.

Results. Three experimental series were performed. Macroscopically all the effects of hypervitaminosis D as described by GILLMAN *et al.*[7] could be observed.

Series A. A dose of 30 000 u. of Calciferol per day was fed for 6 days to experimental animals. Control rats were maintained on normal food intake, while the experimental animals refused food after the 4th day of calciferol feeding. All the animals were killed the day after the last vitamin D administration. In comparison with the control rats it was evident that the treated animals were starved. Macroscopically, no definite changes of the aortae could be observed. There was no difference in the lipoproteinolytic activity of the aortae or hearts between the two groups, either on a wet weight or fat-free dry weight basis. The free fatty acid content of serum and epididymal fat was markedly increased in the calciferol-fed rats. It is obvious that starving of the experimental animals in all probability interferred in this series and this circumstance had to be eliminated in the next series.

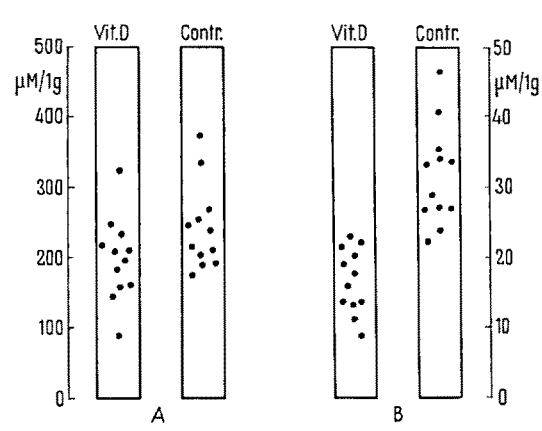
Series B. The experimental animals were fed calciferol in the same way as in series A for 5 days. The daily food intake of controls was adjusted exactly according to the consumption of the calciferol-treated animals, to eliminate the effect of the diminished food intake of the latter animals. After normalization of the food consumption, rats of both groups were allowed to eat *ad libitum*. The rats were killed 15 days after the last dose of vitamin D, all food having been removed 15 h before decapitation.

Most of the aortae of the calciferol-treated animals revealed heavy macroscopic changes, and a loss of elasticity, their chemically determined calcium content being increased. Despite an almost doubled wet weight, their fat-free dry weight and nitrogen content was only slightly decreased (2%) in comparison with controls. The lipoproteinolytic activity of the aortae after calciferol treatment was significantly decreased on a wet weight ($P < 0.01$), dry weight ($P < 0.01$), fat free dry weight ($P < 0.001$) or nitrogen content ($P < 0.05$) basis. On the other hand, the lipolytic activity of heart tissue remained unchanged. An elevation of about 20% in the serum cholesterol level of Vitamin D treated rats was also observed.

Series C. A dose of 45 000 u. per day of calciferol was fed for 7 days to the experimental animals in the same way as in series B, the rats being killed 21 days after the last dose. The macroscopically visible changes and mineralization of the aortae of vitamin D-treated animals were even higher than in those of series B, and their nitrogen content (related to dry weight) dropped on the average to 9.3%, as compared with 13.8% of the controls. The lipolytic activity of these aortae was again significantly decreased on a wet weight basis ($P < 0.001$), and the same applies when calculating the results on dry weight ($P < 0.001$) or fat free dry weight ($P < 0.001$) basis, or nitrogen content basis ($P < 0.05$).

Discussion. The lipolytic activity of aortae, as determined by the above methods, is unequivocally decreased in vitamin D fed animals after a delay of 2 or 3 weeks following the last dose of calciferol. The question arises whether the increase of extracellular material in the diseased aortae may not interfer with the lipolytic activity, probably associated with the cells of the vessel wall. This seems to be improbable in the light of our further experiments in collaboration with LOJDA and MRHOVÁ[8], in which a very definite decrease of histochemically and chemically determined non-specific esterase activity of aortae was found, irrespective of their desoxyribonucleic acid content.

The mechanism of the above decrease of the lipoproteinolytic activity in the aortae of vitamin D treated animals is not clear. In the light of our previous work[1-5], it is possible that disturbed calcium metabolism may play a decisive role in this decrease as well. On the other hand, the well known changes in the connective tissue components of these aortae could also be of great and as yet un-known significance, in connection with the lipolytic activity of the vessel wall. Further detailed studies in this direction are in progress.



Lipolytic activity of aortic tissue in control and vitamin D treated rats (series C). Activity expressed in μM of fatty acids released per 1 g of: (A) nitrogen of mean fat free dry weight. (B): postincubation dry weight.

Zusammenfassung. Nach Calciferolzufuhr verkleinerte sich die lipolytische Aktivität der Rattenaorta, während die Aktivität des Herzgewebes unverändert blieb, wobei das Serumcholesterol anstieg. Diese Ergebnisse werden im Zusammenhang mit der Pathogenesis der Atherosklerosis diskutiert.

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