Inhibition of Lens Regeneration in the Eye of the Adult Newt, *Diemictylus viridescens*[1]

Numerous experiments have demonstrated that, in certain Urodeles, a new lens forms from iris tissue after removal of the original lens from the eye (STONE[2]). Several investigators have shown that reimplanted lenses inhibit lens regeneration (FROST[3], REYER[4]). It has also been found that at least two fractions of electrophoretically separated lens proteins are capable of inhibiting lens regeneration (SMITH[5]). It has been reported that when chick lens extract is tested against rabbit chick-lens antiserum by double diffusion in agar gel three precipitin lines are formed which can be identified with precipitin lines formed with isolated α-, β- and γ-crystallins (MAISEL and LANGMAN[6]). These authors also reported that amphibian (frog) lens extract tested with the chick lens antiserum formed only 2 precipitin lines. These lines showed partial identity with the α- and γ-crystallin precipitin lines of the chick. The amphibian lens apparently does not contain an antigen comparable to the β-crystallin of the chick lens. The present study was undertaken to discover whether this antigenic difference extended to the newt and if so whether it had any significance for the suppression of lens regeneration in this species.

Rabbits were immunized against chick lens extract in order to obtain lens antiserum. The lens antiserum was tested against chick lens extract and against newt lens extract by the accepted method of immunoelectrophoresis in agar gel. The results obtained (Figure 1) confirm that the α- and γ-crystallins of the lenses of both species are antigenically similar, although the γ-crystallin arc formed by the newt lens extract is weaker than that of the chick lens extract, and that there is no β-crystallin arc formed between newt lens extract and chick lens antiserum.

Both chick lens extract and newt lens extract were separated electrophoretically on agar, stained with Amidol Black-10-B, and the regions corresponding to the α-, β- and γ-crystallins identified. Plugs of agar about 1 mm³ were removed from the β-crystallin areas of companion slabs and inserted in lentectomized eyes of adult newts.

The lenses of the right eyes of 20 healthy adult newts were extirpated as follows. The animals were anesthetized in MS 222 and placed on gauze in open petri dishes containing 10% Holtfreter's solution. The cornea was slit with a fragment of razor blade in a holder and the lens forced out by pressure with fine forceps. Care was taken not to injure the iris. In 10 animals the lens was replaced by a plug of agar containing chick β-crystallin and in 10 others with a plug of agar containing newt β-crystallin. On the 10th day the original plugs of agar were removed from the eyes and fresh plugs inserted. On the 20th day the animals were sacrificed and the operated eyes were fixed in Bouin fluid, embedded in paraffin, sectioned, and stained in H and E. Throughout the experiment the animals were maintained in tap water at 21 °C.

Histological examination of the eyes revealed that in 8 of the 10 lentectomized eyes bearing newt lens β-crystallin in agar gel no new lenses were regenerated from the iris, and in 2 eyes there was a poor beginning of lens regeneration. However, in all of the 10 eyes bearing chick lens β-crystallin in agar new lenses were regenerated from the iris (Figure 2). It is obvious that the inhibition was not caused by the mechanical presence of the agar plug (see also SMITH[5]).

Although FROST[3] demonstrated that there are quantitative differences in the inhibitory action of regenerating lenses of different ages inserted in lentectomized newt

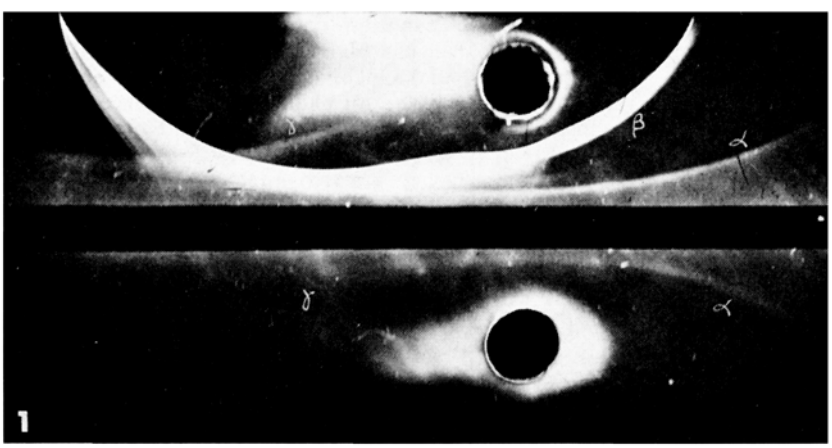

Fig. 1. Immunoelectrophoretic comparison of chick and newt lens extracts by means of chick lens antiserum. Upper well, chick lens extract; lower well, newt lens extract.

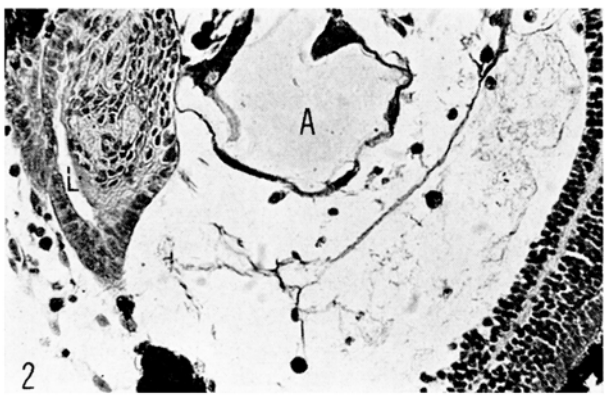

Fig. 2. Section of an adult newt eye 20 days after extirpation of lens and containing an agar plug of chick β-crystallin. L, regenerated ens; A, agar plug of chick β-crystallin.

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eyes he could find no evidence of any qualitative differences. It was his opinion that the existence of any inhibitory substance of lenticular origin remained speculative. Subsequently, SMITH[5] found that of 7 fractions obtained by electrophoretic separation of newt lens proteins on starch gel at least 2 fractions inhibited lens regeneration and at least 1 fraction stimulated lens regeneration. It is of interest, therefore, to note that the significant difference between newt lens extract and chick lens extract is in the antigenic nature of the β-crystallins which seem to be class-specific. This is in complete agreement with the observation of MAISEL and LANGMAN[6] for the frog. The electrophoretic fraction of newt lens extract which contains the β-crystallin (and perhaps other substances not revealed by immunological methods) inhibits newt lens regeneration, whereas the same fraction of chick lens extract does not inhibit newt lens regeneration. Thus, chick lens extract is not only immunologically different from that of the newt, but it is also physiologically different.

Résumé. La comparaison immunoéléctrophorétique d'extrait du cristallin de salamandre avec de l'extrait du cristallin de poulet a montré que la différence essentielle réside dans le caractère antigénétique du β-cristallin. La régénération du cristallin fut inhibée par les matières extraites du cristallin de salamandre mais non pas par celles qui provenaient du cristallin de poulet.

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Über den Einfluss des Penicillamins auf das Skeletsystem wachsender Ratten

D-Penicillamin ist ein Kupferchelator und bewirkt eine Verarmung des Organismus an Kupferionen[1,2]. Es hemmt in vitro eine kupferhaltige Aminooxydase des Knorpel-Knochengewebes, die bei der intramolekularen Tropokollagenvernetzung benötigt wird[3-5]. In vivo tritt nach Penicillamin-Verabreichung bei der Maus und bei der Ratte ein Osteolathyrismus auf, der durch Kupfergabe reversibel ist[4,6]. Im Gegensatz zum lathyritischen Kollagen nach β-Aminopropionitril ist der Aldehydgehalt des Kollagens nach Penicillamin-Behandlung grösser als derjenige des Normalkollagens[7]. Es war deshalb von Interesse zu erfahren, ob Penicillamin die Struktur und die mineralbindenden Eigenschaften der fibrillenhaltigen Grundsubstanz im Knochen- und Knorpelgewebe verändert.

Material und Methodik. 15 ca. 125 g schweren Wistarratten wurde täglich 0.2 g D-Penicillamin (DISTA, Liverpool) peroral verabreicht. 6 Ratten dienten als Kontrolle. Als Parameter für den Mineralumsatz im Skeletsystem wurde 9 Wochen nach Behandlungsbeginn 7 Tieren 10 μCi Sr^{85} i.v. injiziert. 30 h danach wurde ein Photoszintigramm und Röntgenbild angefertigt und zusätzlich 1, 3, 4, 6, 10, 14 und 21 Tage nach der Sr^{85}-Injektion die Sr-Aktivität über dem Tibiakopf bestimmt.

8 Tieren wurde nach der 9. Behandlungswoche die proximale Tibiaepiphyse entnommen und nach Vorfixierung in cacodylat-gepuffertem Glutaraldehyd (6,25%) und Nachfixierung in cacodylat-gepuffertem Osmiumtetroxyd (4%) in Epon 812 für die ultrastrukturelle Untersuchung eingebettet.

Befunde und Diskussion. Das gesamte Skeletsystem der mit Penicillamin behandelten Tiere ist gegenüber den Kontrollen strahlentransparenter, und die langen Röhrenknochen sind in ihrem Wachstum gegenüber den Kontrollen zurückgeblieben (Figur 1).

Die auffällige Verbreiterung der Epiphysenfuge wird vor allem durch eine Zunahme des Blasenknorpels verursacht. In ungewöhnlicher Weise werden in der Grundsubstanz um die hypertrophischen Blasenknorpelzellen 2000 Å dicke Kollagenfibrillen gefunden, die in einer Periodik von 640 Å gebändert sind. Sie entstehen durch seilartige Zusammenlagerung dünnerer Fibrillenelemente der Umgebung. Solche Kollagenfibrillen werden nie im hyalinen Knorpel einer normalen Epiphysenfuge be-

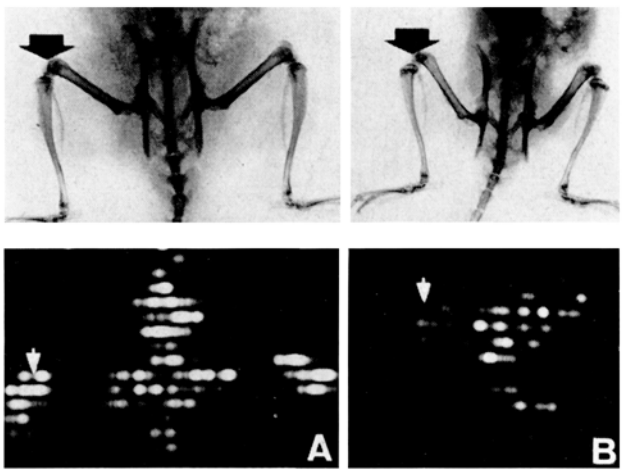

Fig. 1. Röntgenbild und Photoszintigramm nach Sr^{85}-Injektion. a) Kontrolltiere, b) penicillaminbehandelte Ratten: Retardiertes Wachstum der Röhrenknochen, verbreiterte Tibiaepiphysenfuge, fehlende Sr^{85}-Akkumulation über dem Kniebereich (Pfeil).

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