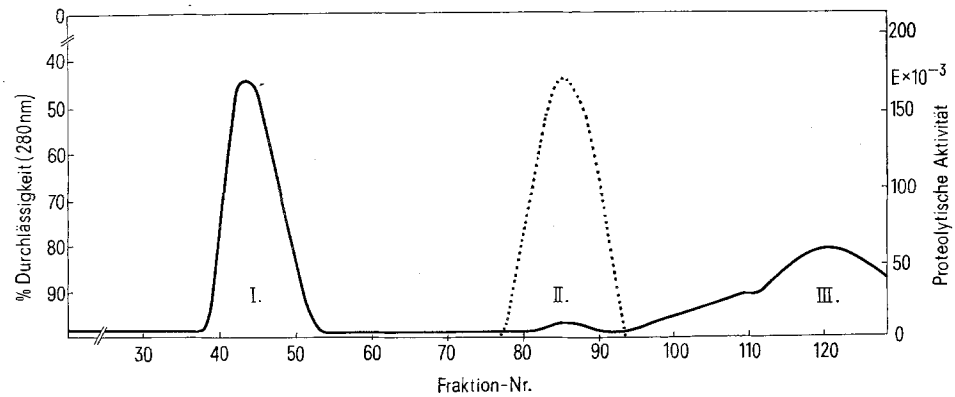

Gelfiltration des Schlüpf-Sekretes (s. Text). Sephadex G-75 in 0,1 *M* NH_4HCO_3-Puffer, pH 8.0. Säule: 2.8 × 74 cm. Jede Fraktion enthält 3 ml, Durchflussrate 15 ml/h. I, III = inaktives Material, II = Schlüpf-Protease. Linke Ordinate —, Durchlässigkeit im Eluat bei 280 nm; rechte Ordinate ·····, proteolytische Aktivität mit Casein als Substrat in O.D. bei 280 nm.

Aufgrund von laufenden Untersuchungen über die Struktur und die chemische Zusammensetzung der Eihülle sollte man bestimmte Enzyme, wahrscheinlich Glycosidasen und Proteasen, im Schlüpfsekret erwarten. Da für entsprechende enzymatische Tests der Rohextrakt herangezogen werden muss, dürfen positive Befunde aber erst dann dem Schlüpfsekret zugerechnet werden, wenn sich eine enge Substratbezogenheit zur Eihülle aufzeigen lässt bzw. wenn die Stoffe in isolierter und gereinigter Form ihre Wirksamkeit auf die Eihülle als natürliches Substrat beweisen. Entsprechende Versuche in dieser Richtung weisen das Vorhandensein einer α-Amylase auf, die mit Amylose als Substrat (0.02 *M* Phosphat-Puffer, pH 7,1; 0.008 *N* Jodlösung, bei 623 nm, d = 1 cm) zu 6 Street-Close-Einheiten/100 ml bzw. 34,2 mU/ml bestimmt werden konnte.

Bei der vorgenommenen Prüfung auf Hexosaminidasen bedienten wir uns der entsprechenden Nitrophenylsubstrate und bestimmten das freigesetzte Nitrophenol im Spektralphotometer bei 430 nm[4]. Nachweisen liessen sich hierbei die acetylierten Formen der β-D-glucosaminidase und der β-D-galactosaminidase.

Da sich die Einhüllen besonders durch einen relativ hohen Proteingehalt auszeichnen, vor allem die Zona radiata, erschien uns das Vorhandensein einer Protease als sehr wahrscheinlich. Tüpfel-Versuche mit dem Rohextrakt auf eine Gelantine-Schicht und deren Transparent-Werden an diesen Stellen erhärteten die Richtigkeit der Vermutung. Bei der Suche nach weiteren Substraten erwiesen sich neben der Gelantine das Casein und die B-Kette des Insulins als positiv. Eine erste Reinigung des Rohextraktes mittels einer Gelfiltration über Sephadex G-75 in Ammoniumbicarbonat-Puffer (0,1 *M*; pH 8,0) erbrachte eine einzige proteolytische Fraktion, die Casein und die B-Kette als Substrate spaltete und damit die Einordnung als Endopeptidase erlaubt.

Lässt man nun diese isolierte Proteinase auf Eierschalen einwirken, so erfolgt wie beim natürlichen Schlüpfen eine Auflösung der Hülle, die den Gedanken nahelegt, dass diese Proteinase möglicherweise allein die einzige, wirksame Komponente des Schlüpfsekretes darstellen könnte. In dem Fall wäre die Bezeichnung «Schlüpfsekret» in «Schlüpfenzym» zu ändern.

Summary. A method is described to get the secrete of the hatching glands together with the perivitelline fluid by stimulating the eggs of *Salmo gairdneri* with dissolved gaseous nitrogen in various buffers. Characterizing this fluid by various methods there could be shown some enzymes of those a proteinase alone seems to be the really 'hatching enzyme'.

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Sequential Appearance of α, β, and γ-Crystallins in Embryonic and Metamorphic *Rana pipiens*

The reported times of first appearance of the lens-specific proteins, the crystallins, in vertebrate development have had a controversial history (for reviews see [1, 2]). It is currently accepted that in birds [3, 4], δ crystallins are the first to appear, while in mammals the first crystallin class to be detectable is α crystallins [5, 6]; however, γ crystallins are predominant in the mammalian embryonic lens [5, 7].

The situation with amphibians is less clear. Yamada[8] has suggested with reservations that α-and β-crystallins appear before γ-crystallins in the regenerating newt lens. In the normal developing newt lens, Ogawa[9] found α and β-crystallins first to appear, although the antisera and test antigens utilized were not specific (discussed in [10]). Immunofluorescence results obtained with the developing lens of the anuran *Rana pipiens*, the American grass frog[10], suggested, however, that γ-crystallins are among the first, if not the first crystallins to appear in normal amphibian lens development. To better define the ontogeny of the crystallins in this amphibian, classical direct immunological techniques have been employed in the present study to corroborate and elucidate the immunofluorescence profiles previously obtained[10].

Materials and methods. Adult *R. pipiens* were kept in tap water at 5 °C until used. *R. pipiens* eggs were obtained by the method of induced ovulation[11], artifically

inseminated by a sperm suspension in 1/10 Holtfreter's solution, and maintained in this same medium at 18 °C until SHUMWAY Stage 25 (feeding stage)[12]. Embryos were then transferred to an aquarium with aeration and filtration and reared at 22 ± 1 °C in pond water to obtain metamorphosing specimens.

To obtain antigen for production of antibody, adult *R. pipiens* lenses were removed, cleaned of any extraneous eye tissue, and processed at 5 °C as previously described[13]. The total-lens-protein antigen sample thus obtained was fractionated by successive DEAE-cellulose and Sephadex G-100 chromatographies[10] to provide a purified γ-crystallin test antigen. Embryonic and metamorphic lens antigens were obtained by microsurgical removal and cleaning of the lenses from SHUMWAY Embryonic Stage 25 and TAYLOR-KOLLROS Stages I, VI, and XXIV[14]. The lenses were then pooled, homogenized by hand in a chilled micro-homogenizer, and centrifuged at 12,000 *g* for 30 min. The supernatants were used as reacting antigens in the immunological investigations. Protein concentrations, determined by the method of WARBURG and CHRISTIAN[15], were adjusted to 6 mg/ml.

Antibodies directed against total lens protein were prepared by immunization of virgin female rabbits with a 1:1 (v/v) mixture of complete Freund's adjuvant and total lens protein. The γ-globulin fraction of the immune sera was obtained by dialysis against 40% saturated ammonium sulfate at 5 °C. The antibodies were absorbed before use[16]. Micro-immunoelectrophoresis[17] was performed in 1% agar (Difco Special Agar-Noble) followed by incubation of the slides with antibody at 18 °C for 24 h. Immunodiffusion analyses[18] were carried out with 1% agar in phosphate-buffered saline, pH 7.0.

Results and conclusions. Immunoelectrophoretic analysis of adult total lens proteins employing absorbed antibodies directed against adult total lens protein results in a complex of precpiitin arcs. These can be identified as α-, β-, and γ-crystallin arcs by analogy with agar and immunoelectrophoresis patterns obtained with isolated lens protein fractions[10]. Total lens protein samples from SHUMWAY Stage 25 and TAYLOR-KOLLROS Stages I, VI, and XXIV were therefore tested in immunoelectrophoresis and Ouchterlony immunodiffusion analyses against the absorbed antibodies prepared from adult lenses. The results of the immunoelectrophoresis indicated that only a single small arc, with γ-crystallin mobility, is visible in the Stage 25 pattern. Additional components with mobilities greater than γ-crystallin begin to appear in the TAYLOR-KOLLROS stages, until at Stage XXIV the lens has apparently the same antigenic composition as the adult lens, as seen by the identical immunoelectrophoretic patterns obtained (Figure 1, A–E).

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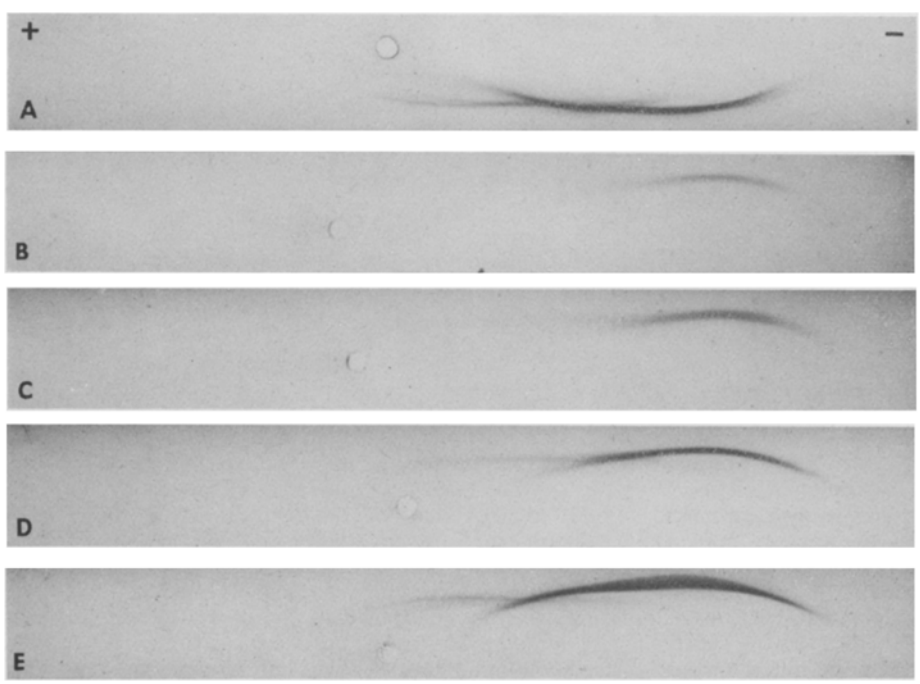

Fig. 1. Immunoelectrophoresis of embryonic and larval (metamorphic) *R. pipiens* total lens proteins vs. absorbed antibodies directed against total lens protein. Electrophoresis in 0.05 *M Tris*-glycine buffer, pH 8.3, carried out at 22 °C for 60 min (320 V, 2.5 mA). A) Adult *R. pipiens* total lens proteins; B) SHUMWAY Stage 25 total lens proteins; C) TAYLOR-KOLLROS Stage I total lens proteins; D) TAYLOR-KOLLROS Stage VI total lens proteins; E) TAYLOR-KOLLROS Stage XXIV total lens proteins.

Tests identical to those performed in immunoelectrophoresis were carried out in Ouchterlony immunodiffusion, in which the antigen dilution effect present in immunoelectrophoresis is not a factor. Immunodiffusion analysis has revealed the presence of two distinct antigenic components in lenses at SHUMWAY Stage 25 (weakly visible) and TAYLOR-KOLLROS Stages I and VI, corresponding to γ- and β-crystallins, and the presence of three distinct antigenic systems in lenses at TAYLOR-KOLLROS Stage XXIV, the additional component being identical with adult α-crystallins (Figure 2).

To determine the degree of tissue specificity of the absorbed antibodies, they were tested in Ouchterlony immunodiffusion against extracts of adult *R. pipiens* tissues other than lens; no reaction could be noted. This indicates that the antibodies used in this study are directed solely against components present in lens but absent in other tissues.

In conclusion, the results of this investigation indicate that the lens of *R. pipiens* contains detectable amounts of both γ- and β-crystallins by SHUMWAY Stage 25 and, together with previous immunofluorescence data[10], suggest that they appear simultaneously. Since γ-crystallins are not present in the lens epithelium at SHUMWAY Stage 25, the immunofluorescence observed there at that time using an absorbed anti-total-lens-protein antibody[10] can now be ascribed to the presence of β-crystallins. α-crystallins are the last of the lens-specific proteins to be detected during normal development of the anuran amphibian lens[19, 20].

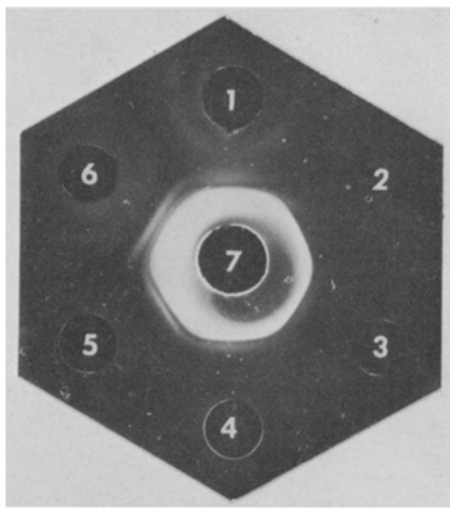

Fig. 2. Ouchterlony analysis of embryonic, larval (metamorphic), and adult *R. pipiens* lens proteins vs. absorbed antibody directed against total lens protein. Well 1, adult total-lens-proteins; Well 2, DEAE-G-100 γ crystallins; Well 3, SHUMWAY Stage 25 total lens proteins; Well 4, TAYLOR-KOLLROS Stage I total lens proteins; Well 5, TAYLOR-KOLLROS Stage VI total lens proteins; Well 6, TAYLOR-KOLLROS Stage XXIV total lens proteins; Well 7, anti-adult-total-lens protein antibody (absorbed). Incubation was for 48 h at 37 °C.

Zusammenfassung. Immunelektrophoretische und Immundiffusions-Studien zeigen, dass die Linse von *Rana pipiens* während der Entwicklung (Embryonal-Stadium 25 nach SHUMWAY) α- und β-kristalline Substanzen enthalten. Diese und frühere Ergebnisse[10] sprechen dafür, dass beide Substanzen gleichzeitig auftreten, und beweisen, dass β-kristalline Substanzen in diesem Entwicklungsstadium alleine im Linsenepithel vorkommen, während die Kristalline die am spätesten nachweisbaren linsenspezifischen Proteine (TAYLOR-KOLLROS-Metamorphosestadium VI) in der Linsenentwicklung von *R. pipiens* sind.

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Alteration of the Prospective Fate and the Inductive Power of the Definitive Streak Node in the Chick

While the young node may induce either a primitive streak or a neural structure, it is well established that the node of the definitive streak always elicits neural induction (for review, see GALLERA[1]). This change in its inductive power is correlated with some modifications of its prospective fate: at stage 2, it gives rise essentially to embryonic endoblast; later its inherent tendencies to give axial and paraxial mesoblast increase, so that no more than 50% of the node cells take part in the endoblast formation at the definitive streak stage (NICOLET[2]). Accordingly, it has been revealed that the node, whatever its age may be, does not produce a streak induction, if it differentiates partially into axial and paraxial mesoblast (GALLERA and NICOLET[3]). Hence we had suspected that this mesoblast may suppress the ability to induce a streak. Presently we see that the situation is more complex than previously thought, because, in a very peculiar environment, the definitive streak node was entirely converted into embryonic endoblast and induced a streak.

The experiments were performed on chick embryos cultured in vitro (GALLERA et NICOLET[4]) and staged after the tables of HAMBURGER and HAMILTON[5]. In the first set of experiments, 24 nodes of definitive streaks were transplanted on the posterior end of the host streak. The stages of the hosts went from stage 2 to 4. In order to recognize unequivocally the structures yielded by the grafts, 9 nodes were removed from donors, which have been previously incubated for 8 h on a medium containing 10 μC of tritiated thymidine. This time is sufficient to label all the nuclei (NICOLET[6]). In the second series, 11

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