and postfixed in 1% osmium tetraoxide in the same buffer. After dehydration in a gradient of alcohol water, and alcohol propylene oxide, the blocks were embedded in epon 812 and polymerized at 60 °C.

Gold sections were mounted on nickel grids, and floated for 20 min on a drop of 10% hydrogen peroxide solution, in order to eliminate the excess osmic acid.

The sections were extensively washed with distilled water and incubated on a drop of antibody directed towards human calcitonin diluted 1/50 in phosphate buffer 0.2 M ph 7.5 containing 0.2% human albumin and 1% sodium merthiolate. Excess antibody was removed by repeated washing of the sections in phosphate buffered saline (PBS) and the washed sections were incubated on a drop of anti-rat Ig immunoglobulin (rabbit) labelled with peroxidase and purified by molecular sieving[4]. After further washings with PBS, the sections were stained for peroxidase by incubation with continuous agitation for 20 min in the following substrate: phosphate buffer 0.2 M pH 7.2 containing 0.075% diamino benzidine and 0.001% hydrogen peroxide. The treated sections were washed twice with distilled water and post-fixed in 1% osmic acid before examination in an electron microscope[5,6].

The following controls were used in order to establish the specificity of the immunoperoxidase stain: a) sections incubated in the presence of normal rat serum, b) sections incubated in the presence of specific anticalcitonin antibody saturated with synthetic human calcitonin, c) sections directly incubated in the substrate for the control of normally occurring peroxidase in the thyroid gland.

Results. In sections of the thyroid gland treated with lead and uranium salts, C cells are easily demonstrated by the presence of numerous electron dense granulations (figure a). Treatment with hydrogen peroxide destains the cellular organellae, the membranes and the electron dense granules. In the thyroid sections stained by the double immunoperoxidase technique, the C cells contain numerous granules revealed by the presence of a dark precipitate of reaction product (figure c). This reaction is specific as sections incubated with normal serum or with the antibody saturated with synthetic calcitonin do not show a positive reaction (figure b). Naturally occurring peroxidase is limited to the mast cells and red blood corpuscles.

Discussion. In electron microscope studies, C cells have been identified principally by their high content of electron dense granules. The work presented here confirms that these granules do contain specifically immunoreactive calcitonin. The fact that a part of these granules do not show a positive reaction with the immunoperoxidase technique may be due to a partial denaturation of the calcitonin affecting its immunoreactivity, or to the presence of a second type of granules containing a polypeptide-s of unknown nature synthetized by the C cells. The naturally occurring peroxidase present in the thyroid does not hinder specific labelling of the granules of the C cells, as the enzyme occurs only in the mast cells and red blood corpuscles which are morphologically quite distinct from the C cells. The calcitonin content of the C cells is located in the granules, and separation of cellular components, by density gradient centrifugation, showed that calcitonin is present predominantly in the granular fraction[7].

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Eye lens development and γ crystallins in Discoglossus pictus (Anura)

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Summary. The ontogeny and localization of the γ crystallins in Discoglossus pictus lens development has been determined. Using antibody specific for amphibian γ crystallins in the immunofluorescence technique, it was found that γ crystallins first appear in primary lens fibre cells in the lens rudiment, and continue to be restricted to the fibre area as lens development progresses. Thus the role of γ crystallins as indicators of a differentiated state remains constant in amphibian evolution, having been demonstrated in the most archaic anuran superfamily, as well as in others more recently evolved.

Discoglossus pictus, the painted frog, is a member of the family Discoglossidae and native to southwestern Europe and northwestern Africa. Although ranid in appearance and size, this anuran is considered to be evolutionarily primitive. Previous studies to detect the time of first appearance and localization of the γ crystallins, those structural lens proteins specific for lens fibre formation in amphibians, have consistently associated their appearance with the differentiation of primary lens fibre cells in the embryonic lens rudiment[3–5]. In order to determine the presence of this association in the archaic anurans, the otogeny and localization of the γ crystallins in D. pictus development was elucidated by means of the immunofluorescence technique.

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2 The authors are grateful to Prof. W. J. van Doorenmaalen for use of the laboratory facilities; and to the Hubrecht Laboratory, International Embryological Institute, Utrecht, The Netherlands, for the gift of live D. pictus eggs. We thank Miss C. M. J. Stuifsand for the normal histology and Mr Th. Hulskes for the illustrations.

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Fertilized D. pictus eggs were kindly provided by the Hubrecht Laboratory, International Embryological Institute, and reared in the laboratory. Embryos of D. pictus were staged externally according to Shumway[6] and fixed in cooled 95% ethanol for use in immunofluorescence[3], or in Bouin's fluid for normal histology. Sections 5 μm in thickness through the developing eye region were processed for immunofluorescence according to the technique of McDevitt, Meza and Yamada[3]. Antibody to amphibian γ crystallins (derived from Rana pipiens[3,4,7]) was incubated with the sections containing brain and eye rudiment in the indirect technique of Weller and Coons[8], as described by McDevitt et al.[3]. The resulting apple-green immunofluorescence was observed and photographed using dark-field conditions, with the sites of immunofluorescence interpreted as the sites of corresponding antigen (γ crystallins) in the tissue section.

When sections through the developing brain and eye region of D. pictus were subjected to the immunofluorescence technique, no positive reaction of any kind could be observed until Lens Developmental Stage VI[3], at which time the lens rudiment has attained the vesicle stage and exhibits elongation of those cells adjacent to the optic cup. This selective elongation foreshadows the formation of lens fibres, and thus these cells are termed primary lens fibre cells. The immunofluorescence is localized in the cytoplasm of only those cells adjacent to the optic cup (prospective retina), and not in the external layer of the lens rudiment (figure, a). This localization of fluorescence indicative of γ crystallins remains restricted to the fibre region up to Lens Developmental Stage X, at which time the lens has attained its definitive histology (figure, b and c). At no time did immunofluorescence appear in the external layer (later, lens epithelium) or in other eye tissues.

These results suggest that the concept of γ crystallins as indicators of a differentiated state, i.e. lens fibre formation, is a valid one, and that this association of a developmental event with a biochemical event is conserved in evolution. Anuran amphibia are considered to have widely varying degrees of primitiveness[9,10], with the superfamily Ascaphoidae (family Ascaphidae and family Discoglossidae) the most archaic in terms of evolution. Fossil records date the Discoglossidae as late Jurassic, while the Ranidae are considered modern or advanced anurans (Oligocene)[10]. Thus D. pictus is the most ancient anuran yet investigated in this regard, and the γ crystallin immunofluorescence profile obtained coincides exactly with that established for other, more highly advanced anurans[3–5].

The general outlines of lens morphogenesis are also very similar to higher anurans, i.e. ranids. Lens development in D. pictus is not aberrant, as it is in its younger (early

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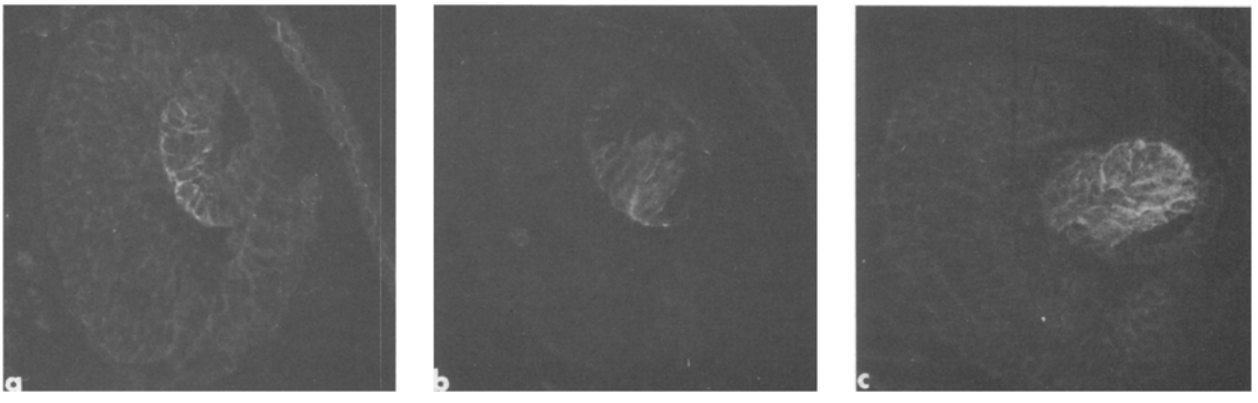
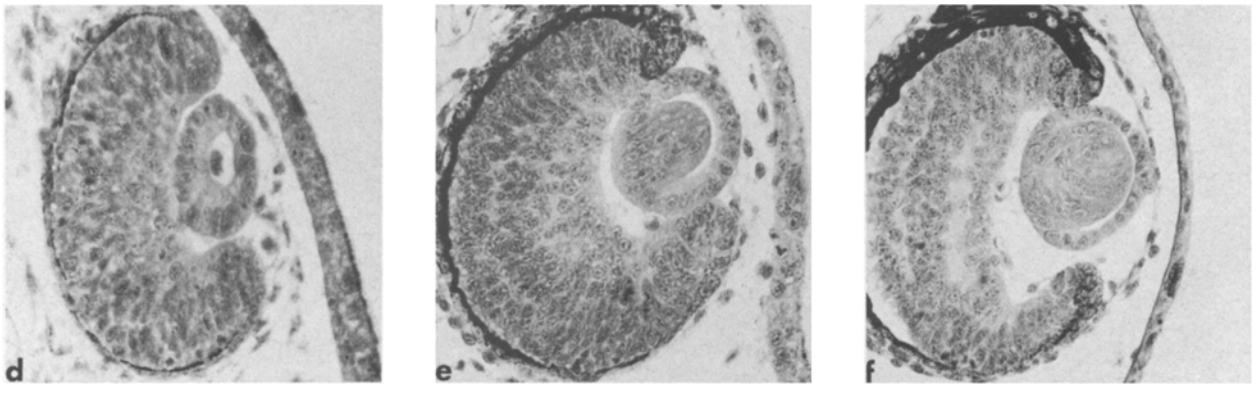


a–c Selected lens developmental stages[3] of embryonic Discoglossus pictus, incubated in the indirect immunofluorescence technique with anti-γ crystallin antibody: *a* Stage VI; *b* Stage VIII; *c* Stage X.



d–f Lens developmental stages of embryonic D. pictus, for histological reference purposes: *d* Stage VI; *e* Stage VIII; *f* Stage X. Hematoxylin and eosin.

Cretaceous) and purely aquatic relative, Xenopus, family Pipidae[4]. Exceptions to the ranid mode of lens formation[3] noted by us include small size (e.g. at Stage V, the lens rudiment of D. pictus is only $^1/_4$ the diameter of that of the corresponding R. pipiens lens stage); and late persistance (after Stage X) of a lens vesicle remnant. These did not alter, however, the consistent immunofluorescence profile for γ crystallins that we obtained; we submit that overwhelming evidence, both intra- and interphyletic, confirms this class of lens-specific structural proteins as one of the most valuable probes available to the developmental biologist today.

On the biomechanical function of the liver capsule[1]

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Summary. We have carried out histomechanical studies on the human and bovine liver capsule under tension loading. Results: Nonlinear force-time curve under extension with constant speed, initial upper and lower summit decrease of the dynamic relaxation curve, amplitude diminution phenomenon of the dynamic relaxation in the lower nonlinear part of the extension-time curve, dynamic (cyclic) force recovery.

The main components of the liver capsule are connective tissue cells, nerves, collagen, and elastic fibres. The physiological intrahepatic tissue pressure interacts with the tension of the capsule[3–6]. The liver capsule expands in consequence of an acute gain in volume with certain diseases of the liver. Impacts in a blunt abdominal trauma can result in high tensions of the liver capsule as well as in ruptures of the capsule and parenchyma. In this context, the question arises which mechanical properties the liver capsule embodies[7–10].

Materials and methods. We have studied the human and bovine liver capsule. The histomechanical examinations were performed 24–36 h after death. The specimens were stored about + 3 °C. A rectangle area was marked on the surface of the liver. The marginal lines of this rectangle were cut with a razor blade. After that the designated capsule area was carefully dissected. Then the specimen was once more measured and attached to the clamps of the testing machine. The histomechanical loading examinations were performed in physiological solutions of defined temperatures with the Dynatron after Meskat, Rosenberg and Hoffmann[11] for dynamical stress-strain curves and a statical universal testing machine[12, 13].

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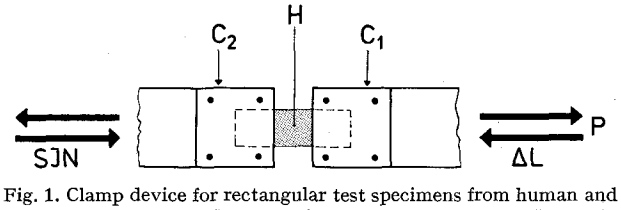

Fig. 1. Clamp device for rectangular test specimens from human and bovine liver capulse. C_1 right clamp which induces a change in length (ΔL) at the capulse specimen (H) with a certain preselected constant speed. P force (load) measurement device attached to the right clamp. C_2 left clamp which brings about sinus-shaped changes in length.

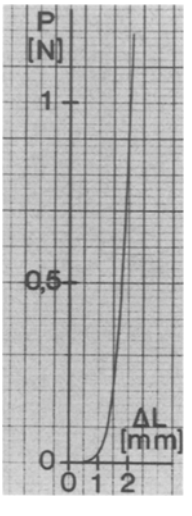

Fig. 2. Force-extension diagram from the bovine liver capsule. Size of the capsule test specimen before dissection 'in situ' 30 × 10 mm. After the piece of capsule had been dissected, a certain contraction could be observed. The mechanical test was performed in Ringer solution. The diagram was taken from a steady state series (after preconditioning). Abscissa: increase in length ΔL in mm. Ordinate: force (load) P in Newton. The initial length between clamps before preconditioning and in a perfect release from tension was 10 mm.