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Study of the Soluble Lens Proteins from Different Amphibian Species

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Soluble lens proteins from five species of amphibia have been studied by zone electrophoresis and other immunochemical methods. Their patterns, as revealed by electrophoresis, do not differ markedly though the numbers of bands and subunits vary. The γ-crystallin appears to be the predominant lens protein in all the species. Immunodiffusion tests showed a reaction of complete identity of lens antigens of different species with respect to *Xenopus laevis* lens antiserum; while *X. laevis*, *Triturus cristatus*, *Ambystoma mexicanum* lens antigens revealed partial identity when tested against *Rana esculenta* and *Bufo bufo* lens antisera.

1. Introduction

Soluble lens proteins of different vertebrates have been widely investigated. Bon (1958), Bon, Swanborn, Ruttenberg and Dohrn (1964), Bon, Ruttenberg, Dohrn and Batnik (1968), Cobb, Carter and Koenig (1968a), Manski and Halbert (1964), and Rabacy (1964) examined the soluble lens proteins of different species of fish. Swanborn (1966), and Björk (1968) studied the immunological relationship of the α-crystallins from different vertebrates and mammals. However, information derived from the comparative study of the soluble lens proteins from different species of amphibia is scanty. For that reason, we selected five species according to their habitat, and studied the soluble lens protein by agar and starch gel electrophoresis, immunoelectrophoresis, immunodiffusion, and immunosmophoresis. The results are given in the present report.

2. Materials and Method

Lenses were used from adult frog (*Rana esculenta*), toad (*Bufo bufo*), South African clawed toad (*Xenopus laevis*), newt (*Triturus cristatus*) amd axolotl (*Ambystoma mexicanum*). After removal, the lenses were cleaned and homogenized in cold saline. The homogenate was centrifuged at 32700 g for 30 min in a Lourdes centrifuge and the supernatant was stored at −25°C until needed.

For agar electrophoresis a 3% solution was made with Difco Bacto agar in distilled water and boiled. It was then poured on a glass tray and cooled. Later, solidified solution was cut into small blocks and washed extensively in deionized water at 4°C. When required for use, it was mixed with an equal volume of buffer to make it 1·5%.

Antibodies against *R. esculenta*, *X. laevis* and *B. bufo* were prepared from rabbits according to the methods followed in this laboratory. Each rabbit received five injections, each injection containing 60 mg wet weight of the lens homogenate in 1 ml saline incorporated in an equal volume of adjuvant.

It was not possible to prepare antisera against *T. cristatus* and *A. mexicanum* lens as the material available from these was inadequate. Agar electrophoresis was performed on glass plates (17 × 4 cm) in Veronal buffer at pH 8·6 according to the procedure of Wieme

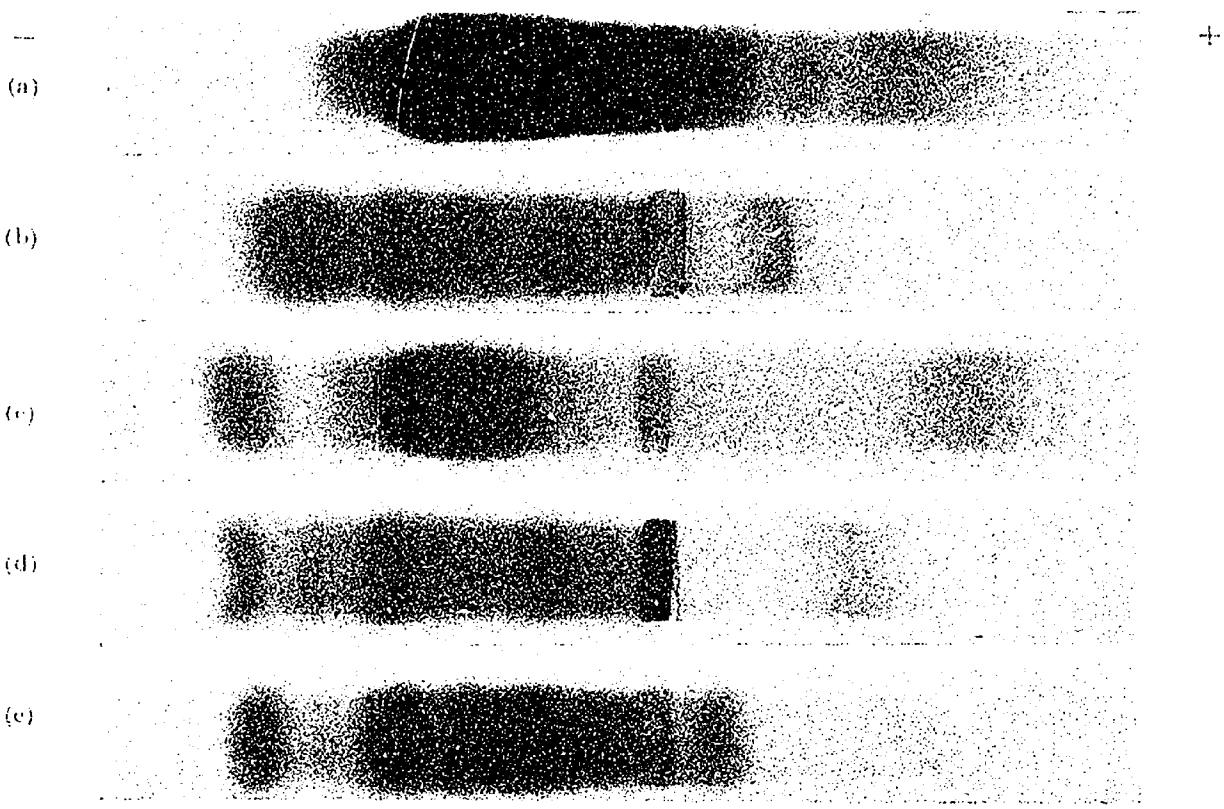

PLATE 1. Agar electrophoresis pattern of soluble lens proteins in Veronal buffer at pH 8.6, 6 v/cm, 7–8 mA current for nearly 6 hr.
(a) *B. bufo*, (b) *A. mexicanum*, (c) *R. esculenta*, (d) *T. cristatus*, (e) *X. laevis*.

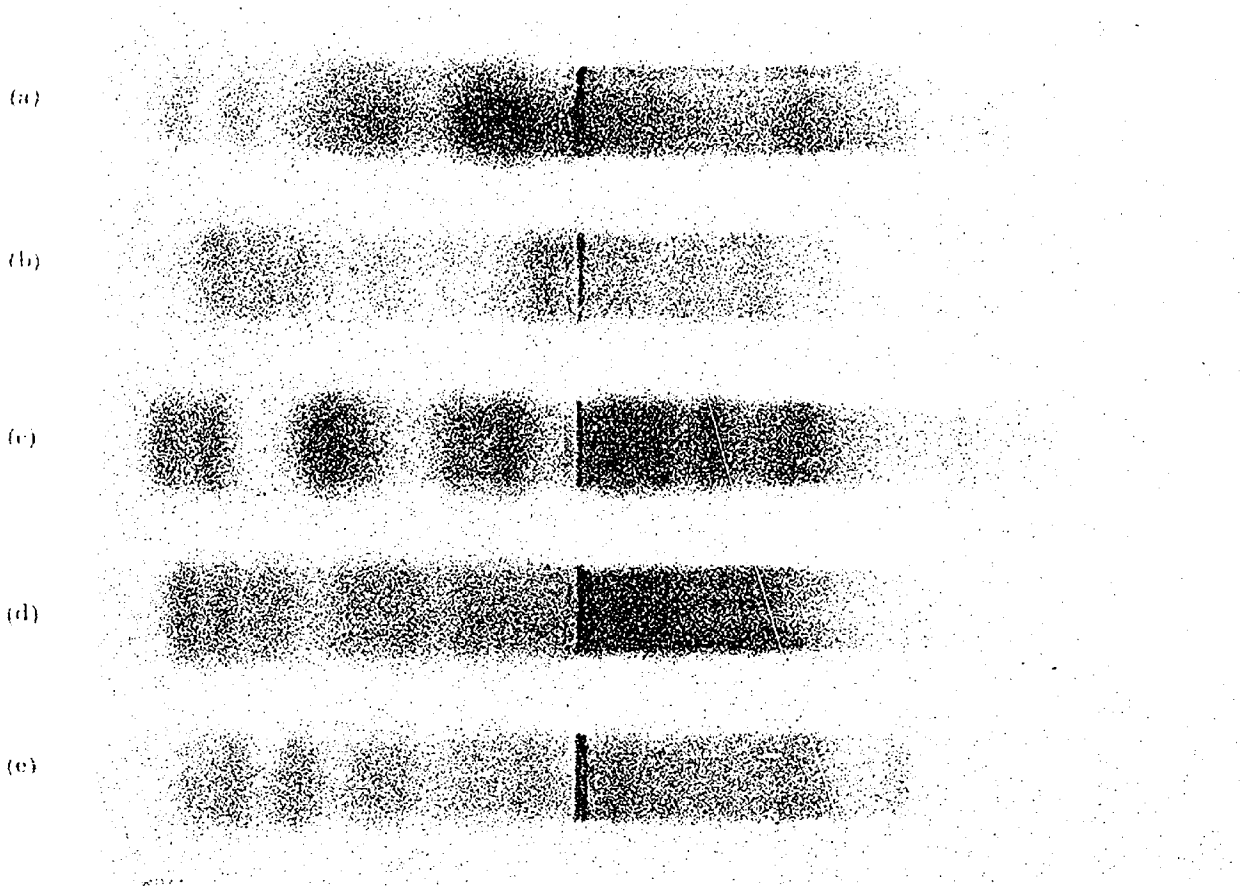

PLATE 2. Starch gel electrophoresis of soluble lens proteins in 0·03 M Tris and 0·5 M boric acid buffered at pH 8·6, 175 V and 10 mA current for 18 hr.
(a) *B. bufo*, (b) *A. mexicanum*, (c) *R. esculenta*, (d) *T. cristatus*, (e) *X. laevis*.

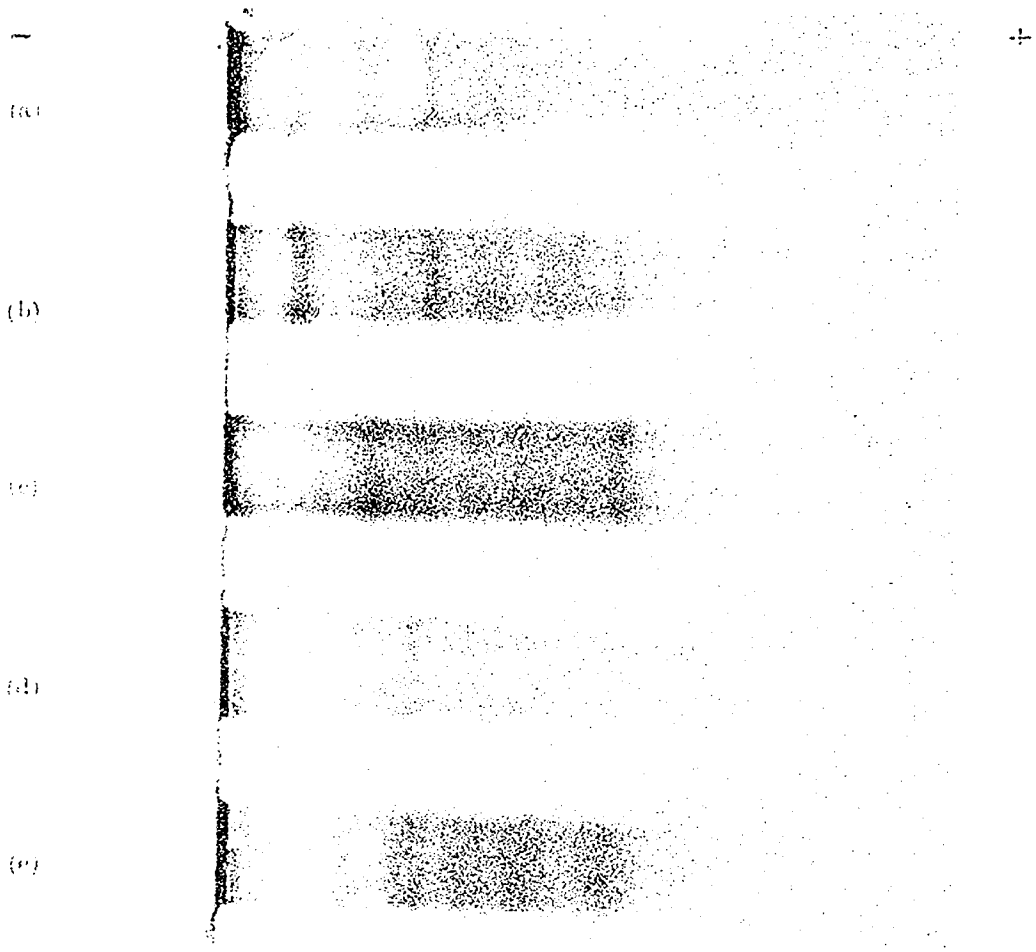

PLATE 3. Starch gel electrophoresis of the soluble lens proteins in presence of 8 M urea, in 0·76 M Tris and 0·3 M boric acid buffered at pH 8·6 for 20 hr with 250 V and 16 mA current.
(a) *N. laevis*, (b) *T. cristatus*, (c) *R. esculenta*, (d) *A. mexicanum*, (e) *B. bufo*.

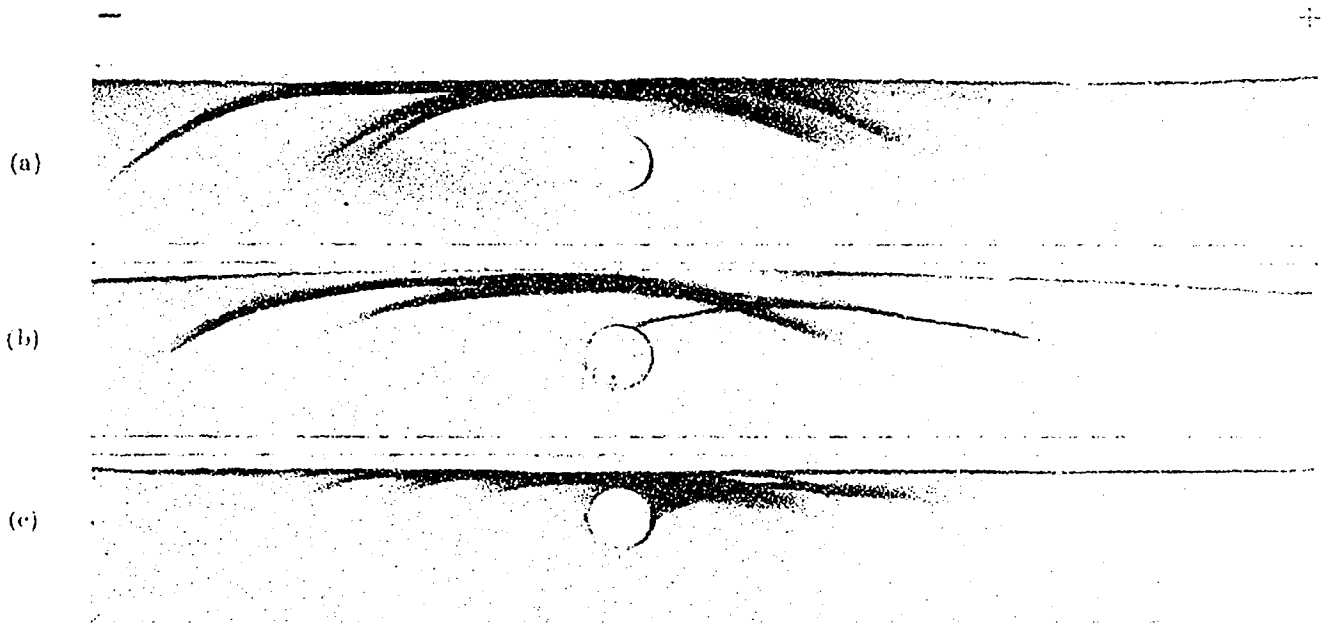

PLATE 4. Immunoelectrophoresis of soluble lens proteins against homologous lens antiserum in Veronal buffer at pH 8·6 for 90 min with 40 v and 25 mA current.
(a) *X. laevis*, (b) *R. esculenta*, (c) *B. bufo*.

(1959). Samples to be tested were applied to the glass plates according to Schalekamp's method (1963). The electrophoresis was run for nearly 6 hr with 6 v/cm, and an average of 7–8 mA current per glass plate. The whole process was carried out under petroleum ether at 4°C. The glass plates were then fixed in acetic acid-alcohol, dried and stained with amido black. As control, a mixture containing human serum albumen, dextran and siderophilin in the proportions of 1 : 1 : 1 was subjected to electrophoresis for the same length of time. The position of dextran was marked by an incision in the gel after fixation.

Starch gel electrophoresis was carried out according to the methods of Smithies (1959), and hydrolysed starch (Connaught Research Laboratories) was used. The gel was prepared with 0·03 M Tris buffered at pH 7·6 with 0·5 M boric acid. The bridge solution was also buffered at pH 7·6 with 0·03 M boric acid and 0·06 M NaOH. Samples were subjected to electrophoresis for 18 hr at 4°C at 175 v and 10 mA current. Gel blocks were then sliced into pairs and stained with amido black.

Starch gel electrophoresis in presence of 8 M urea was also done at an alkaline pH after the method of Wake and Baldwin (1961) as modified by van Dam (1967), i.e. the gel concentration was raised from 11.4 to 14%, and the gel was set up vertically (Smithies, 1962). The gel was prepared at pH 8·6 with Tris borate buffer (0.76 M Tris and 0·3 M boric acid). The bridge solution was also buffered at pH 8·6 with 0·3 M boric acid and 0·05 N NaOH. The electrophoresis was carried out for 20 hr at 4°C at 250 v and 10 mA current. At the end of the experiment the gel was sliced into pairs and stained with amido black.

Immunoelectrophoresis was performed according to the micromethod of Scheidegger (1955) in Veronal buffer at pH 8·6 for 90 min with 40 v and a current intensity of 25 mA at 4°C. Immunodiffusion was allowed for 48 hr at room temperature. Slides were then washed in normal saline, dried and stained with Ponceau S.

For immunodiffusion, lens extracts from five species were tested against lens antisera of *X. laevis*, *R. esculenta* and *B. bufo* according to the method of Ouchterlony (1948). The agar was mixed with Veronal buffer at pH 8·6 and the reaction was carried out at room temperature for 72 hr.

For immunosmophoresis we followed the methods of Bon and Swanborn (1963) on glass plates with 20 v/cm for 25 min. The agar was mixed with an equal volume of Aronsson and Grönwall (1957) buffer at pH 8·9.

3. Results

Electrophoretic patterns of the soluble lens proteins, as revealed by agar and starch gel electrophoresis, are shown in Plates 1 and 2. It appears that by the latter method more bands could be observed. The number of bands, however, varied with the different species. All these bands could be classified under three main groups of proteins, namely, the α-, β- and the γ-crystallins, according to their mobilities with reference to the control sample, and most of these appeared on the cathodic side. In *R. esculenta* a band could be observed in front of the α-crystallin [Plate 2(c)] and seems to represent the so-called pre-α. Its presence in the same animal could also be demonstrated by immunoelectrophoresis [Plate (4b)] and immunosmophoresis. This particular band was found to be absent in all the other species studied.

Gel electrophoresis in 8 M urea showed the subunit bands present in the soluble lens fractions (Plate 3). No investigation has yet been done by us with isolated lens proteins from the species used in the present experiment.

Lens antigens from all the species used showed reactions of complete identity with respect to *X. laevis* lens antiserum; whereas *X. laevis*, *T. cristatus* and *A. mexicanum* lens antigens showed partial identity by producing spurs against *R. esculenta* and *B. bufo* lens antisera, *R. esculenta* and *B. bufo* showed great similarity with one another in immunodiffusion reactions. Immunoelectrophoretic patterns of *X. laevis*, *R.*

esculenta and *B. Bufo* lens antigens against their homologous antiserum are shown in Plate 4(a), (b) and (c). The pre-α-line could only be observed in the *R. esculenta* material. The long line in *X. laevis* as reported by Campbell, Clayton and Truman (1968) is also to be found in *R. esculenta* and *B. bufo* material as well as in *X. laevis*.

4. Discussion

In the agar electrophoresis, which is a rapid method for the determination of relative mobility of proteins, separation of the protein components was poor on the anodic side, which seems to contain the α- and β-crystallins, in comparison with the reference mixture used. With starch-gel electrophoresis, separation on the anodic side was also not good. This is probably due to retardation of large molecules in the starch gel (Smithies, 1962). Separation of low molecular weight proteins on the cathodic side was good with agar-gel and even better with starch-gel methods. It appears that these low molecular weight cathodic proteins form the bulk of lens crystallins, and they are most likely the γ-crystallins. McDevitt (1967) and Cobb, Carter and Koenig (1968b), also reported that the γ-crystallins are the predominant lens proteins in *Rana pipiens* and *R. clamitans*, respectively.

Gel electrophoresis in presence of 8 M urea showed the subunit band present in the lens crystallins of all these species. Immunosmophoresis (Bon and Swanborn, 1963; Swanborn, 1966) seems to be an excellent method for the detection of pre-α, and by this technique we could detect this line only in *R. esculenta*, though it is said to be present in *X. laevis* (Campbell, Clayton and Truman, 1968). The discrepancy is not clearly understood. It would appear that the pre-α is not restricted to mammals, as already suggested by Swanborn (1966).

Immunological reactions of complete identity of *T. cristatus* and *A. mexicanum* lens antigens against *X. laevis* lens antiserum could possibly be due to a similarity in the habitats of these animals, and this similarity could possibly explain the appearance of spurs when *X. laevis, T. cristatus* and *A. mexicanum* lens antigens reacted against *R. esculenta* and *B. bufo* lens antisera. It seems difficult to explain the reaction of complete identity of *R. esculenta* and *B. bufo* lens antigens against *X. laevis* lens antiserum. Since *X. laevis* shares serologically identical antigens with both of these anurans, it would appear to be intermediate between the two species.

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REFERENCES

Aronsson, T. and Grönwall, A. (1957). *Scand. J. Clin. Lab. Invest.* **9**, 338.
Björk, I (1968). *Exptl Eye Res.* **7**, 129.
Bon, W. F. (1958). *Publ. Staz. Zool. Napoli* **30**, 373.
Bon, W. F., Swanborn, P. L., Ruttenberg, G. J. M. and Dohrn, A. (1964). *Publ. Staz. Zool. Napoli* **34**, 59.
Bon, W. F. and Swanborn, P. L. (1963). *Anal. Biochem.* **11**, 16.
Bon, W. F., Ruttenberg, G., Dohrn, A. and Batnik, H. (1968). *Exptl Eye Res.* **7**, 603.
Campbell, J. C., Clayton, R. M. and Truman, D. E. S. (1968). *Exptl Eye Res.* **7**, 4.
Cobb, B. F., Carter L. and Koenig, V. L. (1968a). *Comp. Biochem. Physiol.* **24**, 817.
Cobb, B. F., Carter, L. and Koenig, V. L. (1968b). *Comp. Biochem. Physiol.* **26**, 519.
Manski, W. and Halbert, S. P. (1964). Protides of the Biological Fluids. *Proc. Colloq.* **12**, 117.

McDevitt, D. S. (1967). *J. Exp. Zool.* **164**, 21.
Ouchteriony, Ö. (1948). *Arkiv Kemi* **26B**, 1.
Rabaey, M. (1964). Protides of the Biological Fluids, *Proc. Colloq.* **12**, 273.
Schalekamp, M. A. D. H. (1963). Thesis, University of Utrecht.
Scheidegger, J. I. (1955). *Int. Arch. Allergy Appl. Immunol.* **7**, 103.
Smithies, O. (1959). *Biochem. J.* **71**, 585.
Smithies, O. (1962). *Arch. Biochem. Biophys.* (suppl.) **1**, 125.
Swanborn, P. L. (1966). *Exptl Eye Res.* **5**, 302.
van Dam, A. F. (1967). Thesis, University of Utrecht.
Wake, R. G. and Baldwin, R. L. (1961). *Biochim. Biophys. Acta* **47**, 225.
Wieme, R. J. (1959). Thesis, University of Ghent.