

Nosiheptide exhibits no toxicity when administered to the mouse by the oral, s.c. and i.p. routes at the dose of 2.5 g/kg. The bacteriostatic activity of nosiheptide against some microorganisms is shown in the table. The minimum inhibitory concentration (MIC) determinations were carried out by the dilution method in the appropriate medium for each microorganism and after incubation for 18 h at 37 °C. Nosiheptide is mainly active in vitro against gram-positive cocci and bacilli, including staphylococcal strains resistant to penicillin, streptomycin, tetracyclin and numerous other antibiotics such as chloramphenicol, novobiocin, spiramycin and erythromycin. It is also active against certain gram-negative bacteria such as *Neisseria catarrhalis* and *Pasteurella multocida*. In vitro, nosiheptide reveals an activity against staphylococci and streptococci which is similar or superior to that of penicillin G. In vivo, nosiheptide does not exhibit any systemic activity either p.o. or s.c. in mice infected experimentally with staphylococci or streptococci. However, in mice with a s.c. or i.p. infection by staphylococci, it is active when injected immediately after the germs into the site of inoculation.

Used as a feed additive on the proportion of 2.5–10 g per ton, nosiheptide shows a favourable effect on the growth and conversion index in chickens^{30,31} and pigs³²; it is not detected in the flesh of chicks maintained during 8 weeks on a diet containing 200 g/t³³.

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Polypeptides of the lens fibre cell intracellular matrix¹

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Summary. The cytoskeletal proteins of the vertebrate lens fibre cell comprise polypeptides ranging in mol.wt from 43,000 to 250,000 daltons. The main intermediate filament polypeptide of the pickerel, Northern frog, chick, bovine, and human lens has a mol.wt of 54,000 daltons. Peptide analysis revealed that the chick 54,000 dalton protein was more similar to the bovine protein than to the pickerel protein.

The water-insoluble protein of the lens can be fractionated by 8 M urea into urea-soluble and urea-insoluble components². The urea-insoluble fraction is enriched in membrane, while the urea-soluble fraction contains lens crystallins (water-soluble protein) as well as the noncrystallin proteins of the fibre cell matrix or cytoskeleton².

This study presents a comparative electrophoretic analysis of the urea-soluble protein of lenses of different species. The isolation and peptide analysis of one of the noncrystallin polypeptides is also reported.

Lenses were obtained from the following mature animals immediately on sacrifice of the animal: bovine (*Bos taurus*), chick (*Gallus domesticus*), Northern frog (*Rana pipiens*), and fish (pickerel; *Esox vermiculatus*). Human lenses obtained from 2 newborns (set A and B) were removed within 24 h after death. The cortex fibre cell mass was dissected from each lens and homogenized in standard salt buffer (designated SM) consisting of 0.1 M KCl, 0.005 M MgCl₂, 0.006 M sodium phosphate buffer pH 7.2, to which 0.001 M 2-mercaptoethanol was added.

After centrifugation at $37,000\times g$ for 20 min at 4°C to obtain the water-soluble protein, the water-insoluble material was collected and washed as previously described². The insoluble material was then homogenized in 8 M urea (200 mg/ml). After 2 h extraction at room temperature, the homogenate was diluted at 4 M urea with SM buffer and centrifuged at $77,000\times g$ for 20 min at 10°C . The urea-soluble fraction (USF) was retained for further study.

The procedure for the isolation of intermediate filaments of the lens fibre cells has been described³. The 4 M urea-soluble lens fraction was exhaustively dialyzed against SM buffer to remove the urea. After dialysis the lens fraction was centrifuged at $37,000\times g$ for 20 min to remove any remaining membrane vesicles. The resultant supernatant was then centrifuged at $77,000\times g$ for 1 h. The $77,000\times g$ pellet was enriched in intermediate filaments. The chick lens epithelial cell filaments were similarly isolated. Electrophoresis was performed in polyacrylamide slab gels ($T=5.13\%$, $C=2.5\%$) in the presence of 1% SDS as previously described⁴.

Peptide fingerprints were prepared in the laboratory of Dr J.C. Brown (University of Virginia, Charlottesville, Virginia). After fixation and staining of the gel, specific bands were cut out with a scalpel and the protein iodinated with

^{125}I while still in the gel. Iodination was performed by the chloramine-T technique and the iodinated proteins digested with trypsin⁵. The resulting mixture of ^{125}I -labelled peptides was then analyzed by 2-dimensional chromatography on 20×20 cm Silica gel G plates (250 μm thickness; Analtech, Newark, Delaware) which had previously been activated by heating to 100°C for 30 min. The 1st dimension was developed in 7:3 n-propanol: NH_4OH and the 2nd in the organic phase formed from 6:1:4 n-butanol:acetic acid:water. ^{125}I -labelled peptides were localized on the developed chromatogram by radioautography for 1–7 days. The SDS pattern of the water-soluble crystallins of the lens is shown in figure 1. The highest mol.wt crystallin polypeptide is that of chick delta crystallin (48,000 daltons, figure 1, c). The SDS pattern of the urea-soluble proteins is more complex (figure 3). In addition to water-soluble crystallins, noncrystallin polypeptides are prominent. Although there are differences in mobility of polypeptides from one species to another, a general similarity of patterns is evident. In all samples there is a polypeptide with estimated mol.wt of 250,000 daltons, as well as a polypeptide with mol.wt 82,000–105,000 daltons depending on the species. Several polypeptides are found in the mol.wt range of 43,000–54,000 daltons with a major component at 54,000 daltons. A polypeptide with mol.wt of 47,000 daltons is present in the human and bovine lens, while a prominent 45,000 dalton polypeptide is present in the frog lens. The 43,000 dalton polypeptide is more prominent in the human and chick lens, although it is present in all species analyzed. Actin comigrates with the 43,000 dalton polypeptide.

The SDS pattern of isolated pickerel, bovine and chick intermediate filaments shows in each case mainly the 54,000 dalton polypeptide (figure 2). Peptide fingerprints of this polypeptide are shown in figures 4–7. The peptide maps of the 54,000 dalton polypeptide from the chick epithelial and fibre cells are identical (figures 4 and 5). The

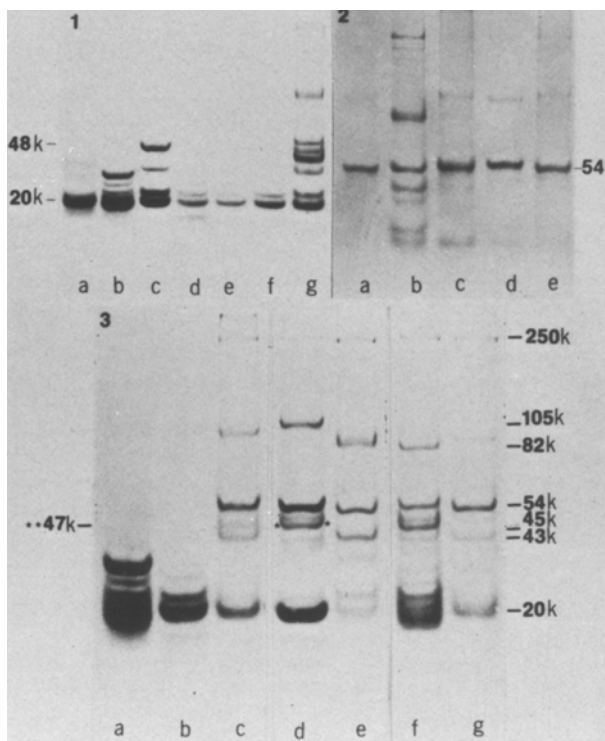


Fig. 1. Electrophoretic patterns in 5.13% polyacrylamide gels – 1% sodium dodecyl sulphate (SDS) of lens fibre cell water-soluble protein: a, pickerel; b, Northern frog; c, chick; d, human (set A); e, human (set B); f, bovine. The urea-soluble protein of the chick lens is seen in g. The letter k represents kilodaltons.

Fig. 2. SDS-PAGE gel pattern of isolated intermediate filaments of: a, chick lens epithelial cell; c, pickerel fibre cell; d, bovine fibre cell; e, chick fibre cell. The pattern of the chick fibre cell USF is shown in (b).

Fig. 3. SDS-PAGE patterns of lens water-soluble (WSF) and urea-soluble (USF) protein. a, Northern frog WSF; b, bovine WSF; c, human USF (set A); d, bovine USF; e, chick USF; f, Northern frog USF; g, pickerel USF. The asterisks indicate the 47,000 dalton polypeptide. The USF patterns of human set A and B were the same.

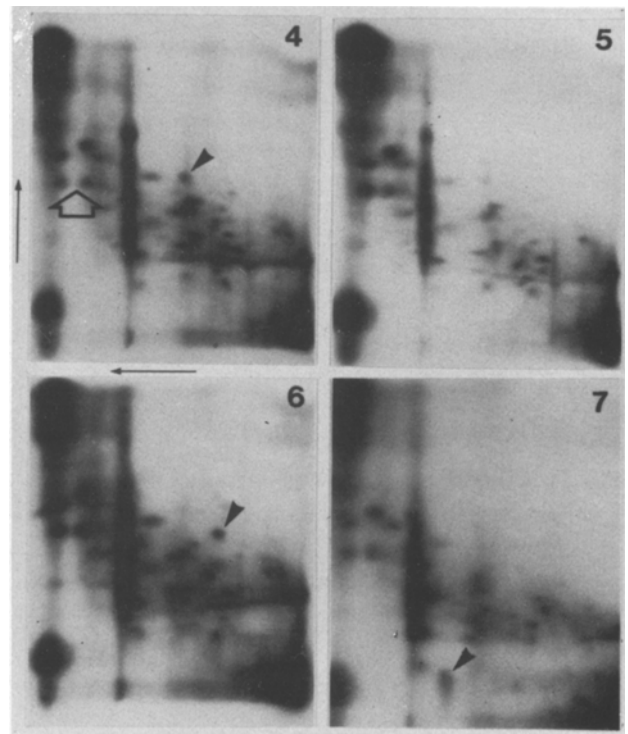


Fig. 4–7. Peptide maps of the 54,000 dalton polypeptide of chick epithelial cells (figure 4), chick fibre cell (figure 5), bovine fibre cell (figure 6), and pickerel fibre cell (figure 7).

bovine, pickerel and chick fibre cell polypeptide all share the constellation of peptides indicated by the open arrow in figure 4. Differences are however evident, and peptides unique to each species are indicated by the closed arrows. There is a greater correspondence between bovine and chick peptide patterns than between these and the pickerel pattern.

The results obtained in this study show that the proteins of the lens fibre cell cytoskeleton are a complex of unique polypeptides distinct from the crystallins. Aside from species differences the noncrystallin polypeptides fall in 3 major mol.wt categories: 250,000 daltons, 82,000–105,000 daltons, and 43,000–45,000 daltons.

The 54,000 dalton polypeptide is identified as the major component of the lens intermediate filament. Similar results have been reported for the bovine lens⁶. Peptide analysis showed a great similarity between the chick and bovine polypeptides. The pickerel protein has a more

distant relationship. The 43,000 dalton polypeptide comigrates with actin under the electrophoretic conditions described here. The presence of actin in the bovine lens has been documented⁶. The role of cytoskeletal proteins in the lens fibre cell awaits further study.

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Maternal regulation of wing polymorphism in *Pyrhocris apterus*: Effect of cold activation

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Summary. In a macropterous strain of *Pyrhocris apterus* the offspring of females kept under long-day conditions are invariably mostly macropterous, whereas the offspring of females from short-day conditions become macropters under long-day and brachypters under short-day conditions. The brachypterizing effect of short days was removed by the chilling of mothers for 70 days.

In *Pyrhocris apterus* L. (Heteroptera, Pyrrhocoridae) the membranes of the forewings are either reduced (brachypters) or fully developed (macropters). In wild population in Central Europe the penetration of this character is environmentally controlled (largely by photoperiods)¹. We selected a strain whose members developed into macropters under any photoperiodic conditions, provided the parents lived under long-day conditions². Under short-day conditions the imagines of this strain are in diapause, but after some time (30–50 days) some females spontaneously begin to lay eggs. The reactions of the offspring of these short-day mothers differ from those of mothers from long-day conditions. The 29th–31st generations of the strain selected for macropterism were used. The insects were kept in groups in plastic boxes and supplied with linden seed and water. They were kept either under a 12 h-light:12 h-dark photoperiod (short-day) or a 16 h-light:8 h-dark photoperiod (long-day) and at a temperature of 25–27 °C. In short-day conditions the females were either left to lay eggs spontaneously or they were activated by a) transfer to long-day conditions, b) chilling at temperatures of 3–10 °C for 70 days, or c) by injury – cutting of the wings. The line kept under long-day conditions served as the control.

The offspring of females reared under long-day conditions consists of about 90% of macropters and 10% of brachypters if the larvae are reared under long-day conditions or there are about 60–70% of macropters if they are reared under short-day conditions. There was no difference among the offspring of mothers reared continuously under long-day and those transferred into long-day as imagines. The progeny of females reared under short-day conditions mostly become brachypters if held under short-day conditions as larvae (about 25% of macropters). The production of brachypters lasts for the whole life of short-day mothers (about 2–3 months). Under long-day conditions the offspring of

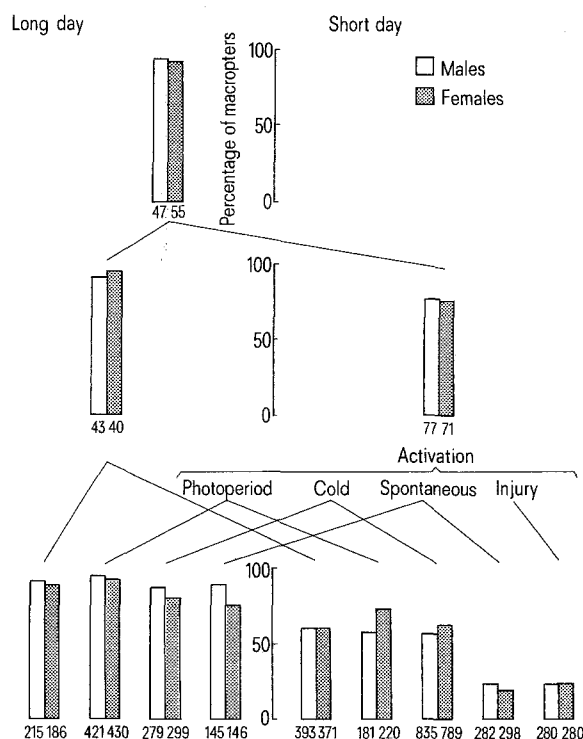


Fig. 1. The percentage of long-winged individuals (in 3 successive generations) when parents and progeny at the larval stage have been subjected to different treatments. Number of individuals in each sample given below each column.