# Isolation of filaments of the chick lens[1]

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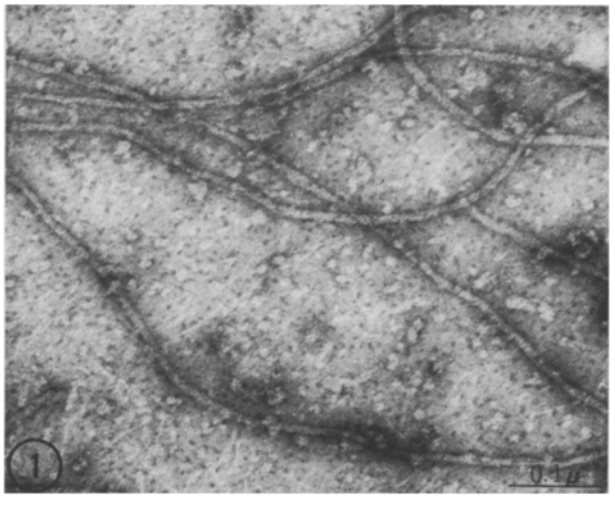
*Summary.* A preparation of intermediate filaments isolated from the chicken lens is enriched with a 50,000 dalton polypeptide.

The presence of intermediate filaments (10–12 nm in diameter) in the water-insoluble intracellular matrix of the vertebrate lens has previously been reported[2]. In this study a method for the isolation of these filaments is described.

Lenses obtained from 3–4-month-old chickens immediately after death were decapsulated and the epithelial and annular pad cells removed. The fibre mass was homogenized in standard salt solution (SEM) containing 0.1 M KCl, 0.005 M $MgCl_2$, 0.006 M sodium phosphate buffer pH 7.2 to which 0.01 M 2-mercaptoethanol was added. The homogenate was centrifuged for 20 min at 37,000 × g at 4 °C and the water-insoluble pellet retained. This pellet, which contains large numbers of filaments and the plasma membrane of the fibre cells, was washed repeatedly in the buffer with homogenization and centrifugation as previously described[3]. The pellet was then homogenized in 8 M-urea prepared with SEM and allowed to extract for 3 h. After centrifugation at 78,000 × g for 20 min at 10 °C, the urea-soluble fraction (8M-USF) was separated from the membrane-rich urea-insoluble pellet. The urea-soluble (8M-USF) was then exhaustively dialysed against frequent changes of SEM to remove the urea and is designated 8M-USFPD (PD = post dialysis). Filaments are soluble in 8M-urea but reform after removal of the urea[4]. The action of urea thus permits the separation of filaments from the urea-insoluble membrane material.

The 8M-USFPD was centrifuged at 37,000 × g for 15 min to remove membrane not pelleted by centrifugation of the original high density 8M-urea solution. The 37,000 supernatant was then centrifuged at 78,000 × g for 1 h



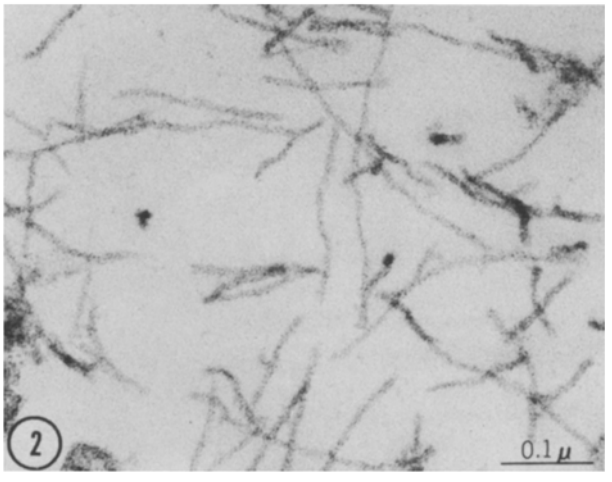




Fig. 1. Negative stain analysis of the 78,000 × g lens pellet shows mainly intermediate filaments. × 120,000.

Fig. 2. Section of the 78,000 × g lens pellet to show intermediate filaments. × 120,000.

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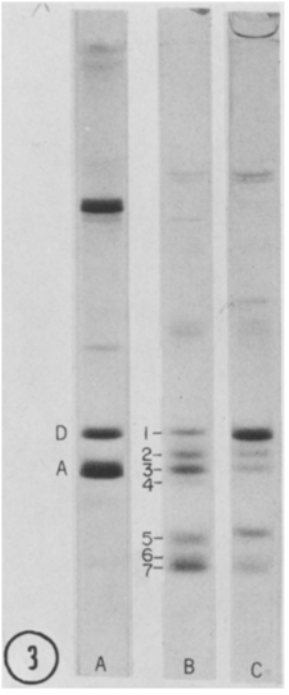


Fig. 3. Electrophoretic patterns in 5.13% polyacrylamide gels containing 1% sodium dodecyl sulphate. *A* Chick smooth muscle desmin (D) and actin (A). *B* Chick lens 8M-USF fraction. *C* Chick lens 78,000 × g pellet enriched with intermediate filaments.

at 4 °C in an SW 65K rotor in the L3-40 Beckman ultracentrifuge. A portion of the resultant pellet was fixed for electron microscopy, a portion was negatively stained with 1% uranyl acetate and the remainder electrophoresed in 5.13% polyacrylamide gel containing 1% sodium dodecyl sulphate[3]. Actin and desmin were isolated from chick gizzard smooth muscle as described by Lazarides and Hubbard[5]. The 8M urea-soluble fraction of gizzard smooth muscle which had been extracted with 0.6 M KCl, and 0.6 M Kl was used for gel analysis. Desmin represents the 50,000 dalton polypeptide that is considered a subunit of the intermediate filaments of chick smooth muscle[5].

Microscopic analysis showed that the lens 78,000 × g pellet consisted mainly of intermediate-sized filaments (10–12 nm in diameter) (figures 1 and 2). A few membrane profiles were observed in the 78,000 × g pellet, and free particles were evident on the negative stain. The electrophoretic patterns of smooth muscle actin and desmin, and of the lens 8M-USF and 78,000 × g pellet of intermediate filaments are shown in figure 3. The lens 8M-USF (figure 2, B) contains all the crystallin polypeptides (bands 2, 4, 5, 6 and 7) present in the lens water soluble fraction[3], and many noncrystallin components previously identified and characterized by mol. wt[3]. The most prominent noncrystallin components (bands 1 and 3) correspond in position to that of muscle actin and desmin. Although the 78,000 × g pellet contains the major polypeptides present in the 8M-USF, it is markedly enriched in the amount of band 1 of mol. wt previously estimated at 49,000 daltons[3].

These results strongly suggest that the intermediate filaments of the lens contain a polypeptide of mol. wt identical to that of desmin. Further study is required to determine whether the lens polypeptide and desmin are identical in biochemical structure.

## Association of DNA replication with nuclear membrane in larvae of *Chironomus thummi*[1]

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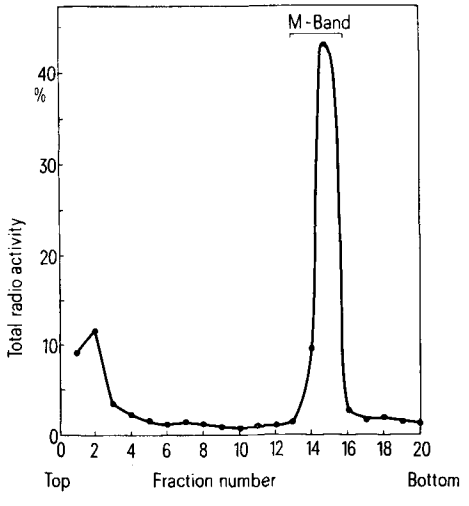
*Summary.* In the larvae of *Chironomus thummi*, the newly replicating DNA has been found to be associated with the nuclear membrane, as evidenced by the isolation of DNA nuclear membrane complexes (M-band) of $^3$H-thymidine labelled larvae.

In bacteria it has been shown that chromosomal DNA is attached to the cell membrane at various points, and DNA replication is intimately associated with the cell membrane[3,4]. Although there are a few studies indicating absence of any association between nuclear membrane and DNA replication in eukaryotic cells[5,6], several recent reports present compelling biochemical and electron microscopic evidence to suggest a close association between DNA replication and nuclear membrane in both animal and plant cells[7–11]. Thus scanning electron microscopic studies of the chromosomes within the nuclei of the salivary glands of the larvae of *Chironomus thummi* have revealed that the chromosomes are connected to the inner surface of the nuclear membrane[12]. In the experiments to be reported here, we have attempted to examine whether, in the larvae of *Chironomus thummi*, the newly replicated DNA is associated with the nuclear membrane complexes isolated by sedimentation in biphasic sucrose gradients using the M-band technique[13].

*Chironomus thummi* larvae (4th instar) were incubated for 24 h at 28 °C in distilled water containing 0.1 mCi/ml thymidine-methyl-$^3$H (sp. act. 6 Ci/mM) to label DNA. After incubation the larvae were washed, suspended in Krebs-Ringer-phosphate buffer (KRP)[14] containing 0.25 M sucrose and homogenized at 0–4 °C. The homogenate was filtered through a cheese cloth (8 layers). The filtrate was centrifuged at 500 × g for 10 min and the pellet was suspended in the same KRP-buffered sucrose solution. This suspension (1 ml) was layered on 10% Ficol in KRP (25 ml) and centrifuged at 3000 × g for 30 min. The pellet




Sedimentation profile of labelled DNA of nuclear lysate from *Chironomus* larvae. Total radioactivity of the nuclear lysate loaded on the gradient was 5.52 × 10$^4$ cpm.

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