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]

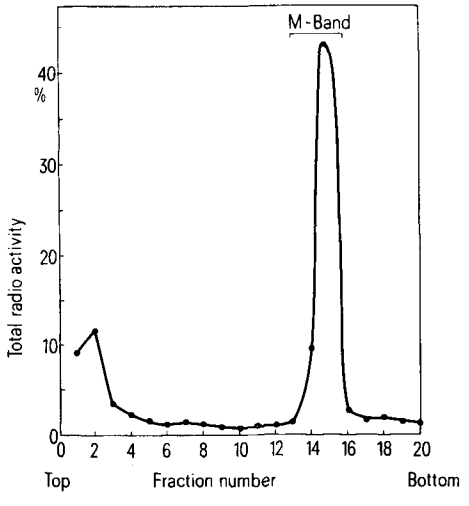
C. K. K. Nair and H. N. B. Gopalan[2]

Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Bombay 400 085 (India), 11 June 1977

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.

1 Acknowledgment. The authors thank Dr D. S. Pradhan for discussions and encouragement.

2 Botany Department, Kenyatta University College, Nairobi, Kenya.

3 A. Klein and F. Bonhoeffer, A. Rev. Biochem. *41*, 301 (1972).
4 M. L. Pato, A. Rev. Microbiol. *26*, 347 (1972).
5 J. A. Huberman, A. Tsai and R. A. Deich, Nature *241*, 32 (1973).
6 D. E. Comings and T. A. Okada, J. molec. Biol. *75*, 609 (1973).
7 C. E. Hilderbrand and R. A. Tobey, Biochim. biophys. Acta *331*, 165 (1973).
8 P. Binkerd, T. Roach and A. Toliver, Physiol. Chem. Physics *6*, 245 (1974).
9 A. Barrieux, L. Long and L. S. Garren, Biochim. biophys. Acta *312*, 228 (1973).
10 A. A. Infante, R. Nauta, S. Gilbert, P. Nohart and W. Firshein, Nature New Biol. *242*, 5 (1973).
11 W. F. Clay, F. R. H. Katterman and P. G. Bartels, Proc. nat. Acad. Sci. USA *72*, 3134 (1975).
12 W. Trosch and B. Lindemann, Micron *3*, 370 (1973).
13 G. Y. Tremblay, M. J. Daniels and M. Schaechter, J. molec. Biol. *40*, 65 (1969).
14 H. F. DeLuca and P. P. Cohen, in: Manometric techniques, p. 133. Ed. W. M. Umbreit, R. H. Burris and J. F. Stauffer. Burger Publishing Company, Minnesota, 1964.

Radioactivity in DNA of M-bands isolated from *Chironomus* larvae under different conditions

	Percent total ^{3}H-radioactivity (loaded on the gradient) associated with the M-band (± SD)
M-band isolated from *Chironomus* larvae after 2 h labelling with ^{3}H-thymidine	46 ± 2.6
with ^{3}H-thymidine followed by 8 h chase with cold thymidine (1 mg/ml)	16 ± 2.0

Each value is an average of 3 independent experiments. Total radioactivity of the nuclear lysate loaded on the gradient in these experiments ranged from $5–7 \times 10^4$ cpm.

contained nuclei free from other cellular debris. The nuclei so isolated were suspended in TMK buffer[12] (Tris 0.01 M, maganesium acetate 0.1 M and KCl 0.1 M, pH 7.2) and sonicated in an MSE ultrasonic disintegrator Model 60 W at 1.5 mA for 1 min at ice temperature. The sonicated nuclear lysate (0.5 ml) was overlayered on 0.5 ml of 0.2% sarkosyl (NL-97, Geigy) on a biphasic sucrose gradient in TMK buffer (15 ml of 20% sucrose over 5 ml of 60% sucrose shelf) and centrifuged in an MSE Superspeed 50 ultracentrifuge using an SW 3×23 ml rotor at 15,000 rpm at 4 °C for 30 min. A white layer of membrane-complex bound to magnesium sarkosyl crystals banded at the interphase of the sucrose solutions (M-band). After centrifugation, the contents of the tube were collected by siphoning and trichloroacetic acid-insoluble radioactivity in each fraction was determined[15] and expressed as percent of total radioactivity loaded.

The sedimentation profile of the ^{3}H-thymidine-labelled nuclear lysate of the *Chironomus* larvae in the biphasic sucrose gradient is shown in the figure. It can be seen that most of the labelled DNA is associated to the M-band sedimenting at the interphase of the sucrose solutions. Treatment of the nuclear lysate with deoxyribonuclease I (Worthington Biochemical Corporation) prior to sedimentation resulted in the removal of the radioactivity associated with the M-band indicating that the associated radiactivity was due to labelled DNA. Further it was found that most of the radioactivity (80%) was recovered in the thymidine-5′-monophosphate spot when the M-band material isolated from ^{3}H-thymidine labelled larvae was digested with DNase I and snake venom phosphodiesterase (Sigma Chemical Co., St. Louis, USA) and subjected to paper chromatography using isobutyric acid : liquor ammonia : water (66 : 1 : 33) as solvent mixture. To test whether or not the binding of the DNA with the M-band is due to extraneous factors, ^{3}H-labelled *Escherichia coli* DNA (both heat-denatured and native) was sedimented along with unlabelled nuclear lysate. No radioactivity due to bacterial DNA could be seen associated with the M-band. The radioactivity in the M-band decreased when the larvae were first labelled with ^{3}H-thymidine for 2 h and the label was then chased with cold thymidine for 8 h (table). This suggests that the newly synthesized DNA may be associated with the nuclear membrane. Taken together, the findings provide evidence which implies that in *Chironomus* larvae DNA replication may be associated with the nuclear membrane.

15 C. K. K. Nair, D. S. Pradhan and A. Sreenivasan, J. Bact. *121*, 392 (1975).

Isolation and properties of cell walls from *Agrobacterium tumefaciens* B_6[1]

D. Levanon[2]

Department of Biophysics, The Weizmann Institute of Science, Rehovot (Israel), 18 July 1977

Summary. Purified cell walls were prepared from *Agrobacterium tumefaciens* B_6 by extraction of intact cells with hot sodium dodecyl sulfate and digestion with proteases. Such preparations contained peptidoglycan that accounted for about 40% of their dry weight. Electron micrographs of the purified walls showed that they conserved their characteristic shape despite the drastic extraction procedure.

Agrobacterium tumefaciens, a gram negative rod, has long been known as the causative agent of crown gall disease in many plant tissues[3]. So far, only crude wall preparations from this organism have been described in the literature[4,5]. In the present study, electron microscopy of whole cells and of partially purified and purified walls of *A. tumefaciens* B_6 are presented along with their amino acid and amino sugar composition.

Materials and methods. Agrobacterium tumefaciens B_6 was obtained through the courtesy of Dr. A. C. Brown, Rockefeller University, New York. Bacterial cultures were grown on liquid medium that contained: casitone (Difco), 2%; yeast extract, 1%; K_2HPO_4, 0.5%, glucose, 0.2% and $MgSO_4 \cdot 7\ H_2O$, 0.01% at 30 °C. When they reached $^1/_4$ to $^1/_3$ log phase, they were quickly cooled and harvested. Crude walls were obtained by mechanical disruption of fresh or frozen cells[6]. Partially purified walls were obtained by extraction of the crude walls for 22h in boiling 4% sodium dodecyl sulfate[7] followed by trypsin digestion (in 0.05 M Tris-HCl pH 8.5 and 0.02 M $CaCl_2$, at 37 °C overnight) of the insoluble residue obtained after heating in SDS, and extensive washing in water. A second extraction cycle with hot 4% SDS and digestion with pronase (0.05 M Tris-HCl, pH 8.0 and 0.02 M $CaCl_2$, at 37 °C overnight) yielded purified cell walls.

For electron microscopy negatively stained samples of untreated cells or cell walls were deposited on copper grids (400 mesh) previously covered with parlodion membrane. After 1 min they were stained with 2% uranyl acetate and 0.2% lead citrate, and after an additional 45 sec a drop of octadecanol was added and the grids were immediately blotted dry and taken for examination in the Philips 300 electron microscope, operated at 80 kV. For thin sectioning, packed cells were mixed with an equal volume of 3% agar solution and the mixture taken up into narrow part of a Pasteur pipette. After solidification, the gel was exhaled and cut into 1 mm^3. The cubes were dehydrated by passing through a graded series of