antigorite and tremolite represent the late stage derivative. Opaques (magnetite) occur either as euhedral crystals or as clusters in the groundmass. Although there is no protolith mineral preserved in the breccia, nevertheless the dominant antigorite and carbonates as its major constituents suggest it to be derived from either peridotite or pyroxenite.

Opinions regarding the mode of emplacement of the ultramafic bodies in the AFB are divergent. Heron[3] observed them as sills. But later workers argued that these bodies either represent rift-related syn-sedimentary emplacement[2,4] or tectonic slices of obducted oceanic crust emplaced during the closing of the Aravalli basin[5]. The serpentinite and associated chert, carbonates and metabasalt of MMOM occur as tectonic slivers within the Jharol turbidites. Development of mylonite fabric both in the country rock and serpentinite as well as intense fracturing of the latter at the contact zone are the evidences suggesting tectonic emplacement. In the history of an ophiolite complex, serpentinization may occur in varied environments[6], namely before obduction or during obduction and emplacement and some alteration after its emplacement. In the MMOM, the ultramafic breccia is possibly formed after the serpentinization of the ultramafics as a consequence of the tectonic emplacement of the serpentinite during the closing of the Aravalli basin.

1. Gupta, S. N., Arora, Y. K., Mathur, R. K., Iqbaluddin, Balmiki Prasad, Sahai, T. N. and Sharma, S. B., *Publ. Geol. Surv. India*, 1980.
2. Roy, A. B., *Mem. Geol. Soc. India*, 1988, **7**, 3–32.
3. Heron, A. M., *Mem. Geol. Surv. India*, 1953, 79(1), 389.
4. Chattopadhyay, N. and Gangopadhyay, S., *Geol. Surv. India, Spl. Publn.*, 1984, **12**, 17.
5. Sinha-Roy, S., *Mem. Geol. Soc. India*, 1988, **7**, 95–108.
6. Prichard, H. M., *Contrib. Mineral. Petrol.*, 1979, **68**, 231.

ACKNOWLEDGEMENTS. We are grateful to Dr S. Sinha-Roy, for his constant encouragement and guidance. Suggestions of the referees helped in improving the text.

Received 14 September 1992; revised accepted 8 February 1993

Addition of lysine, an amino acid to fix fragile structures, cytoskeleton for electron microscopic studies

Sajid Husain* and Usha Luthra
Institute of Cytology and Preventive Oncology (ICMR), Maulana Azad Medical College Campus, New Delhi, 110 002 India
*Department of Microbiology, Guru Nanak Dev University, Amritsar 143 005, India

Addition of lysine in Karnovski's modified fixative for tissues fixing for electron microscopy gave better results in comparison to without addition of this amino acid. Cytoskeletal elements from the tissues of human uterine cervical carcinoma showed distinct identity of the cytoskeleton and good contrast. Otherwise cytoskeletal structures were not clear and looking like a broom having no identity.

ELECTRON microscope, which has proved a basic tool in different disciplines of science, has revolutionized our understanding of most of the cell structures. Conventional use of glutaraldehyde followed by osmium tetroxide do not fix cytoskeletal elements of a cell. It is seen that visualization and clear identity of the cytoskeletal elements become impossible unless added with some modifying agents. The contractile system of actin filaments and associated proteins are not fully fixed with the routine fixatives. Sometimes it is also seen that the lipids not rich in double bonds are not properly fixed and stained due to not reacting with glutaraldehyde or osmium tetroxide.

In the present study lysine was added to improve fixation and visualization of the cytoskeletal elements, particularly microfibres, which are of immense value in the diagnosis of the carcinoma of the uterine cervix. All confirmed cases of carcinoma *in situ* of human uterine cervix were fixed in modified Karnovski's fixative[1] having 4% formaldehyde, 1% glutaraldehyde and 50 mM lysine in 0.1 m sodium cacodylate buffer for 24 h at 4° C. Secondary fixation was done in ascending grades of acetone whereas propylene oxide was used as clearing agent. Blocks were prepared in araldite resin. The silvery sections were cut on the LKB ultramicrotome model IV. The sections were stained with uranyl acetate and lead citrate.

The performance of lysine in the glutaraldehyde buffer (Karnovski's) to fix cytoskeleton is reported by many workers[2,3]. The addition of lysine was found very effective in fixation and improving staining; the cell appears smoother with intact membrane; the nucleus with circular ring and nuclear pore; the rough endoplasmic reticulum showing fine arrangement of the ribosomes; the mitochondria with internal membrane having high contrast; golgi body showing distinction and clear contrast. Distinct microfibres distinguished like spoke with high contrast were visible otherwise

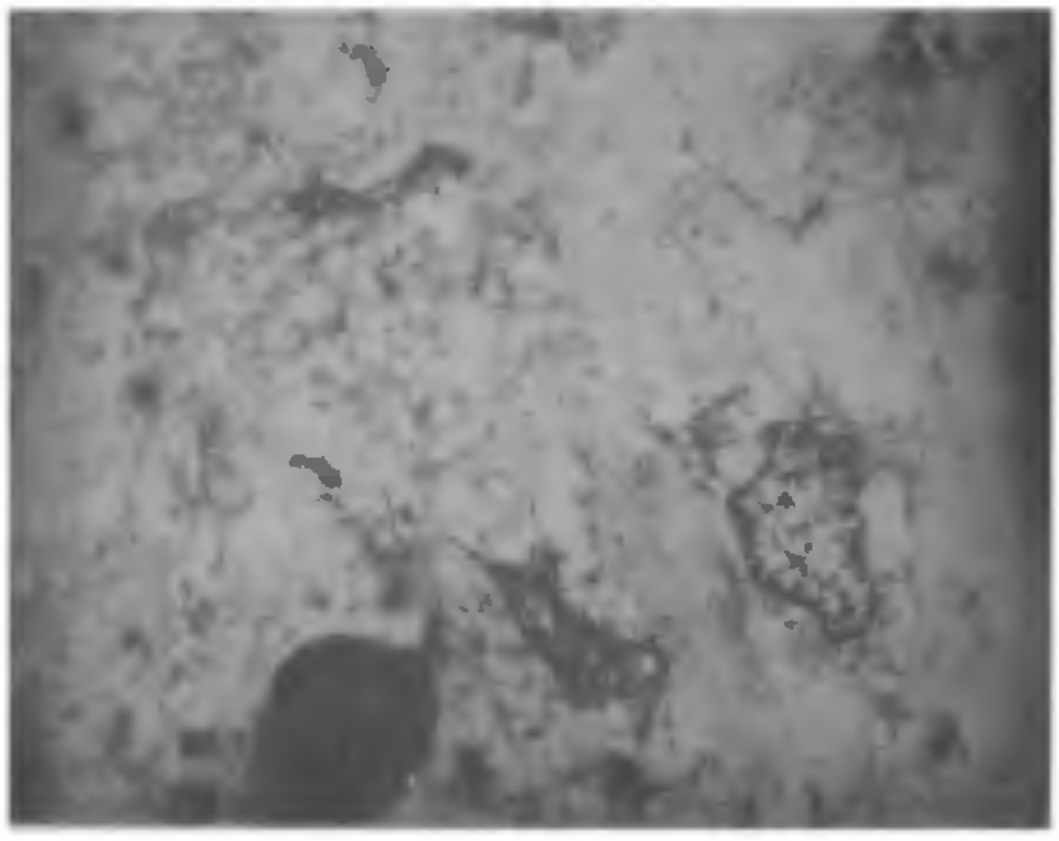

Figure 1. Uterine cervical tissue showing presence of cytoskeleton at 60 kV (×9700).

clumped together without any identity. The addition of lysine to triton extracted cytoskeleton of the cells which has been shown to be useful in demonstrating the nature of the cellular actin filaments. It has been shown by Swift *et al.*[4], that addition of lysine to the fixative allows the lipoprotein particles to be better visualized.

It is suggested that glutaraldehyde reacts with the lysine, forming complex pyridines and pyridiniums. These products are generally weak bases, capable of interacting with osmium tetroxide. During aldehyde fixation and staining weak bases might react with the osmium tetroxide. In aldehyde fixation amines of the tissue are affected. The effect of added amines may act to prevent some of the changes developed as a result of blockade of free amino groups of the tissue. The addition of lysine in glutaraldehyde fixation and its role in preserving internal cell structures were also reported by Boyles[5], Courtoy and Boyles[6].

1. David, G. F. X., Herbert, J. and Wright G. D. S., *J. Anat.*, 1973, **115**, 79.
2. Forbes, M. S., Rennels, M. L. and Nelson, E., *Am. J. Anat.*, 1977, **149**, 47–70.
3. Joyce N., Walter, U., De Camilli, P. and Boyles, J., *J. Cell Biol.*, 1981, **91**, 354a.
4. Swift, L., Soule, P. D., Grey, M. E. and Lequire, V. S., *J. Lipid Res.*, 1983
5. Boyles, J. K., *J. Cell Biol.*, 1982, **95**, 287a.
6. Courtoy, P. J. and Boyles, J., *J. Lipid Res.*, 1983, **83**, 258–273.

Received 18 January 1993; accepted 8 February 1993.

Restriction enzyme digestion patterns of DNA from an Indian isolate of equine herpes virus-1

P. K. Uppal, E. Plateau* and M. P. Yadav
National Research Centre on Equines, Sirsa Road, Hisar 125 001, India
*Laboratoire Central De Researches Veterinaires, Paris, France

The DNA fingerprints of EHV-1 isolate 'UPPAL', encountered in a respiratory disease outbreak in horses at Turf Club in India in 1989, were compared with two known abortigenic and one respiratory EHV-1 isolates using *Bam*H1 and *Pst*1 restriction endonucleases. All the four viruses comprising two from respiratory tract (UPPAL' and 'Sheva') and two from abortion cases (4056A and 14208A) had the same pattern as abortigenic strains. The two isolates, 14208A and Sheva were comparable whereas, 'UPPAL' and 4056A were close but not identical. However, minor differences observed in the *Bam*H1 as well as *Pst*1 fingerprint patterns can be expected as these viruses were of different epizootiological origin.

Four distinct herpes viruses, viz. equine herpes virus-1, 2, 3 and 4 (EHV-1, EHV-2, EHV-3, and EHV-4) have been isolated from horses[1,2]. EHV-1 is responsible for causing abortions in mares, still birth, neonatal foal mortality, paralysis and respiratory infections[3,4]. Two antigenic subtypes of EHV-1, viz. subtype-1 and 2, which were previously recognized on the basis of serological tests, major differences in structural proteins and restriction endonuclease cleavage patterns are now designated as EHV-1 and EHV-4 respectively[2,5,6]. EHV-1, though encountered both from respiratory as well as abortion and paralytic (myeloencephalitic) syndromes, is mostly associated with abortions. On the other hand, EHV-4 mainly causes respiratory disease but at times has been found to cause abortions as well[7].

Since both EHV-1 and EHV-4 can be isolated from the respiratory or abortion syndromes, laboratory tests are necessary to identify the type involved in a particular syndrome. Restriction endonuclease fingerprints of viral DNA not only provide a powerful tool to differentiate between these two EHV types but also an important basis for the study of molecular epizootiology of the disease as the two types have been shown to possess distinct fingerprints[5]. The present study was undertaken to compare the DNA fingerprints of a recent EHV-1 isolate, viz. 'UPPAL', encountered in a respiratory disease outbreak in race horses[8] during 1989 with two known abortigenic EHV-1 strains and one EHV-1 strain isolated from respiratory tract of a horse.

The isolates of EHV-1 analysed are listed in Table 1. These were grown in CCL-64 cell monolayers at a low multiplicity. The cells were maintained in RPMI 1640