of *K. barbitarsis* as estimated by this method. Assimilation (60.3%) and net production (Pe_2) efficiencies (66.4%) of *K. barbitarsis* are far higher than those reported for other aquatic detritivorous insects fed on nutritionally poor detritus organic matter and the causative factors have been discussed in detail elsewhere[13].

Data on consumption, egestion and assimilation of *Chlorella* detritus obtained by GCT and gut content weight method, gravimetric method ($P+F+U+R$), and isotopic tracer method are provided in Table 3. The differences between the estimates of consumption and assimilation by these methods were less than 7%. Statistical analysis revealed that the differences between these values were not significant ($P>0.5$) (Table 3). Therefore, the proposed method, which considers GCT and faecal energy is recommended for the precise estimation of food consumption of detritivores like *K. barbitarsis*.

1. Waldbauer, G. P., *Adv. Insect Physiol.*, 1968, **5**, 229–282.
2. Scriber, J. M. and Slansky, F. Jr., *Annu. Rev. Entomol.*, 1981, **26**, 183–211.
3. Slansky, F. Jr. and Scriber, J. M., in *Comprehensive Insect Physiology, Biochemistry and Pharmacology* (eds. Kerkuit, G. A. and Gilbert, L. I.), Pergamon Press, Oxford, 1985, pp. 87–164.
4. Muthukrishnan, J. and Pandian, T. J., *Animal Energetics. vol. 1. Protozoa through Insecta* (eds. T. J. Pandian and John Vernberg), Academic Press, New York, 1987, pp. 374–490.
5. Holter, P., *Oikos*, 1973, **24**, 117–122.
6. Johannsson, O. E., *Can. J. Fish. Aquat. Sci.*, 1980, **37**, 1254–1265.
7. Ladle, M. and Hansford, R. G., *Hydrobiologia*, 1981, **78**, 17–24.
8. Dermott, R., *Hydrobiologia*, 1981, **83**, 499–503.
9. Cairns, S. C., *Oecologia (Berl.)*, 1982, **55**, 62–68.
10. Wightman, J. A. and Whitford, D. N. J., Proc. 2nd Australias. Conf. Grassl. Invertebr. Ecol. (eds. Crosby, T. K. and Pottinger, R. P.), Govt. Printers, Wellington, New Zealand, 1979, pp. 32–34.
11. Testerink, G. J., *Ecol. Entomol.*, 1982, **7**, 341–351.
12. Zimmerman, M. C. and Wissing, T. E., *Advances in Ephemeroptera Biology* (eds. Flanagan, J. F. and Marshall, K. E.), Plenum Press, New York, 1980, pp. 231–257.
13. Palavesam, A. and Muthukrishnan, J., *Archiv. Hydrobiol.*, 1993, (in press).

ACKNOWLEDGEMENTS. One of us (A.P.) is grateful to CSIR, New Delhi for the award of a fellowship. We thank Prof. P. K. Chaudhuri (Burdwan) for identification of the insect.

Received 4 June 1991; revised accepted 29 October 1992

Electron microscopic observations of the Indian isolate of equine infectious anaemia virus grown in equine leucocytes

P. K. Uppal and M. P. Yadav

National Research Centre on Equines, Sirsa Road, Hisar 125 001, India

We have used electron microscopy to demonstrate the presence of viral particles in the leucocytes of naturally infected active symptomatic and asymptomatic equine infectious anaemia-(EIA) carrier horses as well as in the primary leucocyte cultures obtained from a seroconverted asymptomatic donkey after its experimental infection with the blood, spleen and lymph node materials of asymptomatic-carrier horses. Typical retroviral particles resembling to EIA virus were observed in various developmental stages in both asymptomatic and symptomatic cases.

THE occurrence of equine infectious anaemia (EIA) was reported in India in 1987 in Thoroughbred horses[1]. The disease, first suspected clinically, was confirmed serologically by demonstration of specific antibodies by Coggins' test[1] as well as by C-ELISA[2]. There had been apprehensions in the equine industry regarding the infectiousness of asymptomatic EIA-carrier horses. Therefore, the purpose of the present study was to demonstrate the EIA virus by electron microscopy in the ultrathin sections of the leucocytes of asymptomatic seropositive-carrier horses.

Buffy coat cells from active or asymptomatic seropositive natural cases of EIA in horses and 9 days old *in vitro* primary leucocyte cultures of a donkey infected experimentally with blood, spleen and lumph node material from asymptomatic EIA horses as well as from a EIA seronegative healthy pony were prepared as described elsewhere[3].

For preparation of the material for electron microscopy, the buffy coat cells/leucocytes were centrifuged at 2500–3000 rpm for 30 min in the refrigerated centrifuge (Remi K-70). The pellet obtained was put in 3% glutaraldehyde solution and stored at 4°C. Thereafter, the material was transferred into 2% osmium tetroxide, pH 7.2, for 4–5 h till it became brownish to black. After dehydration in ascending grades of acetone (25, 50, 75 and 100%), the material was kept in 2% uranyl acetate for 15 min for staining.

The material was dehydrated in ascending grades of acetone with embedding mixture (50, 75, 100%) keeping for 30 min in each step. The material was then transferred in the centre of the bottom of gelatin capsule. The capsule was filled with 100% embedding solution and kept at 60°C for 36–48 h. When the preparation became solid, sections of 1–2 millimicron thickness were cut, stained with methylene blue for 30 sec and examined under a light microscope. From the selected portion retained on the block, thin sections (60–80 nm thick) were cut, picked upon 200 mesh

unsupported copper grids and stained with uranyl acetate and lead citrate[4].

The specimens thus prepared were examined with a JEOL 100CXII electron microscope at the Department of Pathology, Laboratoire Central De Researches Veterinaires, Paris.

All the three samples processed from EIA-positive animals, comprising one each from active EIA horse, asymptomatic EIA seropositive horse and leucocyte culture from experimentally infected seropositive donkey showed the presence of characteristic retrovirus particles indistinguishable from human immunodeficiency virus (HIV) in morphology. The particles were observed in all the cases in cytoplasmic vacuoles and within the

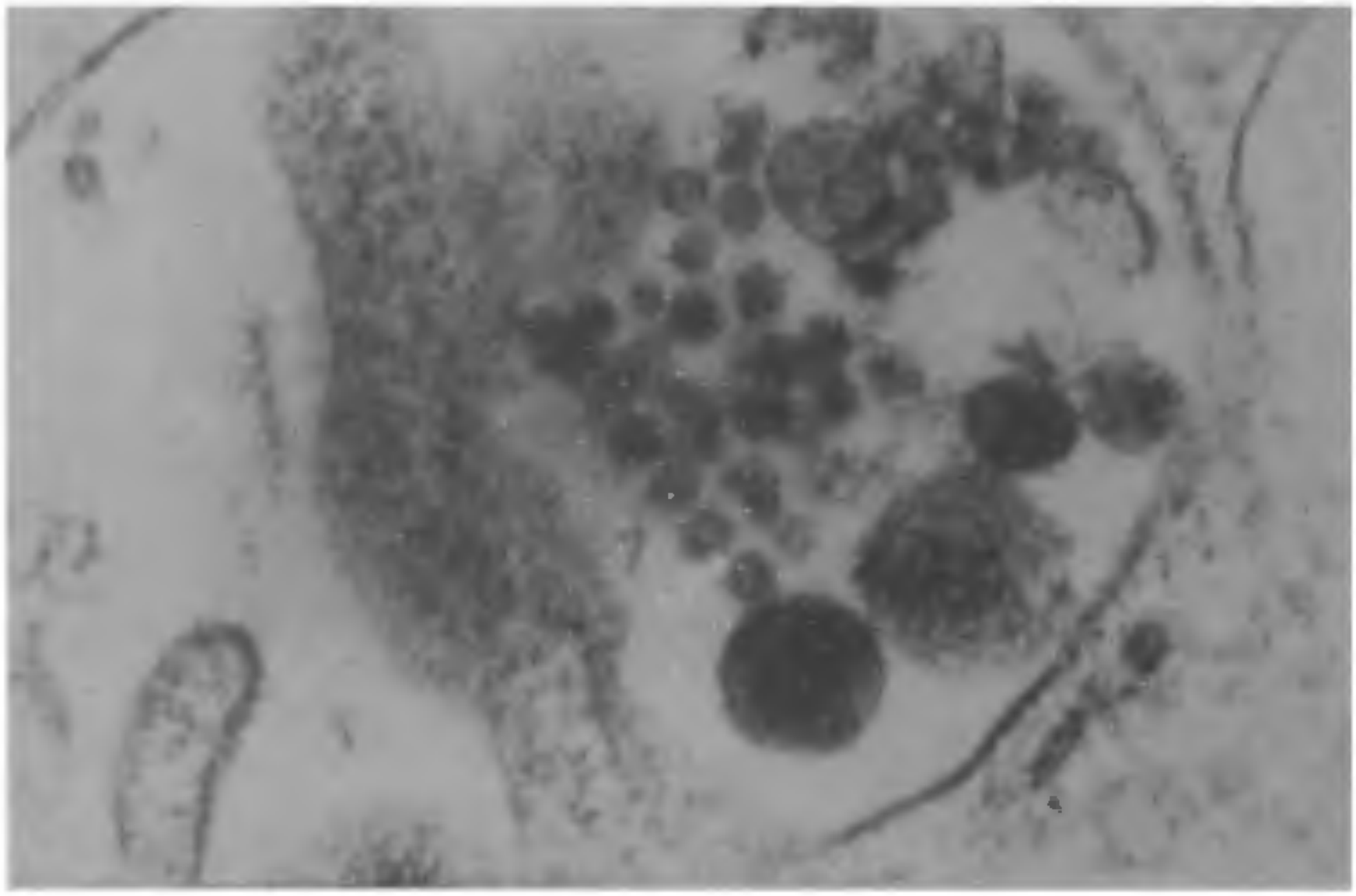

Figure 1. Electron micrograph of mature virus particles of equine infectious anaemia in the cytoplasm of lymphocyte.

Figure 2. (*a*) Electron micrographs of immature virus particle in the process of budding through plasma membrane, (*b*) and (*c*) polygonal to round mature virus particles attached to the plasma membrane.

cytoplasmic membrane (Figure 1). The virus particles of varying sizes were observed. The shape was polygonal to round. Both mature and immature virus particles were observed in the process of budding from plasma membrane (Figure 2). The virus particles were more in the *in vitro* cultured cells compared to the leucocytes obtained from naturally infected horses. Similarly, the mature virus particles were seen in large numbers in the cytoplasm of *in vitro* cultured cells.

Mature virus particles after budding were also observed exterior to the plasma membrane. The mature enveloped EIA viral particles at the cell surface showed electron dense core and were found under the bulging plasma membrane. The virus particles which were of crescent shape in the initial stages assumed round shape subsequently. Most of the particles showed electron dense shell. The morphological features and process of budding were similar to those described by other workers for EIAV[5–7] and HIV. No virus particles were observed in the electron micrograph of buffy coat cell preparation from EIA-negative healthy pony.

It is thus apparent that both active and asymptomatic EIA horses had typical retrovirus particles in leucocytes. The *in vitro* grown leucocytes of a seroconverted but asymptomatic donkey which was experimentally infected with the materials from EIA-carrier horse also showed the presence of the virus particles, thus confirming the serological diagnosis.

1. Uppal, P. K. and Yadav, M. P., *Vet. Rec.*, 1989, **124**, 514–515.
2. Uppal, P. K. and Yadav, M. P., *Curr. Sci.*, 1992, **63**, 332–333.
3. Uppal, P. K. and Yadav, M. P., Annual Report, National Research Centre on Equines, Hisar, 1988, p. 33.
4. Uppal, P. K. and Yadav, M. P., Annual Report, National Research Centre on Equines, Hisar, 1989–90, pp. 35–36.
5. Kono, Y. and Kobayashi, K., *Arch. Gesamte Virusforsch.*, 1969, **28**, 411–416.
6. Weiland, F., Matheka, H. D., Coggins, L. and Hartner, D., *Arch. Virol.*, 1977, **55**, 335–340.
7. Gonda, M. A., Charman, H. P., Walker, J. L. and Coggins, L., *Am. J. Vet. Res.*, 1978, 731–740.

Received 14 October 1992; accepted 19 October 1992