The Haemocytes of the Mosquito *Aedes albopictus* and their Comparison with Larval Cells Cultured in vitro

In cell cultures it is extremely difficult to determine which cells from the parent tissue proliferate in vitro, mainly because the morphology and behaviour of the cells change remarkably when they are put to in vitro conditions. The degree of morphological variation increases proportionately with the period for which the cells are kept in vitro[1]. In some young primary cultures, the morphological features could to some extent be considered to refer to their origin in the parent tissue. Though many cell lines have been established from various species of mosquitoes, very little is known about the origin of the cells proliferating in vitro in these cultures. KITAMURA[2] reported that the cells proliferating in ovarian cultures of *Culex molestus*, *Aedes albopictus* and *A. aegypti* were probably derivatives of haemocytes. In our laboratory tissues from different species of mosquitoes are grown[3–6]. Thus, it is extremely difficult to determine the origin of the cells that proliferate in these cultures. However, the general morphology and behaviour of the cells growing in *Aedes albopictus*, *A. w-albus* and *A. novalbopictus* cell

[1] E. N. WILLMER, *Cells and Tissues in Culture* (Academic Press, London, New York 1965), Vol. 1.
[2] S. KITAMURA, Kobe J. med. Sci. *12*, 63 (1966).
[3] K. R. P. SINGH, Curr. Sci. *36*, 506 (1967).
[4] U. K. M. BHAT and K. R. P. SINGH, Curr. Sci. *39*, 388 (1970).
[5] K. R. P. SINGH and U. K. M. BHAT, Experientia, *27*, 142 (1970).
[6] U. K. M. BHAT and P. Y. GURU, Exp. Parasit. *33*, 105 (1973).

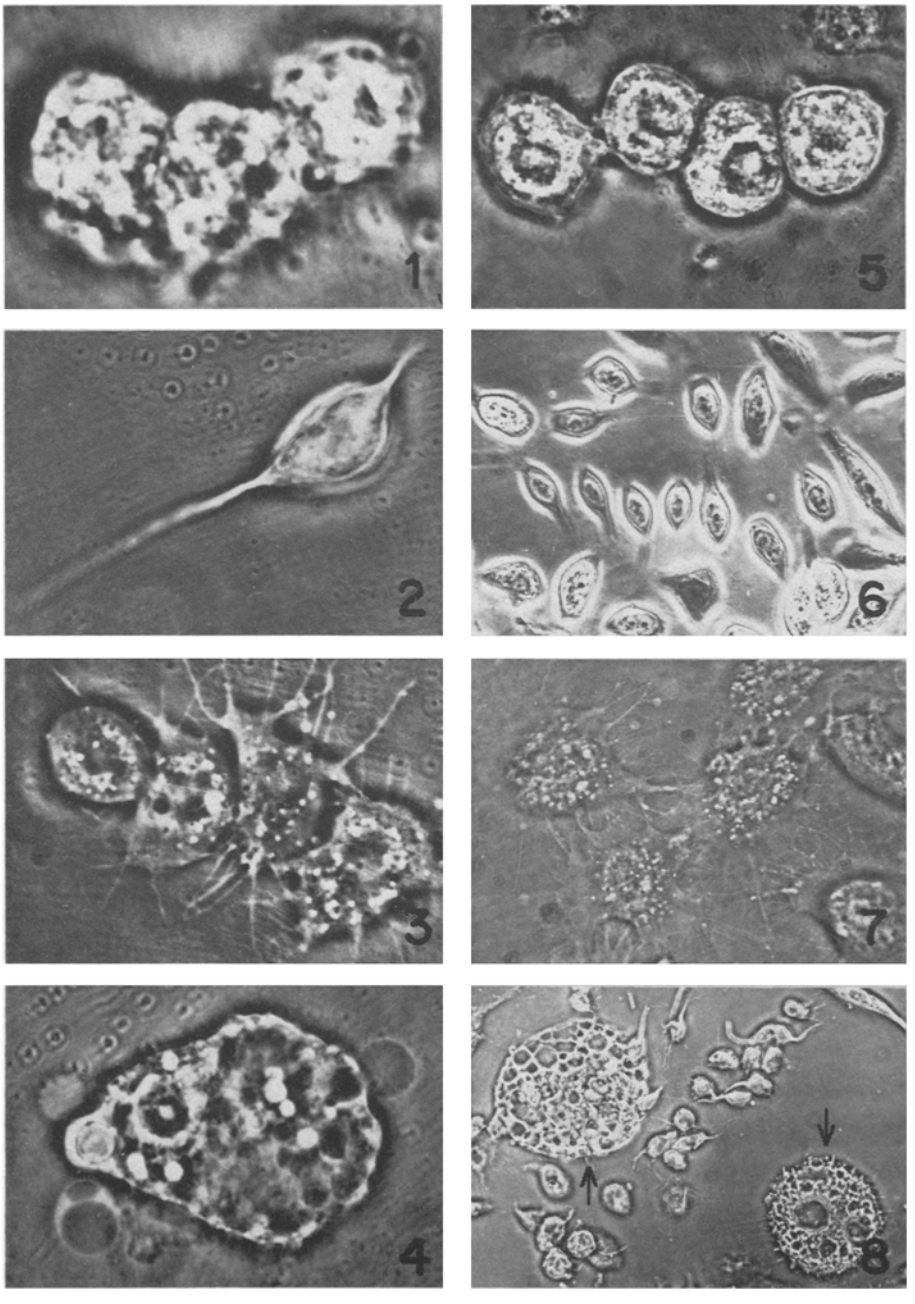

Fig. 1–4. Haemocytes of larvae of *Aedes albopictus* (all living, phase-contrast). 1. Prohaemocytes. ×2,250. 2. Plasmatocyte. ×1,125. 3. Podocytes, small. ×1,350. 4. Spherule. ×1,200.
Fig. 5–8. Some common types of cells growing in larval cell culture of *Aedes albopictus* (all living, phase-contrast). 5. Type A, epithelial-like cells. ×1,500. 6. Type A, fibroblast-like cells. ×337. 7. Type B, epithelial-like cells. ×900. 8. Round vacuolated cell. ×600.

Percentage of the haemocytes and the corresponding cell types of *Aedes albopictus* primary culture

Haemocytes	%	Cell types from primary culture	%
Prohaemocyte	60–70	Type A (ephithelial-like)	10–20
Podocyte, small	15	Type B (epithelial-like)	50–70
Podocyte, large	10	Type C (epithelial-like)	5–10
Plasmatocyte	10	Type A (fibroblast-like)	5–10
Spherule	1–2	Round vacuolated	< 1

cultures resemble the haemocytes and haemocyte cultures of other insects[7–11]. As no published information is available on the haemocytes of any of the mosquitoes, we studied and compared the haemocytes of the mosquito *A. albopictus* with the cells of the same mosquito cultured in vitro.

Fourth instar larvae of *A. albopictus* reared in the laboratory were washed twice with glass distilled water and kept in a drop of medium[3] on a glass slide. The heart of the larva, which was clearly visible through the cuticle, was ruptured at the thoracic region with the help of 2 fine-pointed needles. By applying slight pressure, starting from the posterior to anterior end, the haemolymph was made to flow out into the medium. Haemolymph from 4–5 larvae provided an adequate number of haemocytes. Live haemocytes were studied under phase-contrast illumination. Haemocytes that attached to the glass were also studied after staining with Wright-Giemsa stains. Haemocytes were classified according to the descriptions given by MUNSON[12]. The percentage of different types of haemocytes was calculated from 142 cells scored in the drop of medium in which the haemocytes from 5 larvae were collected.

Five types of haemocytes were detected in *A. albopictus* larval haemolymph. They were prohaemocytes and plasmatocytes, 2 types of podocytes and spherules.

Prohaemocytes were generally spherical with well defined cell boundary (Figure 1), and measured upto 8 µm in diameter. Cytoplasm was densely granular and compact. Each cell had a prominent nucleus and a nucleolus. They formed 60–70% of the total haemocytes observed.

Plasmatocytes were oval or fusiform (Figure 2), and had compact cytoplasm with distinct cell boundary. They measured about 6 µm at the widest point. They formed about 10% of the total haemocytes.

Both the types of podocytes were thin and flat with finger-like cytoplasmic processes. They readily attached to the glass surface. Cytoplasm was thinly granular and the cell boundary was diffuse. Each cell had a prominent nucleus and a nucleolus. They formed about 20% of the total haemocytes. The first type of podocyte (Figure 3) was in majority. They were smaller (upto 15 µm diam.) and had a large number of cytoplasmic processes. The second type was larger (upto 30 µm diam.) and contained a few vacuole-like cytoplasmic inclusions. They had fewer cytoplasmic processes than the first type.

Spherules were fewer in number, 1–2% of the total number of haemocytes observed. They were spherical and their cytoplasm had many vacuole-like inclusions of varying sizes (Figure 4).

A comparison of different types of haemocytes of *A. albopictus* and the cells growing in its larval cell cultures indicated that the prohaemocytes, two types of podocytes, plasmatocytes and spherules of larval haemolymph corresponded with the types A, B and C epithelial-like cells, type A fibroblast-like cells and vacuolated round cells of the culture respectively (Figures 5–8)[13]. Even the relative abundance of the different types of haemocytes, in many instances, was comparable to those of corresponding cell types in primary cultures (Table). However, the several other types of cells observed in primary cultures of *A. albopictus* could not be correlated with the haemocytes. In all primary cultures only a few types of cells were detected during the first one or two days of culture. On subsequent days larger numbers of cell types were detected in these cultures. Therefore, it is possible that some of the haemocytes observed in the larvae change their morphology and behaviour and subsequently appear as distinct types in cultures. Frequent locomotory and phagocytic activities of the cells growing in cultures of *A. albopictus* larval cells[13] and their morphological resemblance to the larval haemocytes further support the hypothesis that the cells proliferating in these cultures were the derivatives of haemocytes.

Summary. Five types of haemocytes were detected in the haemolymph of *Aedes albopictus* larva. Their comparison with the cells growing in the larval cell cultures of this mosquito indicated that the prohaemocytes, two types of podocytes, plasmatocytes and spherules of larval haemolymph correspond with the types A, B and C epithelial-like cells, type A fibroblast-like cells and vacuolated round cells in the culture respectively.

U. K. M. BHAT[14] and K. R. P. SINGH[15]

Virus Research Centre,
Indian Council of Medical Research, P.O. Box 11
Poona 411001 (India), 1 April 1975.

[7] J. C. JONES, J. Morph. *99*, 223 (1956).
[8] J. C. JONES, Am. Zoologist *2*, 209 (1962).
[9] J. MITSUHASHI, Nature, Lond. *215*, 863 (1967).
[10] J. CHAO and G. H. BALL, Curr. Topics Microbiol. Immun. *55*, 28 (1971).
[11] S. S. SOHI, Can. J. Zool. *49*, 1355 (1971).
[12] S. C. MUNSON, *Insect Physiology* (John Wiley & Sons, Inc, New York 1953).
[13] U. K. M. BHAT and K. R. P. SINGH, Indian J. exp. Biol. *9*, 153 (1971).
[14] Present address: U. K. M. BHAT, NIH Visiting Fellow, Rocky Mountain Laboratory, Hamilton, Montana 59840, USA.
[15] Present address: K. R. P. SINGH, Senior Scientist, WHO/ICMR Project on Genetic Control of Mosquitoes, 2-Ring Road, Kilokri, New Delhi-110014, India.