CYTOLOGY

Establishment of a Line of Cells from the Silkworm *Bombyx mori*

FOLLOWING the establishment of lines of cells from tissues of the moth *Antheraea eucalypti* Scott[1] and the mosquito *Aedes aegypti* L.[2], a line of cells from the silkworm *Bombyx mori* L. has now been established.

The method of culturing the cells and the medium were the same as for the *A. eucalypti* cells[1]. All the cultures were incubated at 29°–30° C. The cultures were set up in 1964 and consisted either of six whole ovaries torn apart or of dissociated cells from six ovaries suspended in 3·0 ml. of medium in Petri dishes. The ovaries were obtained from larvae which had started to spin their cocoons. Of the six cultures of whole ovaries, five died after 6 months and one survived for 12 months after it had been set up.

In the cultures of dissociated cells, most cells became attached to the substrate within 12 h. During the next 4–5 days many cells died, but by the tenth day the cultures were extremely healthy and dividing cells were common. Some cells formed sheets, very like syncytia, which attached firmly to the substrate. Short lengths of undissociated ovariole showed muscle contractions at 12 days. The sheaths surrounding these pieces of ovariole formed long processes that contracted regularly (Fig. 1). At the end of 6 months only two of the cultures had survived. Three months later, cells in one of these cultures rapidly increased in number. Most cells did not attach to the substrate. Two weeks later a subculture was made by transferring the cells to a new culture vessel along with half the medium. The remaining culture died 2 months later. The culture which had "transformed" and the subcultures continued to proliferate and further subcultures were made at 2–3 week intervals for 3 months. Since this time subcultures have been made at 6 day intervals.

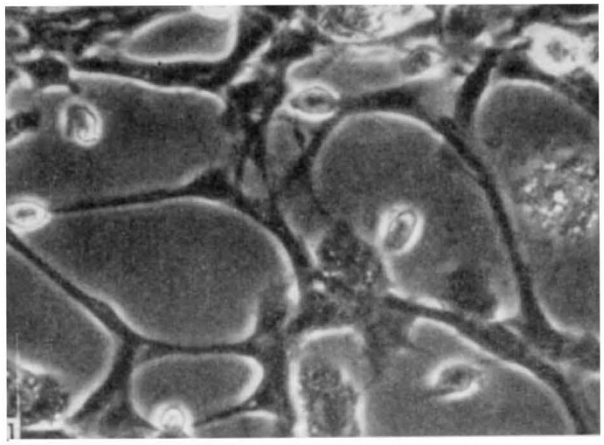

Fig. 1. Protoplasmic extensions which have formed a network, in a culture of silkworm ovarian cells. The network was contractile, 8 months in culture.

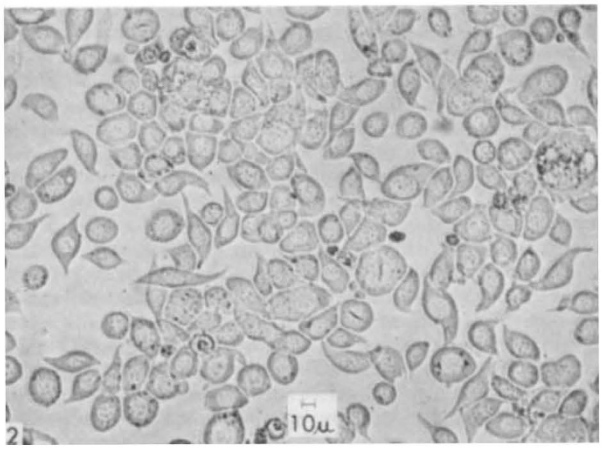

Fig. 2. Cells from the established line of silkworm cells 29 months in culture.

The most common type of cell in the line is spindle-shaped, 12–25μ wide and 50–70μ long. The only other type of cell significant in number is slightly spindle-shaped to about 18–30μ in diameter (Fig. 2). Many of these latter cells form small sheets on the surface of the culture vessel. The network of contracting tissue persisted after the "transformation", but by 16 months only single cells remained.

The generation time of the cells, measured 2 yr after the culture was set up, was 48 h. The cells have been adapted to grow in medium containing 1 per cent bovine plasma albumin and 1 per cent heat treated haemolymph from *Antheraea pernyi* (Guer.). The cells have been deep frozen in medium containing 10 per cent glycerol and have proved to be still viable after 10 months at −180° C.

Although the diploid number of chromosomes in the silkworm is 56, most cells examined 15 and 18 months after the line was established contained many more than 100 chromosomes.

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1 Grace, T. D. C., *Nature*, **195**, 788 (1962).
2 Grace, T. D. C., *Nature*, **211**, 366 (1966).

Metaphase Arresting Compounds in Embryos

COLCHICINE, demecolcin (N-desacetyl-N-methylcolchicine) and vinblastine are all metaphase arresting agents[1]. The first two compounds have been used extensively to determine various parameters of cellular dynamics, such as the mitotic index and the turnover time. In adults good agreement has been found between data obtained using colchicine and isotopes[2]. Vinblastine is not used to examine these cell parameters, probably because it has been shown to affect DNA synthesis[3]. The effect of these compounds on embryonic rats *in utero* has been examined. In this study, we found that the mitotic index obtained using the different compounds differed profoundly.

Mitotic indices were obtained by counting one thousand cells in sections of the lining of the cerebral ventricles of twelve day embryo rats after administering the metaphase arresting agent intraperitoneally to the mother. The animals were killed 4 h after the administration of the drug. The number of mitotic figures found was expressed as cells in division per hundred cells over a period of 4 h. Nine embryos from three litters were examined at each dose level. In the absence of any metaphase arresting agent 7·4 per cent of the cells of the inner surface of cerebral ventricles were found in various stages in division.

Wegner *et al.*[4] using ^{3}H-thymidine reported that the generation time in different ectodermal and endodermal tissues in the rat embryo varied between 12 and 18 h. They also noted that the source of variation was in the $G1$ period, the S, $G2$ and M periods being fairly constant. The mitotic time was 1 h. We used this figure in this experiment and a generation time of 13·5 h is found. If the generation time is 13·5 h, then some 30 per cent of the cells should be found blocked in division after 4 h treatment with the arresting agent. The data pre-

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