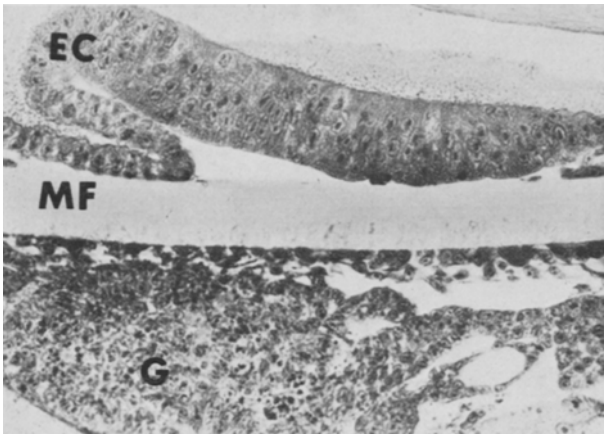

Fig. 4. Cellular processes extending into a Millipore filter (MF) incubated for 24 h in a chick embryo between a graft of Hensen's node (G) and the host ectoderm (EC). Note that more processes emerge from the graft towards the host ectoderm than vice versa, and that neural induction has occurred.

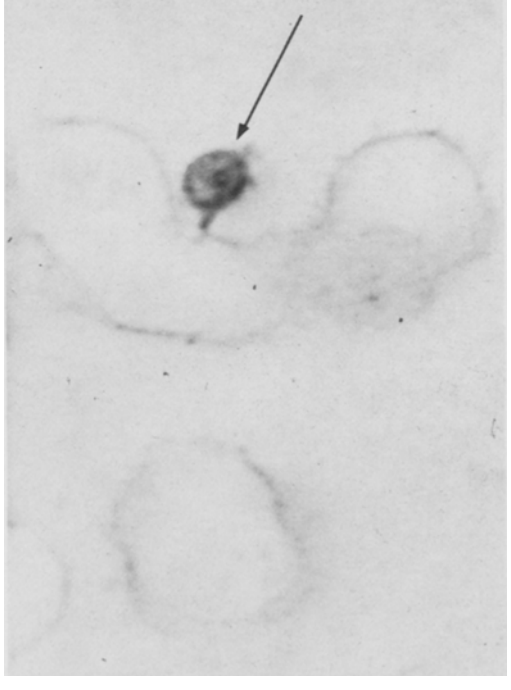

Fig. 5. A Millipore filter following immersion in a solution of saline and Hensen's nodes cut into smaller pieces. The filter is filled with cytoplasmic droplets (arrow). ×185,000.

remain in the filter, however, and are present even after 16–24 h incubation (Figure 3).

Many cellular processes can be seen by light microscopy in both of the filter types incubated 24 h in the chick embryos. Usually more processes emerge from Hensen's node toward the host ectoderm than vice versa. Several of the processes appear to project into the filter to about half of the thickness of the filter (Figure 4). The remainder of the filter appears to have darkened contents.

In the electron microscope the pores of the filters in the chick embryos are clearly outlined and cellular processes are present in the pores adjacent to the node and host ectoderm. Throughout these filters small droplets of cytoplasm were present.

In addition, stage 4 Hensen's nodes were excised and minced in saline. Millipore filters were added to this preparation and incubated at 37.5 °C for 10 min. The pores of the filters were outlined and cytoplasmic droplets similar in appearance to those in Figure 3 were present throughout the filter (Figure 5).

Microsurgery causes damaged cells and cell debris. This cell debris appears as cytoplasmic droplets and readily passes into the filters. Cellular processes can also extend from cell populations on either side of the filter and contact this debris without penetrating very far into the intervening filter. Thick membrane filters do not, therefore, prevent cellular contact[11].

Résumé. La microchirurgie cause des dommages aux cellules. Les débris cellulaires ont l'apparence de gouttelettes cytoplasmiques qui pénètrent dans les filtres Millipore. Dans un système d'induction neurale primaire, les polpulations de cellules d'un côté ou de l'autre du filtre étendent des processus cellulaires qui prennent contact avec ces débris sans pénétrer très loin dans le filtre. Par conséquent même les filtres épais n'empêchent pas le contact cellulaire.

MARJORIE A. ENGLAND

Department of Anatomy, University of Leicester, University Road, Leicester LE1 7RH (England),
2 October 1974.

[11] I should like to thank Miss V. COWPER, the Bedford College Electron Microscope Unit, London, who kindly produced the stereoscanning photographs.

Some Ultrastructural Observations on the Cytotoxicity of an Alcoholic Extract of *Suberites inconstans* on HeLa Cells

Some marine sponges are known to produce antitumour substances[1]. In our studies of local marine sponges, we have shown that the species, *Suberites inconstans*, contained an alcohol-soluble principle which was cytotoxic to HeLa cells[2]. This alcoholic extract, at a concentration of 60 µg/ml and above, irreversibly inhibited the growth of HeLa cells; and by means of phase contrast microscopy, it was shown that cell death was caused by the rupture of both nuclear and cell membranes. This paper describes further studies of the cytotoxic effect of this sponge extract on HeLa cells at the ultrastructural level and attempts to correlate the electron microscope observations with those of our previous results.

Materials and Methods. An alcoholic extract was made of the sponge, *Suberites inconstans*, as described previ-

[1] R. F. NIGRELLI, M. F. STEMPIEN JR., G. D. RUGGIERI, V. R. LIGUORI and J. T. CECIL, Fedn. Proc. *26*, 1197 (1967).
[2] C. H. TAN, C. K. TAN and Y. F. TEH, Experientia *29*, 1373 (1973).

ously[2]. HeLa cells grown in Beem capsules were treated with the extract and processed for electron microscopy as described by TAN et al.[3]. The cells were exposed to the extract at a concentration of 120 µg/ml of McCoy's 5a medium for 30, 60 and 120 min respectively. Control cells were exposed for similar times with McCoy's 5a medium containing 0.5% ethanol.

Observations. No deleterious effects were observed when HeLa cells were grown in McCoy's 5a medium containing 0.5% ethanol for 30, 60 or 120 min (Figure 1). Many cells in various stages of division were observed. In cells which had been exposed to an alcoholic extract of the sponge for 30 min, only the mitochondria showed vacuolation and loss of cristae (Figure 2). After being exposed to the extract for 60 min, many of the cells showed rupture of the nuclear membrane with extrusion of nuclear material into the cytoplasm (Figure 3, double ringed arrows). None of the usual cytoplasmic organelles could be identified with certainty. Many vesiculated profiles (Figure 3, V) were present in the cytoplasm. Dense bodies (Figure 3, DB) and lipid droplets (Figure 3, L) did not appear to be affected. These cytoplasmic structures tended to accumulate around the nucleus and leave a clear zone at the periphery (Figure 3, CZ). After 120 min of exposure to the extract, the cells showed rupture of both the nuclear (Figure 4, double ringed

[3] C. K. TAN, H. L. CHAN and C. H. TAN, submitted for publication (1974).

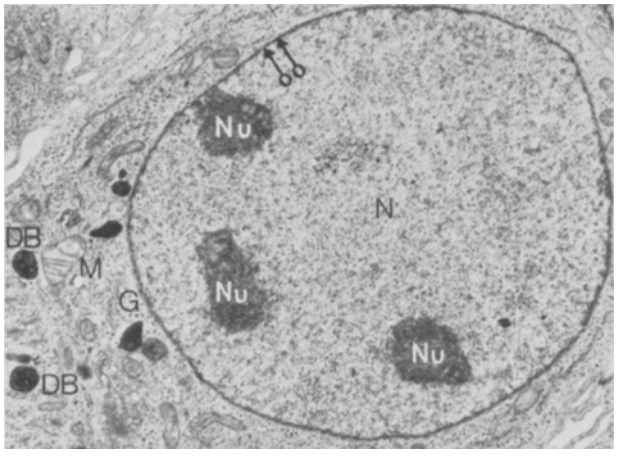

Fig. 1. Control. HeLa cell in McCoy's 5a medium without sponge extract. Note, well-preserved nucleus (N) with 3 prominent nucleoli (Nu) and an intact nuclear membrane (double ringed arrows). The cytoplasm contains a prominent Golgi complex (G), mitochondria (M) and several dense bodies (DB). ×8,400.

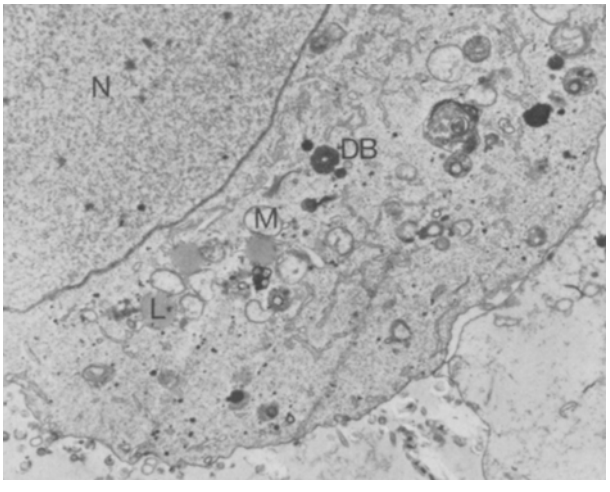

Fig. 2. 30 min after exposure to sponge extract. Mitochondria show vacuolation and loss of cristae. The cell and nuclear membranes appear intact. Nuclear morphology still appears unaffected. ×12,600.

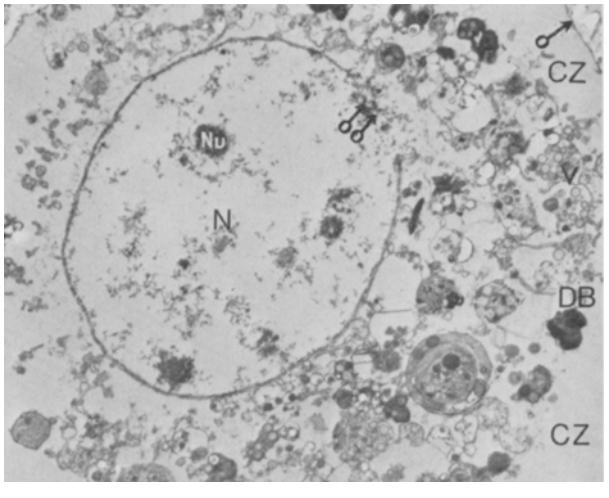

Fig. 3. 60 min after exposure to sponge extract. The nucleus shows loss of nucleoplasm (N) and nucleolus (Nu) appear to have fragmented. The nuclear membrane (double ringed arrows) is ruptured, but the cell membrane (ringed arrow) is still intact. Cytoplasmic organelles cannot be identified. Several dense bodies (DB) are present amidst numerous vesicular profiles (V). These structures accumulate in the perinuclear zone, leaving a clear zone (CZ) between them and the cell membrane. ×9,000.

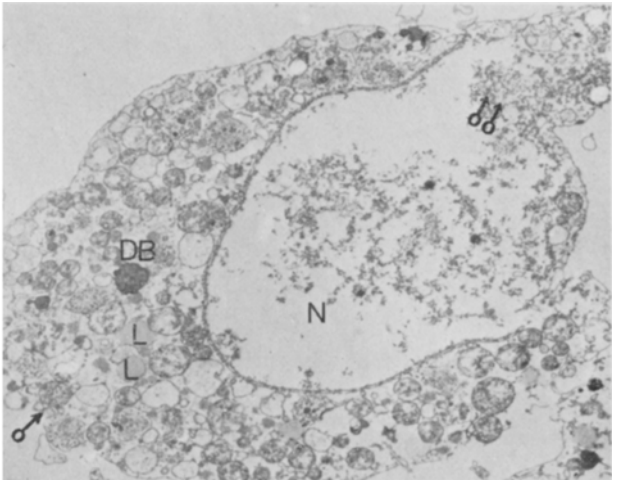

Fig. 4. 120 min after exposure to sponge extract. The nuclear membrane has ruptured (double ringed arrows) resulting in the extrusion of nuclear material into the cytoplasm. The cell membrane has also ruptured (ringed arrow) and the cytoplasmic contents extruded out of the cell. The cytoplasmic contents consist of many vesicular elements, a few lipid droplets (L) and dense bodies (DB). ×12,600.

arrows) and the cell membranes (Figure 4, single ringed arrow) together with extrusion of their cytoplasmic contents.

Discussion. The present ultrastructural study of the effects of an alcoholic extract of the sponge, *Suberites inconstans*, has confirmed some of the findings obtained by means of phase contrast microscopy. The earliest effect of the extract appeared to be exerted on mitochondria. Since mitochondria are very sensitive cell organelles which succumb rapidly to various forms of adverse conditions, e.g. anoxia and cytotoxic chemicals, such changes in the ultrastructure of the mitochondria would be expected to be the first observable effect of cytotoxicity.

The reaction of HeLa cells to colloidal gold[4], hydroxyurea[5], and several antibiotics, notably actinomycin D[6,7], toyocamycin[8,9], daunomycin[10], and phleomycin[11–13], have been reported. The effect of most of these substances on the viability of HeLa cells treated with them appear to be related to their ability to inhibit nucleic acid synthesis. The effect of the sponge extract on HeLa cell growth has been shown by our previous study[2] to be related to inhibition of DNA synthesis.

The present ultrastructural study has suggested that the extract may also have a profound effect on cell membrane permeability. The appearance of a clear zone beneath the cell membrane (Figure 3, CZ) would suggest an intracellular accumulation of fluid which had leaked into the cell. This could possibly account for the cytoplasmic contents being pushed into a perinuclear position. The mechanism of rupture of both nuclear and cell membranes is at present unknown.

Résumé. Des observations ultrastructurales ont été faites sur les cellules HeLa de l'éponge *Suberites inconstans* extraites par une solution alcoholisée. Aux degrés toxiques de concentration, les mitochondries furent les premières à présenter des vacuoles et à perdre leurs cristae. Il s'en suivit une rupture de la membrane nucléaire, l'apparition de véhicules cytoplasmiques, une accumulation de fluide dans le cytoplasme et finalement une rupture de la membrane cellulaire due à la perte du contenu cytoplasmique.

C. K. Tan, C. H. Tan and Y. F. Teh

Departments of Anatomy, Biochemistry and Pharmacology, University of Singapore, Sepoy Lines, Singapore 3 (Republic of Singapore), 2 October 1974.

[4] C. G. Harford, A. Hamlin and E. Parker, J. biophys. biochem. Cytol. *3*, 749 (1959).
[5] J. H. Kim, A. S. Gelbard and A. G. Perez, Cancer Res. *27*, 1301 (1967).
[6] M. N. Goldstein, I. J. Slotnick and L. J. Journey, Ann. N.Y. Acad. Sci. *89*, 474 (1960).
[7] L. J. Journey and M. N. Goldstein, Cancer Res. *21*, 929 (1961).
[8] A. Tavitian, S. C. Uretsky and G. Asc, Biochim. biophys. Acta *157*, 33 (1968).
[9] U. Heine, Cancer Res. *29*, 1875 (1969).
[10] A. Rusconi and A. Dimarco, Cancer Res. *29*, 1507 (1969).
[11] N. Tanaka, H. Yamaguchi and H. Umezawa, Biochem. biophys. Res. Commun. *10*, 171 (1963).
[12] K. Kajiwara, J. H. Kim and G. C. Mueller, Cancer Res. *26*, 233 (1966).
[13] B. Djordjevic and J. H. Kim, Cancer Res. *27*, 2255 (1967).

Alleviation of Inhibitory Action of 5-Bromode-oxyuridine by Methionine in Early Chick Embryos

Numerous reports have shown that the thymidine analogue, 5-bromodeoxyuridine (BrdU), can suppress the synthesis of cell-specific macromolecules, alter the cell surface of differentiating cells[1–4], and interfere with embryonic development[5–8]. Lee et al.[7] reported that the inhibitory action of BrdU could be alleviated by subsequent treatment with excess thymidine in explanted streak stage chick embryos. We now report that the BrdU effect can also be alleviated by methionine, a methylating agent known to occur in animal cells.

Materials and methods. Fresh and fertile White Leghorn eggs were incubated at 37.5 °C for 17–19 h to obtain stage 4 embryos[9]. The embryos were explanted by New's[10] technique. Nutrient medium (thin albumen) with or

[1] J. Abbott and H. Holtzer, Proc. natn. Acad. Sci., USA *59*, 1144 (1968).
[2] R. H. Stellwagen and G. M. Tomkins, J. molec. Biol. *56*, 167 (1971).
[3] W. Ostertag, T. Crozier, N. Kluge, H. Melderis and S. Dube, Nature New Biol. *243*, 203 (1973).
[4] M. C. O'Neal and F. E. Stockdale, Devel. Biol. *37*, 117 (1974).
[5] M. Gontcharoff and D. Mazia, Expl Cell Res. *46*, 315 (1967).
[6] R. Tencer and J. Brachet, Differentiation *1*, 51 (1973).
[7] H. Lee, A. K. Deshpande and G. W. Kalmus, Wilhelm Roux Arch. EntwMech. Org. *75*, 102 (1974).
[8] H. Lee, A. K. Deshpande and G. W. Kalmus, J. Embryol. exp. Morph., in press (1974).
[9] V. Hamburger and H. L. Hamilton, J. Morph. *88*, 49 (1951).
[10] D. A. T. New, J. Embryol. exp. Morph. *3*, 326 (1955).

The development of stage 4 chick embryos, subcultured for 20 h on 4 different media following pretreatment with BrdU (3×10^{-4} *M*) for 4–5 h (Groups I–III).The embryos of Group IV were treated the same except that plain nutrient medium was used throughout cultivation

Group No.	No. of embryos at subculturing	Subculture medium	Embryos (%) showing abnormalities in			
			Brain	Neural tube	Heart	Somites
I	32	BrdU (3×10^{-4} *M*)	100.0	84.4	21.9	100.0
II	42	Methionine (3×10^{-4} *M*)	33.3[a]	16.7[a]	14.3	19.1[a]
III	36	Homocysteine (3×10^{-4} *M*)	86.1	77.8	27.8	91.7
IV	34	Nutrient medium	14.7[a]	11.8[a]	11.8[a]	14.7[a]

[a] Statistically significant at the 0.01 level with the same structure in Group I.