Electron Microscopic Observations of Neocarzinostatin Treated Burkitt Lymphoma Cells

Neocarzinostatin (NCS), a recently developed acidic, polypeptide antibiotic isolated from a streptomyces culture filtrate[1], was used by SAIRENJI et al.[2] on the P3HR-1 Burkitt lymphoma cell line in an effort to improve the productivity of the Epstein-Barr virus (EBV). NCS induced the formation of giant cells, and more EBV particles, as compared to the untreated control cell cultures. Others have reported in HeLa cells[3] and in *Sarcina lutea*[4], that NCS inhibits DNA synthesis selectively, leaving RNA unaffected. KAWAI and KATOH[5] have shown in the HR1K Burkitt lymphoma cell line, that NCS exerts a differential effect on DNA and RNA synthesis. At a dose abolishing 90% of DNA synthesis within 1 h after treatment, RNA synthesis was unaffected. In this paper, we describe electron microscopic observations of the morphological alterations induced in the HR1K cells when continuously cultured in the presence of NCS.

Material and methods. The culture conditions for the HR1K cells used in this study have been described previously[5]. NCS was prepared in a 1 mg/ml stock solution in culture medium, and diluted and dispensed to yield 0.1, 1.0, and 10.0 µg/ml solutions in 15 ml volumes containing 8.5×10^6 cells/ml. Cells were sampled after 1,

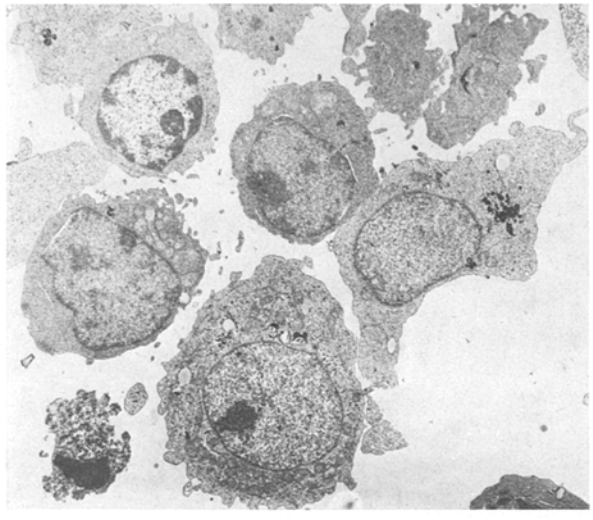
[1] N. ISHIDA, K. MIYAZAKI, K. KUMAGAI and M. RIKIMARU, J. Antibiot. Ser. A *18*, 68 (1965).

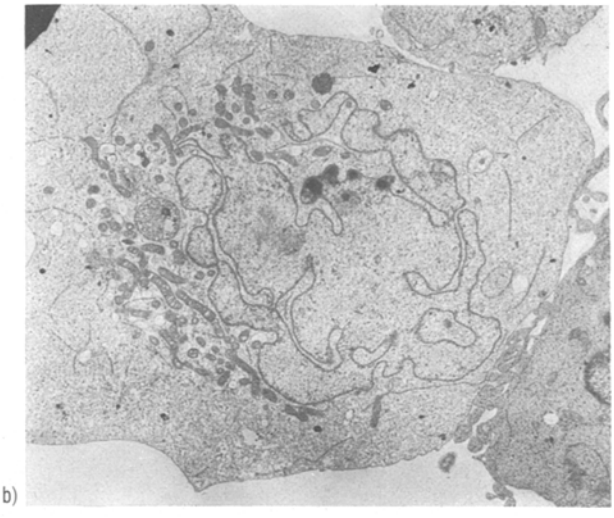
[2] T. SAIRENJI, J. YAMAGUCHI, S. KATAGIRI, and Y. HINUMA, Gann *61*, 451 (1970).

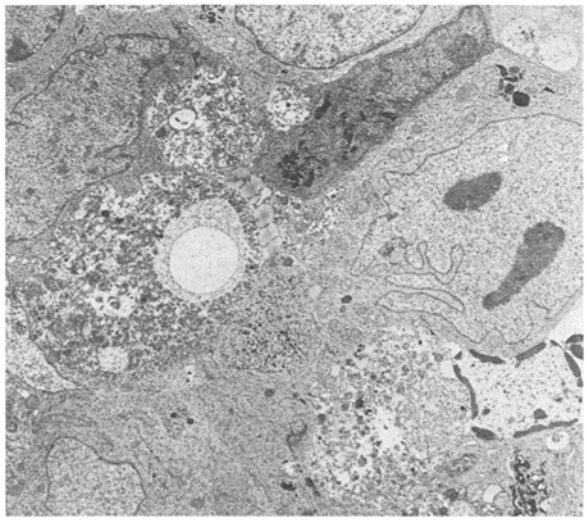
[3] M. HOMMA, T. KOIDA, T. SAIDO-KOIDE I. KAMO, M. SETO, K. KUMAGAI and M. ISHIDA, 6th Int. Congr. Chemother. (1969), p. 115.

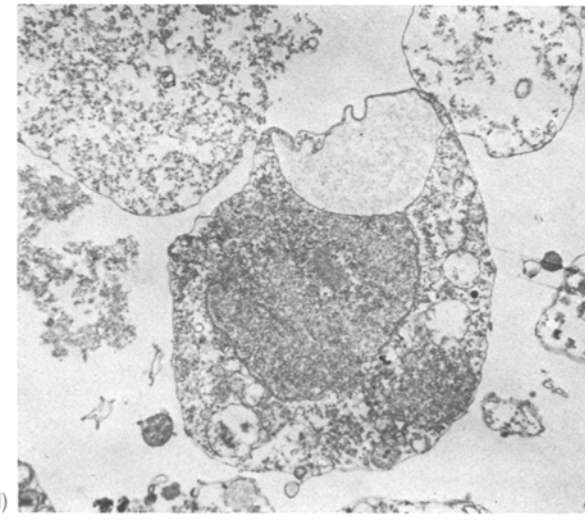
[4] Y. ONO, Y. ITO, H. MAEDA and N. ISHIDA, Biochim. biophys. Acta *119*, 46 (1966).

[5] Y. KAWAI and A. KATOH, J. natn. Cancer Inst. *48*, 1535 (1972).









Electron micrographs of Burkitt lymphoma cells. a) Untreated, control cells, showing large, round, or oval nuclei, with many free ribosomes in the cytoplasm. b) Cells treated with 0.1 µg/ml NCS for 6 days. Prominent enlarged cell shown having many free ribosomes. c) NCS treated cells, 6 days after exposure to 1.0 µg/ml. Enlarged cells and degenerated cell can be seen. Figs. a), b) and c) are all shown at ×3,000 magnification, which serves to emphasize the size difference of untreated cells in (a), and treated cells in (b) and (c). d) Cells treated with 10.0 µg/ml NCS for 2 days. All cells are degenerated. Large vacuoles can be seen. Some of the cells still contain nuclei, but their nuclear membranes are not clearly outlined in certain places. × 6,000.

2, 4, and 6 days. At each sampling, 5 ml was taken for light microscopy, and 10 ml for electron microscopy.

Cell smears on slides were stained by the May-Grunwald-Giemsa technique, and examined by light microscopy for all measurements. For electron microscopy, cells were centrifuged at $500 \times g$ for 10 min, washed once with cold phosphate buffered saline, and centrifuged again. The cell pellets were fixed in 4% glutaraldehyde in 0.1 *M* phosphate buffer, pH 7.2, for 2 h at 4 °C. After rinsing with 5% sucrose in 0.1 *M* phosphate buffer overnight, and postfixing for 2 h in Millonig's OsO_4 fixative, pellets were dehydrated in ethanol series and embedded in epon. Thin sections were stained with uranyl acetate and lead, and examined in a JEM 100-B electron microscope.

Results. Controls. The cells were usually round, but sometimes elongated or pear-shaped. The pale-staining nuclei were large, round, or oval, and sometimes slightly indented, and contained several prominent nucleoli. Mitoses were common. The cytoplasm contained many free ribosomes and a few mitochondria were grouped together at one pole. Golgi apparatus and endoplasmic reticulum were scanty. Centrioles, vacuoles, lysosome-like bodies, myelin-like figures and intranuclear inclusions were seen. In addition, annulate lamellae, a characteristic component of certain types of cells, including undifferentiated malignant cells, were seen. A few EBV particles were observed, and their ultrastructural features were the same as those previously reported by EPSTEIN, ACHONG, and BARR[6].

NCS, 0.1 μg/ml. The most notable effect was the formation of greatly enlarged cells, observed as early as 24 h after exposure to NCS, and the multinucleate nature of some of these cells. The increase in size reached its maximum on the 4th day of our observations. The diameter of most of these cells was 4–5 times greater than that of the untreated cells. Some cells were round or oval, but most of the others were bizarre in shape, while some of them had evaginations of the cytoplasm. The nuclei were large, irregular in outline and contained several nucleoli. Mitotic figures were frequently observed.

NCS, 1.0 μg/ml. Many enlarged cells were present. The number of degenerated cells increased however, with increasing exposure time to NCS. These degenerated cells had many myelin-like figures and vacuoles in their cytoplasm, and many intranuclear inclusions. The cells varied widely in size distribution. Some cells were the same size as the untreated cells, while others were 2–3 times larger in diameter, and still others were 4–5 times larger in diameter than the untreated cells. The first 2 groups of cells were mostly round, and had round or oval nuclei, but the last group of cells was pleomorphic. Mitotic figures were frequently seen.

NCS, 10.0 μg/ml. Almost all the cells were degenerated by 24 h. Some cells had no nuclei, while others still contained degenerated nuclei. Considerable debris of the degenerated cells were seen. Immature and mature forms of the EBV particles appeared near, or in the degenerated cells, and in the debris of the degenerated cells.

Discussion. The most conspicuous feature of the NCS-treated cells was the increase in size by the dosages of 0.1 μg/ml and 1.0 μg/ml. No cellular degeneration was found in the group treated with 0.1 μg/ml, whereas those treated with 1.0 μg/ml exhibited considerable degeneration from 2 days onward. The diameter of almost all of these cells ranged between 4–5 times greater than that of the untreated cells. Some of the enlarged cells appeared to be very similar to the so-called multinucleated giant cells, which EPSTEIN and ACHONG[7] have previously reported in the EBl culture strain of Burkitt lymphoma cells.

SAIRENJI et al.[2] observed the development of giant cells with the light microscope in NCS-treated Burkitt lymphoma cells. They suggested that NCS, used at 1.0 μg/ml and higher doses, inhibited the synthesis of host cellular DNA and cell division.

While no quantitative studies of viral particles were performed here, it is our distinct impression that EBV productivity was not particularly enhanced by NCS treatment, but nonetheless, were considerably easier to locate in those cultures treated with e.g., 10 μg/ml NCS, chiefly because the visualization of the particles in the extracellular environment was a simpler procedure.

Résumé. Les cellules de Burkitt lymphoma grandissent dans la culture contenant de la néocarcinostatine, un antibiotique, nouveau, polypeptide et acide. La néocarcinostatine a provoqué la formation, en 24 h de cellules très grandes, dans une concentration de 0.1 μg/ml. Si la concentration est plus forte, les cellules dégénerent.

M. SHAMOTO[8], Y. KAWAI[9] and A. KATOH

Oncology Laboratory, Division of Radiotherapy, Mercy Hospital, Pittsburgh (Pennsylvania 15219, USA),
31 July 1972.

[6] M. A. EPSTEIN, B. G. ACHONG and Y. M. BARR, Lancet *1*, 702 (1964).
[7] M. A. EPSTEIN and B. G. ACHONG, J. natn. Cancer Inst. *34*, 241 (1965).
[8] Present address: 2nd Dept. of Pathology, Nagoya University School of Medicine, Nagoya, Japan.
[9] Present address: Dept. of Bacteriology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514, USA.

The Teratogenic Response of the Mouse Embryo to 5-Iododeoxyuridine

The halogenated pyrimidines, 5-chlorodeoxyuridine, 5-bromodeoxyuridine (BUdR) and 5-iododeoxyuridine (IUdR) have a halogen atom substituted for the methyl group of thymidine at the 5′ position of the pyrimidine ring. Because of the similarity to the van der Waals' Radii of the respective halogens to that of the methyl group, they serve as structural analogs of thymidine[1]. As such, they are incorporated into DNA[2,3] and their biological activity is usually associated with this chemical property[4,5]. Chlorodeoxyuridine is teratogenic in the rat embryo[5], while bromodeoxyuridine possesses similar activity against the hamster[6] and mouse[7] embryo. The present study extends the analysis of the effects of these chemically related compounds on mammalian embryonic development utilizing 5-iododeoxyuridine as the teratogen.

Materials and methods. Virgin female mice of the ICR strain[7] were used in this investigation. All animals were housed in an animal facility that was maintained at 21–26 °C and had a controlled lighting regimen of 20 h of light and 4 h of darkness (20 L:4 D), the dark period occurring between 22.00 and 02.00 (Eastern time). All animals were fed Mouse Breeder Chow ad libitum. Females were caged with fertile males of the same strain and were examined daily for evidence of a successful