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

Table I. Resorption rate in litters treated with 5-Iododeoxyuridine

Day of treatment	Dosage (mg/kg)	No. of litters	No. of implantation sites	Resorptions No.	(%)
7	100	10	135	26	(19)
	300	11	135	89	(66)
	500	10	109	109	(100)
8	100	10	144	6	(4)
	300	10	132	81	(61)
	500	11	140	140	(100)
9	100	10	119	7	(6)
	300	10	130	74	(57)
	500	11	140	103	(74)
10	100	11	112	7	(6)
	300	10	105	48	(46)
	500	11	137	126	(92)
11	100	12	144	14	(10)
	300	10	110	49	(45)
	500	11	127	47	(37)

copulation. Females with positive vaginal plugs were isolated and that day was designated day zero (0) of pregnancy.

5-iododeoxyuridine was suspended at a concentration of 20 mg/ml in 0.5% carboxymethylcellulose. The suspension was stored at 4 °C in previously sterilized vaccine bottles and used as needed. Pregnant females were administered single i.p. injections according to 3 separate dosage schedules (100, 300 or 500 mg/kg body wt.) on 5 different days of gestation. These days (7–11) encompassed the entire period of major organogenesis from primitive streak formation through the somite period and coincided with stages 12 through 25 of WITSCHI[8]. Females were killed on day 17 of pregnancy and their uteri examined for both viable fetuses and resorption sites. Live fetuses were removed sequentially according to the protocol developed by WILSON[9], examined for external malformations, and fixed in Bouin's fluid. After an appropriate time interval, the fetal heads were dissected for evidence of cleft palate. In no instance was thoracic or abdominal dissection attempted.

[1] W. H. PRUSOFF, Y. S. BAKHLE and L. SEKELEY, Ann. N. Y. Acad. Sci. *130*, 135 (1965).
[2] M. L. EIDINOFF, L. CHEONG and M. A. RICH, Science *129*, 1550 (1959).
[3] D. W. VISSER, D. M. FRISCH and B. HUANG, Biochem. Pharmac. *5*, 157 (1960).
[4] R. BISCHOFF and H. HOLTZER, J. Cell Biol. *44*, 134 (1970).
[5] S. CHAUBE and M. L. MURPHY, Cancer Res. *24*, 1986 (1964).
[6] P. R. RUFFOLO and V. H. FERM, Lab. Invest. *14*, 1547 (1965).
[7] R. G. SKALKO, D. S. PACKARD JR., R. N. SCHWENDIMANN and J. F. RAGGIO, Teratology *4*, 87 (1971).
[8] E. WITSCHI, in *Growth Including Reproduction and Morphological Development* (Eds. P. L. ALTMAN and S. DITTMER; Fed. Soc. Biol. Washington, D. C. 1962), p. 306.
[9] J. G. WILSON, in *Teratology: Principles and Techniques*, (Eds. J. G. WILSON and J. WARKANY; University of Chicago Press, Chicago 1965), p. 262.

Table II. Malformations produced by 5-Iododeoxyuridine

Day of treatment	Dosage (mg/kg)	Number of		Survivors with					
		Litters	Survivors	Exencephaly No.	(%)	Polydactyly No.	(%)	Cleft palate No.	(%)
7	100	10	109	1	(0.9)				
	300	11	46	22	(48)	1	(2)		
	500	10	0						
8	100	10	138						
	300	10	51					1	(2)
	500	11	0						
9	100	10	112						
	300	10	56			4	(7)	19	(34)
	500	11	37					22	(59)
10	100	11	105			3	(3)	2	(2)
	300	10	57			9	(16)	54	(95)
	500	11	11					4	(36)
11	100	12	130			1	(0.7)	1	(0.7)
	300	10	61					10	(16)
	500	11	80					22	(28)

Results. The resorption rate of litters exposed to 5-iododeoxyuridine in utero is shown in Table I. Over the dose range employed and throughout the developmental periods studied, both 500 mg/kg and 300 mg/kg were highly embryolethal. Only with the lowest dose (100 mg/kg) did intrauterine death approach previously reported control levels[7] and only treatment on day 7 resulted in a significant ($P < 0.005$) increase above these levels. The malformation rate in surviving fetuses is shown in Table II. Three major malformations: exencephaly, polydactyly and cleft palate are produced in sequence. In addition, omphalocele is produced by 300 mg/kg on day 8 (4%), syndactyly by 500 mg/kg on day 9 (3%) and ectrodactyly by 300 mg/kg on day 10 (18%).

Discussion. This study indicates that iododeoxyuridine is a 'classical' teratogen in many respects. First, one can define an embryolethal dose (500 mg/kg), a teratogenic dose (300 mg/kg) and a non-effective dose range (< 100 mg/kg). This is consistent with existing concepts[10,11] but is at variance with previously reported results with 5-bromodeoxyuridine in this strain of mice[7]. Second, the pattern of malformations produced follows a distinct stage-specifity and is qualitatively similar to those malformations observed after transplacental exposure to BUdR[7]. The findings reported here would suggest, therefore, that IUdR is a more potent embryotoxic compound but that it may produce congenital defects by a mechanism similar to its structural analog, BUdR. The difference in toxicity between these two compounds was unexpected but might be related to the vehicle employed (carboxymethylcellulose vs. distilled water), to different transport and incorporation kinetics, or to differences in maternal metabolism. In view of our previous studies which indicated that BUdR is incorporated into the DNA of the embryo on day 10 (stage 18) of gestation[7] coupled with the many studies which demonstrate that IUdR is incorporated into the DNA of susceptible tissues[12,13] leads us to propose that this latter compound is acting as a thymidine analog, and as such may be producing its biological effect by being incorporated into DNA. All of these factors: maternal metabolism, placental transport and incorporation into the DNA of the embryo are presently being evaluated by our laboratory and the final results will be the subject of a future communication[14].

R. G. SKALKO and D. S. PACKARD JR.

Embryology Laboratory, Birth Defects Institute, New York State Department of Health, Albany, New York, 12208 (USA), 29 May 1972.

[10] C. P. DAGG, in *Biology of the Laboratory Mouse*, 2nd edn (Ed. E. L. GREEN; McGraw-Hill, New York 1967), p. 309.
[11] J. G. WILSON, in *First International Conference on Congenital Malformations* (Lippincott, Philadelphia 1961), p. 187.
[12] L. A. DETHLEFSEN, J. natn. Cancer Inst. *44*, 827 (1970).
[13] K. G. HOFER and W. L. HUGHES, Cancer Res. *30*, 236 (1970).
[14] We express our appreciation to Mr. A. M. NILES for his expert technical assistance.

The Finding of Viral Particles in Spontaneous Mammary Adenocarcinoma in Rats

There are descriptions of the presence of viral particles in mammary carcinomas in the rat, experimentally induced with dimethyl-benzo-anthracene, with methylcholanthrene etc.[1]. These observations refer both to primary tumours and to transplantable tumours. Similar findings have been indicated in a mammary carcinoma of the rat obtained by radiation[2], and in cell cultures obtained from 2 mammary carcinomas of the rat induced with chemical means[3]. On the other hand, there have been no reports of viral particles in spontaneous mammary tumours of the same species[1].

We therefore considered it to be of interest to describe a case of spontaneous mammary neoplasia in this species, diagnosed histologically as adenocarcinoma, found in a uniparous 5 month-old female Sprague Dawley rat. The animal was one of a disease-free breed. Its appearance having been noted, the neoplasia tended to grow gradually, eventually reaching, after 7 months, the form and volume of a tangerine. In this period, fragments of tissue were removed, fixed both in osmic acid and in glutaric aldehyde and osmic acid and embedded in araldite-epon. In order to test the acellular transplant-ability of the neoplasia, the following tests were carried out, using both male and female subjects: 1. transplant of fragments of neoplastic tissue in the mammary region; 2. transplant of fragments of neoplastic tissue in the sub-cutis of the back; 3. transplant of neoplastic cells in the peritoneal region; 4. the peritoneal administration of acellular extracts of the neoplasia. Examination under the electron microscope showed type C viral particles, especially in the intercellular lacunae (Figure 1). In this region the particles in some cases appear clustered, but are more often free. They are seen to consist of an outer osmiophil ring and an inner one, the centre of which is rarely homogeneous.

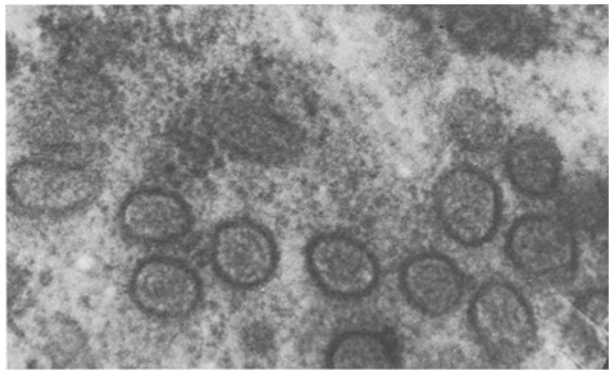

Fig. 1. Viral particles in the intercellular spaces. In some of them the inner membrane is fairly evident. Araldite-Epon; ×100,000.

Particles fundamentally of the same type have also been found in the cytoplasmatic region (Figures 2 and 3) especially in the vicinity of the edge of the cytoplasm. On some occasions particles connected together by an intermediate narrow part have been noted. The viral particles had a diameter of between 80 and 100 millimicrons.

Regarding the transmissibility, the only positive test was found to be that of a transplant of neoplastic tissue

[1] H. C. CHOPRA and R. M. DUTCHER, Biblthca haemat, *36*, 584 (1970)
[2] G. C. ENGLE, S. SHIRAHAMA and R. M. DUTCHER, Cancer Res. *29*, 603 (1969).
[3] V. V. BERGS, M. BERGS and H. C. CHOPRA, J. natn. Cancer Inst. *44*, 913 (1970).