Talbot lagoon is preserved. North of the Ogeechee River there are small remnants of Talbot barrier which generally overlie lagoonal sediments. Throughout coastal Georgia, the Talbot lagoon is seldom more than 2 miles wide.

The Pamlico barrier system is well preserved. Fossil *Callianassa* burrows at several locations indicate that sea level was approximately 24 feet higher than it is today. The highest and best preserved barriers are near Savannah and to the north of the Altamaha River, where they reach elevations as high as 47 feet above sea level, almost 25 feet above the Pamlico sea. The Pamlico lagoon–salt marsh is a broad distinctive feature, ranging from 10 to a maximum of 20 miles in width, east of Hinesville.

Barriers along the Princess Anne shoreline developed only a short distance seaward of Pamlico barriers in the central portion of the coast, but the lagoon–salt marsh widens to both north and south. Sea level was about 13 feet above the present level, as indicated by *Callianassa* burrows at several locations. The barriers have a maximum elevation of 30 feet above present sea level, or 17 feet above Princess Anne sea level, and are fairly continuous except in the area of the Ogeechee River where erosion and dissection have left only small remnants.

The Georgia sea islands are late Pleistocene and Holocene in age. The Silver Bluff (late Pleistocene) parts of the islands were formed when sea level was about 4.5 feet above the present level. The lagoonal salt marsh landward of the Silver Bluff barriers has been reoccupied by Holocene marsh. Along the front of most Silver Bluff islands the Holocene salt marsh is narrow or missing, so the Holocene beach is adjacent to the Silver Bluff barrier. South of the Savannah River, however, the Holocene salt marsh is as much as 6 miles wide.

In southeastern Virginia, Oaks and Coch (*5*) have described shoreline features consisting of barrier islands and associated environments. The elevations of the lower four shorelines that they studied correspond very closely with those reported here, which suggests direct correlation (Table 1). Possible minor warping makes correlation of the upper shorelines more speculative (*9*).

Comparison of modern and Pleistocene shoreline features provides a means of understanding the deposition and modification of the coastal plain sediments. Within the area of this study, the shorelines are approximately horizontal. Morphologic and sedimentologic variation within the preserved segments of the Pleistocene shorelines indicate that detailed study of small areas should be undertaken after thorough regional mapping has located the general position of the shorelines applicable to the local area. Detailed mapping and stratigraphic studies of small areas will give a greater precision for establishing histories of shoreline elevations and deposition.

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## Virus-Like Particles in Established Murine Cell Lines: Electron-Microscopic Observations

Abstract. *Virus-like particles identical in morphology to the RNA tumor viruses have been observed by electron microscopy in six lines of murine L cells and one line of murine liver cells. Control cultures of embryonic mouse cells and 3T3 cells do not contain the particles, and no biologic activity has as yet been associated with them.*

The L-cell line of malignant fibroblasts has been widely used for tissue culture studies since it was established from connective tissue of a $C_3H$ mouse (*1*). In the course of studies on the replication of a murine leukemia virus (Gross) in vitro, virus-like particles were noted in control cultures of L cells. A single similar observation has previously been reported (*2*). We have extended these observations to various lines of L cells and other cultures derived from mouse cells.

We handled cell cultures from various sources (*3*) by a standard procedure to exclude the possibility of introducing contaminating viruses. Upon receipt, the bottle was opened under strict aseptic conditions. The cells were removed from the glass surface with sterile glass beads. One-half of the cells were centrifuged at low speed for 10 minutes to form a pellet for electron-microscopic examination (*4*). Additional samples were taken for electron microscopy from serial subculture in our laboratory. Cells received from the American Type Culture Collection in a frozen ampule were thawed and transferred to prescription bottles in a separate laboratory that was not used for culture of stable murine lines or for virus studies; they were prepared for electron microscopy as soon as a complete monolayer had formed. Mycoplasma was not found in cells or supernatants cultured for its detection (*5*), nor was it indicated by autoradiography with tritiated thymidine (*6*).

Abundant structures resembling RNA tumor viruses (*7, 8*) were seen in six lines of L cells and in one line of NCTC-1469 liver cells (Table 1). Both type-C and type-A particles were present. The type-C particles had an outer membrane and an electron-opaque nucleoid; the total diameter varied from 90 to 115 m$\mu$ (Figs. 1 and 3). The nucleoid had a diameter of approximately 65 m$\mu$. The type-A particles were of the same size, lacked a dense nucleoid, and contained an inner, hollow sphere 65 m$\mu$ wide. An intermediate dense band between the outer membrane and the inner sphere was often observed (Fig. 2); this intermediate band was not observed in type-C structures. The particles were formed by the typical process of budding from the cell membrane into the extracellular space or into vesicles formed by the endoplasmic reticulum (*8*).

The cell lines differed with respect to the amount, location, and morphological type of virus-like particles present. In L-cell clones of L-929 and in the NCTC-1469 liver cells, large numbers of type-C particles were localized in the extracellular space, although budding forms and free type-A particles were occasionally seen both extracellularly and within cytoplasmic vesicles of the endoplasmic reticulum. Abundant particles were

Table 1. Results of electron-microscopic examination of cell lines. Abbreviations for types of media are as follows: 199 FCS10 for 199 with 10 percent fetal-calf serum, MC FCS30 for McCoy's 5a modified medium with 30 percent fetal-calf serum, 199 P for 199 with 0.5 percent Bacto-peptone, and ECS10 (D) for Dulbecco's modification of Eagle's medium with 10 percent calf serum.

| Cell cultures examined | Medium | Virions | | |
|---|---|---|---|---|
| | | No. | Predominant location | Morphological type |
| L-929 | 199 FCS10 | Many | Extracellular | C |
| L-929, clone 5b *(19)* | 199 FCS10 | Many | Extracellular | C |
| NCTC, clone 929 (ATCC) | 199 FCS10 | Many | Extracellular | C |
| NCTC 1469, Liver *(20)* | 199 FCS10 | Many | Extracellular | C |
| L-M (TK$^-$) *(21)* | MC FCS30 | Many | Intracellular | A |
| L-M *(22)* | 199 P | Few | Intracellular | A |
| NCTC 2071 *(23)* | NCTC 109 | Few | Intracellular | A |
| 3T3 *(24)* | ECS10 (D) | Absent | | |
| $C_3H$, Swiss primary cultures | 199 FCS10 | Absent | | |

found in L-M (TK$^-$) cells, but, in contrast, these were predominantly intracellular type-A. Very few virus-like particles were found in the L-M and NCTC-2071 L-cell cultures, grown on serum-free medium, even after repeated examination of serial subcultures. The addition of 5 percent fetal-calf serum to the L-M and NCTC-2071 lines resulted in an increased number of particles in 4 weeks, with the appearance of extracellular type-C in addition to the few intracellular type-A particles seen before the addition of the serum. Control $C_3H$ and embryonic Swiss mouse cultures, as well as the established 3T3 murine line, remained free of the virus-like structures throughout a 4- to 6-month period of repeated electron-microscopic examination.

Following the nomenclature established by Casper *et al.* (*9*), we consider these particles virions rather than viruses because a complete life cycle has not been established for them. For example, infection of primary $C_3H$ mouse or Wistar-Furth rat-embryo cultures with L-929 supernatant fluids for prolonged periods did not transmit morphologically recognizable viral structures to these cultures. Similarly, pellets of ultracentrifuged cell-free supernatants from L-929 cultures had no effects when injected into newborn $C_3H$ mice or Wistar-Furth rats. The animals were observed for 9 months. However, a particulate component of L-929 supernatant fluids labeled with $H^3$-uridine had a density of 1.16 to 1.18 g/cm$^3$ in equilibrium sucrose gradients (*10*), a value reported for murine (*11*) and avian RNA tumor viruses (*12*).

The origin and significance of such virions is speculative at present. Identical structures have been shown in the thymus of both conventional and germ-free mice (*13*) and in chick-embryo cultures (*7*). Moreover, similar virions have been noticed in the NCTC-1469 liver line (*14*), Ehrlich ascites tumor cells (*15*), murine plasma-

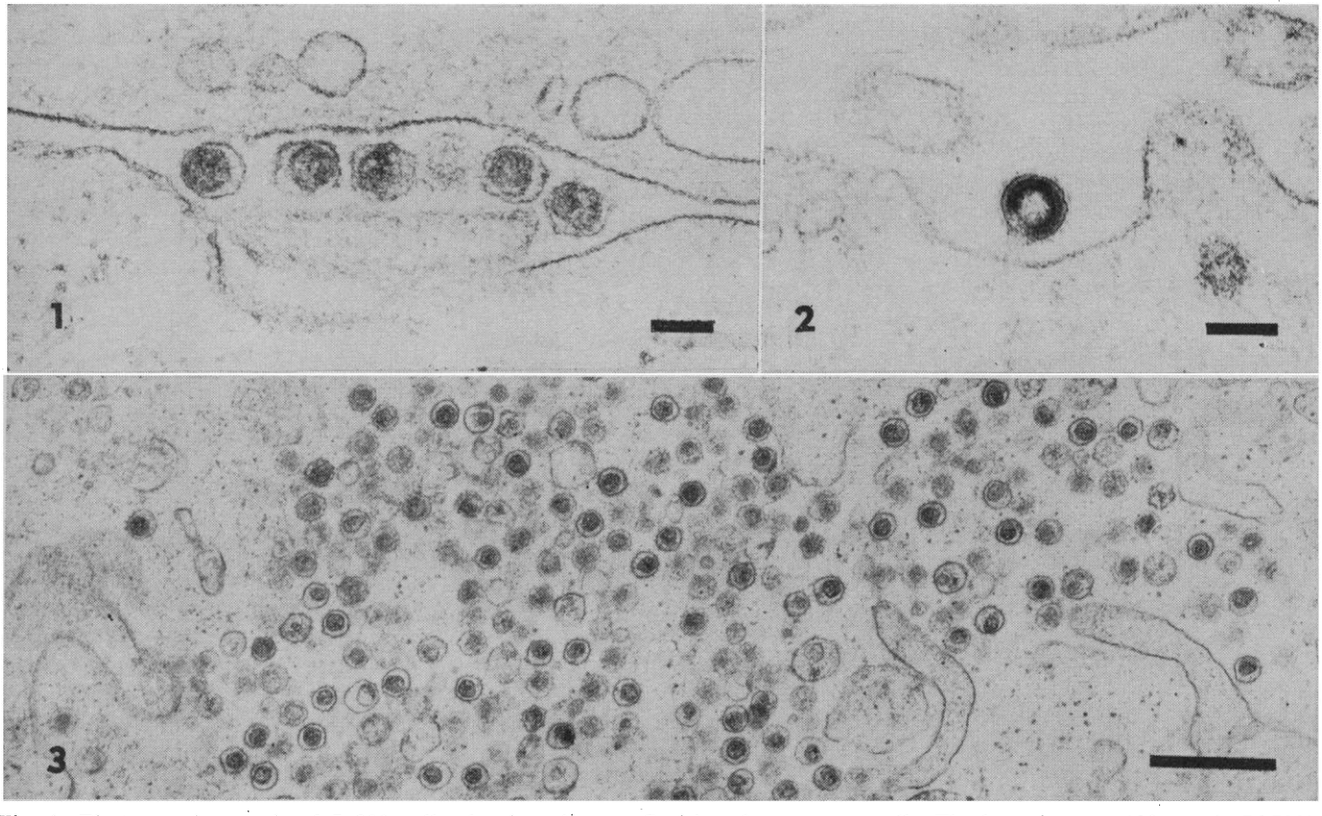


Fig. 1. Electron micrograph of L-929 cells showing six type-C virions between two cells. The bar measures 100 mμ (×76,700). Fig. 2. Electron micrograph of L-929 cells showing a completed type-A particle with an intermediate band budding into the extracellular space. The bar measures 100 mμ (×99,000). Fig. 3. An unusually large accumulation of type-C virions between two NCTC-1469 liver cells. The bar measures 0.5 μ (×33,000).

cell tumors and cell lines (*16*), JLSV$_6$ control cultures of mixed spleen and thymus (*17*), and C$_3$H/HeN cultures (*18*). Even though adequate controls should correct for any effects these particles might produce, their presence may be a significant factor in many of the tissue culture, virological, and biochemical studies in which these established murine cell lines are used.

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## Mutagenesis by Near-Visible Light

Abstract. *Mutants resistant to bacteriophage T5 were produced both in continuous and in stationary cultures of* Escherichia coli *by near-visible light, 320 to 400 millimicrons, at rates greatly exceeding spontaneous rates in the absence of light. Aerobic mutation rates were about twice anaerobic rates, which shows that mutations were induced in either of at least two different processes. Mutations induced by near-visible light involve different photochemical processes than those induced by ultraviolet light.*

Although mutation has been observed with ultraviolet light of wavelengths as long as 315 m$\mu$ (*1*), there has been no definitive report of mutagenesis with the wavelengths in the near-visible region, 320 to 400 m$\mu$. Since light of these wavelengths has been observed to inactivate transforming DNA (*2*) and bacteria (*3*), the possibility of mutagenesis with near-visible light might be anticipated.

This report concerns primarily the production of mutants resistant to bacteriophage T5 in continuous, chemostat cultures of the tryptophan-requiring strain of *Escherichia coli* WP2-HCR$^-$ when these were exposed to near-visible light. These cultures were grown in M9 minimal medium in the presence of excess L-tryptophan (5 $\mu$g/ml) and limiting glucose (100 $\mu$g/ml), giving cell concentrations of 1 to $2 \times 10^8$ per milliliter. The composition of this medium and the techniques used to measure mutation rates have been described (*4*). Growth rates were controlled by the rate of addition of nutrient. Growth and irradiation of cultures was carried out in a dark room at 37°C. Two kinds of light source were used: (i) two 4-watt "black light" fluorescent bulbs (Westinghouse F4T5/BLB, at a distance of 12.5 cm), for which the emission spectrum is largely continuous (Fig. 1), and (ii) a single 100-watt "black light" mercury vapor lamp (Magnaflux CH-4, at a distance of about 25 cm), giving a much purer line spectrum (Fig. 1). Dose rates were determined with a calibrated thermopile and a Keithley 150A microvoltmeter.

Cultures were exposed to an irradiance of about 60 erg/mm$^2$ per second with the 4-watt lamps. (Light output varies from lamp to lamp.) At this irradiance, T5-resistant mutants were induced at a rate of $2.68 \times 10^{-6}$ mutant per bacterium per day. There was neither detectable cell death nor detectable variation in mutation rate over the measured range of growth rates, 3.7 to 12 divisions per day. In the absence of light, mutation rates were growth-rate dependent, and had values some 10 to 20 times smaller. With the much greater irradiances available with the 100-watt lamp, mutations were induced at a rate some 40 times greater than the spontaneous rate in the absence of light. Specific mutation rates per unit irradiance were about the same (Table 1). Mutants have also been produced under similar conditions in a related strain of *E. coli* (*5*).

In addition, it is possible to induce mutation in cells in stationary cultures. Cultures were removed from chemostats for a period of time sufficient to allow for any residual cell division (about 1 hour), and were exposed at about 40 erg/mm$^2$ per sec-

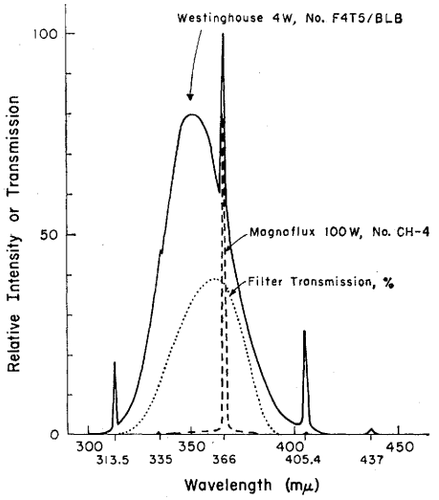


Fig. 1. Emission spectra and filter transmission, as measured with a Zeiss PMQ II spectrophotometer. Spectral intensities are given in arbitrary units and not to the same scale: solid line, for the 4-watt lamps; dashed line, for the 100-watt lamp. The dotted line represents the percentage transmission of the Corning No. 5860 filter.