cytopathogenic effect on almost the entire sheet of cells. The supernatant fluids contained about 100 times more virus than those from nonirradiated cultures (table 1). Increased virus production after ultraviolet irradiation was confined to chronically infected L cells since ultraviolet treatment of normal L cells infected 18 hr previously with egg seed virus did not affect peak titers (table 1). The same dose of ultraviolet irradiation also failed to produce a cytopathogenic effect in uninfected normal L cells.

These results indicate that there are similarities between the state of the chronically infected cultures and lysogeny in bacteria. Continuing investigations are directed at determining whether properties shared in the two phenomena are merely superficial or whether they reflect similarities in basic mechanisms.

IMPROVED METHOD FOR STAINING CELL MONOLAYERS FOR VIRUS PLAQUE COUNTS[1]

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A modification of the plaque technique routinely employed in this laboratory (McLaren, Holland, and Syverton, J. Exptl. Med., **109,** 475, 1959) has been found to give superior results. The following revised procedure is used for demonstrating enterovirus plaques on HeLa cells. Cells are grown in 20 per cent calf serum, 80 per cent yeast extract medium. Calf serum-grown HeLa is preferable to HeLa grown in human serum because the cells adhere more firmly to glass. Cells are dispersed with 0.05 per cent trypsin and dispensed in growth medium in 30 by 30 by 60 mm screw-capped, rectangular bottles to form monolayers of about 2×10^6 cells per bottle. These monolayers are washed, allowed to adsorb virus, rewashed to remove unattached virus, and overlaid with 5 ml of 20 per cent calf serum, 80 per cent yeast extract medium containing 0.6 per cent agar (Difco) (final concentration). Cultures are incubated at 37 C long enough for optimal development of plaques. With poliovirus, this development is reached between 48 and 72 hr following infection. Plaques develop faster under the low-agar overlay; several additional days of incubation are required to obtain plaques of similar size under medium containing more agar. When plaques are optimally developed, as determined by viewing the monolayers with obliquely transmitted light, the medium is removed from each bottle by simply inverting and gently shaking the bottle while the medium is still at 37 C. The gelled medium slides easily out of the bottle into a disposal receptacle. Cytopathically affected cells are usually scraped from the glass as the medium slides out, while uninfected cells remain firmly adherent to the glass. Monolayers are then washed gently with 0.9 per cent saline, and are both fixed and stained by exposure for 2 min to dye solution (1 per cent crystal violet and 20 per cent ethanol in distilled

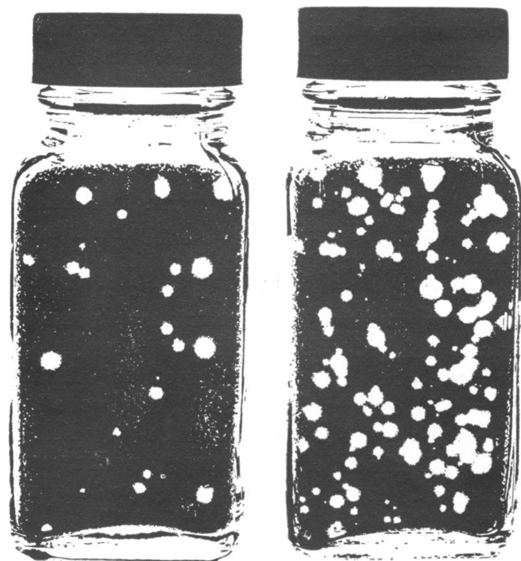

Figure 1. Plaques in HeLa cell monolayer formed by type 1 (Mahoney) poliovirus. Cells stained with crystal violet 42 hr following virus adsorption and agar overlay.

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water). Several drops of crystal violet are sufficient. Although crystal violet is satisfactory, almost any other stain can be employed. When excess dye is rinsed off with tap water, virus plaques stand out as sharply defined, clear areas in a blue cell sheet.

This procedure offers the following advantages: (a) plaques can be stained and observed immediately after they reach optimal size; (b) plaques are sharply defined, and can be counted accurately and easily (figure 1); (c) plaques can be preserved indefinitely after being fixed and stained, and thus can be counted at a convenient time. If desired, representative plaque bottles can be retained as permanent records of plaque size and morphology for comparison with those of later experiments.

This procedure is useful with a wide variety of cell types and viruses. Primary cultures of human amnion and monkey kidney, as well as established human cell lines like HeLa, give good results. Although sharper plaques are obtained when viruses that produce complete cell destruction are used (e. g., poliovirus, Coxsackie A-9 and B-1), less virulent viruses such as vaccinia also yield suitable plaques by this method. Coxsackie viruses, ECHO viruses, and vaccinia virus require from 3 to 5 days for optimal plaque formation. Any standard overlay medium can be employed if the agar concentration is kept low.

MOTILITY OF *RHODOMICROBIUM VANNIELII*

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The isolation, culture, and reproduction of the photoheterotrophic, budding bacterium, *Rhodomicrobium vannielii* has been described previously (Duchow and Douglas, J. Bacteriol., **58,** 409, 1949; Murray and Douglas, J. Bacteriol., **59,** 157, 1950). This organism was described as nonmotile, but we have found that all of the original strains as well as a freshly isolated strain are motile in young cultures.

The substitution of 0.1 per cent sodium lactate for ethanol in the medium described by Murray and Douglas was found to enhance growth of the organism. Fluid cultures after 2 to 3 days of incubation exhibited actively motile clumps of 3 to 4 cells as well as motile single cells. The single cells were observed to arise from terminally attached cells when the filament was disrupted through cell agitation caused by flagellar activity. Mevius (Arch. Mikrobiol., **19,** 1, 1953) has observed that the single motile cells of *Hyphomicrobium vulgare* arise in the same manner.

Electron micrographs of attached and free cells observed in wet mounts are presented in the figures. Figure 1 illustrates an attached cell which possesses a number of flagella, whereas figure 2 presents a single, free-swimming cell with several flagella. Flagellar insertion is peritrichous, in contrast to the polar flagellation observed in the related forms, *Hyphomicrobium vulgare* (Mevius, Arch. Mikrobiol., **19,** 1, 1953) and *Hyphomonas polymorpha* (Pograntz, Schweiz. Z. allgem. Pathol. u. Bakteriol., **20,** 593, 1957). (See page 598 for figs. 1 and 2.)

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