portant components for the PAS positivity of the cell coat. Fucose is of less significance and perhaps less abundant than other PAS positive sugar units. N-acetyl-hexosaminidase from fungi of Genus *Chalaropsis* was also active against the PAS positive cell coat but differed from the glycosidases of *Cl. perfringens* in that it did not require the previous removal of the Hale positive component and was not blocked by N-acetyl-galactosamine, galactose, and fucose.

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Electron Microscopic Observations of Simian Virus-40 in Primary Rhesus Kidney Tissue Cultures. (28763)

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Electron microscopic observations of simian virus 40 (SV-40)(1) have been made in kidney cell cultures from cercopithecus(2,3, 4), and erythrocebus(5) monkeys and have contributed to an accurate description of the cytoplasmic vacuolization which is the characteristic cytopathic effect of SV-40 in these cell systems. Although SV-40 also multiplies readily in rhesus kidney cell cultures, it produces no distinct cytopathic effect and ordinarily may be detected only by subculture in cercopithecus kidney cells. Shein and Levinthal(6), however, using fluorescein-tagged antibody, were able to find SV-40 antigen in rhesus kidney cell cultures. Particles of this virus may also be seen in rhesus kidney cells by direct observation with the electron microscope, as described in the present report.

Materials and methods. Monolayer tissue cultures of primary rhesus kidney were prepared in 2-ounce prescription bottles. The cells were maintained in weekly changes of medium 199 plus 2% calf serum; each bottle contained 6 ml of medium. Experimentally infected tissue cultures were given $10^{5.8}$ $TCID_{50}$ per bottle of a subpassage of SV-40 strain 776(1); the inoculum was contained in 0.1 ml. BS-C-1 cell cultures(7), which were derived from cercopithecus kidney, were used for SV-40 titrations. The cells were held for 28 days, with weekly medium changes. Both the rhesus and the BS-C-1 cells were kept at 36°C.

For electron microscopy, rhesus cells were removed from the bottles, fixed in 1% OsO_4, dehydrated in graded alcohols, embedded in 1:4 methyl:butyl methacrylate, sectioned on a Porter-Blum ultramicrotome, and observed, unstained, in a Philips model 100B electron microscope.

Results and discussion. Cells in the first

experiment were embedded 7 days after addition of SV-40, and 13 days after preparation of the monolayers. On the day of embedding, the titer of SV-40 in the experimental supernatants was $10^{7.0}$ $TCID_{50}$ per bottle; no SV-40 was detected in the parallel control bottles, which were not experimentally infected with SV-40. Examination with the electron microscope failed to reveal viral particles in either the experimental or control cells.

In the second experiment, set up similarly to the first, the cells were embedded 5 days after addition of SV-40 to the experimental bottles, and 12 days after preparation of the monolayers. However, the kidney cells, both experimental and control, were naturally contaminated with SV-40, although this was not known at the time of addition of SV-40 to the experimental bottles. At the time of embedding, the control bottles had a titer of $10^{7.3}$ $TCID_{50}$ per bottle, while the experimental bottles had a titer of $10^{9.2}$ $TCID_{50}$ per bottle. When examined with the electron microscope, both experimental and control preparations contained cells which had round intranuclear particles. These particles were 20 to 30 mμ in diameter, and were present in great numbers (Fig. 1).

The third experiment was performed to determine the effect of long-term incubation on the spread of virus in the cell monolayer. This experiment was similar to the first, in that the rhesus cells were not naturally contaminated with SV-40. The cells were not embedded until 29 days after preparation of the monolayers, and 22 days after addition of SV-40 to the experimental cells. Two days prior to embedding, the experimental cells had a titer of $10^{8.9}$ $TCID_{50}$ SV-40 per bottle, while no SV-40 was detected in the controls. When examined with the electron microscope, the infected preparation had virus-containing cells similar to those seen in the second experiment and shown in Fig. 1. The control cells, however, contained no such particles. In the second experiment, only a small fraction of the cells contained virus; this fraction was larger in the third experiment.

In contrast to the results obtained with cercopithecus and erythrocebus monkey kidney (2,3,4,5), virus particles were not seen in the cytoplasm of infected rhesus cells, but only in the nucleus. This is in keeping with the lack of a prominent cytopathic effect, involving the cytoplasm, in rhesus cells.

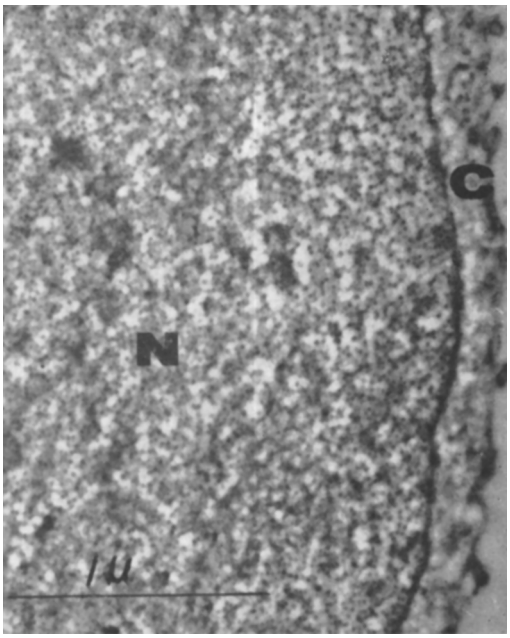

FIG. 1. Electron micrograph of a portion of a rhesus kidney tissue culture cell infected with SV-40. (N), nucleus; (C), cytoplasm. Numerous viral particles are visible in the nucleus. × 34,500.

Infected cells contained a large number of viral particles in their nuclei. On the basis of a comparison between the volume of cell nucleus contained in a thin section, and the total volume of the cell nucleus, it was calculated that one infected cell can contain about $10^{5.7}$ viral particles. For purposes of this calculation, it was assumed that the cell nucleus was a sphere with a radius of 2 μ, the thickness of a section was 50 mμ, and the nuclear content of SV-40 in a section was about 10^4 particles(5).

The titers in the supernatant fluids did not bear a direct relation to the electron microscopic demonstration of virus. The infected cells of the first experiment and the "control" cells of the second experiment both had about the same titer, but viral particles were visible only in the second experiment. The difference between the two lies in the length of time that virus was in contact with the

cells of the respective experiments. The cultures of the second experiment were naturally contaminated, and thus in contact with SV-40 for a longer period than those of the first experiment. The third experiment was similar to the first, only with a prolonged incubation period prior to embedding. The infected cells, in contrast to those of the first experiment, were shown to contain virus particles. The spread of infection through a cell sheet is thus rather slow, and depends on the length of time that virus is in contact with the cells, as well as on the titer of the supernatant. Direct cell-to-cell transfer may play a role in the spread of infection.

These results may be compared with those of other investigators(6), who made use of the fluorescent antibody technique, and found that only 5 to 10 rhesus kidney cells per culture showed evidence for SV-40 antigen 48 hours after addition of virus, and that only 5-10% of the cultured cells did so after 5 days. Assuming that some of this latter antigen has not yet been assembled into a form visible in the electron microscope, and that some infected cells are bound to be missed because the virus does not happen to be present in the particular thin sections cut, these previously described results agree with those of the present investigation.

Summary. SV-40 viral particles were seen in cultures of infected rhesus kidney. SV-40 spreads slowly through the cell sheet, and thus viral particles were not visualized until some time after infection. The SV-40 appears exclusively in cell nuclei, in contrast to its presence in the nucleus and cytoplasm of cell types lysed by the virus.

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Studies on the Mechanism of *Escherichia coli* Resistance to Ethionine.* (28764)

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Ethionine, the ethyl analogue of methionine, inhibits protein synthesis in rats(1) in bacteria(2,3) and protozoa(4). The site of action of ethionine in mammalian and microbial systems has been studied extensively but to our knowledge little attention has been given to mechanisms whereby living systems may become resistant to its toxic or inhibitory effects.

A strain of *Escherichia coli* made resistant to ethionine showed an extended lag period on subculture in a simple medium in the absence of the inhibitor. The present report is concerned with the basis of this lag period and its implications in relation to the nature of ethionine resistance in this microorganism.

Methods. The microorganism used throughout this study was *E. coli* A.T.C.C. 9637. A glucose-salts medium of the following composition was used for growth and maintenance of the organism: NH_4Cl, 1.0 g; K_2HPO_4, 7.3 g; KH_2PO_4, 3.0 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; Glucose, 4.0 g; H_2O, 1.0 liter. Glucose was autoclaved separately before addition to the salts mixture. The final pH of the medium was 7.0.

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