activity against infections with the RO strain but, at the maximum tolerated dose, little capacity to control infections with the RO/PM. Thus contrary to expectations, based on results of earlier studies in bacterial systems and in human infections with *P. falciparum,* there is a considerable degree of cross resistance between trimethoprim and pyrimethamine.

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Absence of Infectious Virus from a Line of SV40-Transformed Human Liver Cells (33862)

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(Introduced by J. F. Enders)

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Morphologic transformation of human cells by simian virus 40 (SV40) has been the subject of a number of reports. Several of these were mentioned in a previous communication (1). More recent studies include the SV40-induced transformation of human cells derived from foreskin (2), astrocytes (3), amnion (4, 5) and the parathyroid glands (6). Certain properties have been found in these studies which distinguish transformed human cells from cells of other species transformed by SV40. These include: (a) a "crisis" period occurring at a variable time after the onset of transformation and evidenced by a decline in cell proliferation and the eventual ability to persist in serial subculture (7, 8). (b) the yielding of virus, detectable in the supernatant, before crisis. (c) a small fraction (0.01–5%) of cells (probably those which yield infectious virus) with nuclear staining in the immunofluorescence test for viral (v) antigen (7, 9).

The present paper reports the transformation by SV40 of cells derived from the liver of a human embryo. Because of a difference in behavior as compared with the known SV40–human cell systems these transformed cells are described in detail.

Materials and Methods. Viruses. SV40, strain VA 45-54, was prepared by serial passage of undiluted stockvirus in BS-C-1 cells and assayed as described previously (1). Sendai virus (10) was obtained from Dr. John F. Enders. It was propagated in the allantoic cavity of 10-day-old embryonated eggs and harvested after 3 days. For fusion experiments, the virus was concentrated and inactivated with propiolactone as described by Neff and Enders (11).

Cell cultures. Cells were prepared from the liver of a human embryo of an estimated 3-months gestation. After one washing in phosphate buffered saline (PBS) the liver was minced, trypsinized, and seeded in 16 × 150-mm tubes. The tubes were incubated at 37° in a stationary position. Three monolayer cultures were inoculated with 0.1 ml of SV40 virus, $10^{6.3}$ $TCID_{50}$/0.1 ml. The BS-C-1 cells were used as indicator cells in cocultivation and fusion experiments.

Media. Cultures were initiated in Eagle's

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basal medium with Hanks' balanced salt solution (bicarbonate 0.35 g/liter), 10% calf serum, 100 μ/ml of penicillin and 100 μg/ml streptomycin. Cultures of transformed cells were changed to Eagle's basal medium with Earle's balanced salt solution (bicarbonate 2.2 g/liter) and calf serum and antibiotics.

Cell fusion. The method was essentially that of Harris and Watkins (12). A suspension of 5×10^6 BS-C-1 cells and an equal number of transformed cells were mixed in 2 ml of Eagle's basal medium in Earle's solution, containing 2% fetal calf serum. To this suspension 1 ml of inactivated Sendai virus containing 5000 HA units was added. The cells were allowed to clump at 4° for 10 min and then were shaken gently at 37° for 20 min. After seeding in 10 tubes (16 × 150 mm), the cells were incubated at 37° in medium containing 10% calf serum. The proportion of fused cells was found to be greater than 70%.

Autoradiography. The BS-C-1 cells were grown with medium containing 1 μCi/ml of Thymidine-^{3}H (sp act 22 Ci/mmole) for 4 days prior to fusion. A 100-fold excess of cold thymidine was added at the time of fusion. Autoradiography was performed as described by Noorduyn and DeMan (13).

Cocultivation experiments, run in parallel with the fusion experiments, were identical, except that 1 ml of medium was substituted for the inactivated Sendai virus.

Test for the detection of T antigen. The indirect test for detection of "tumor" (T) antigen was employed (14). A serum pool was obtained from hamsters bearing SV40-induced tumors. An antihamster globulin of rabbit origin was obtained from Nordic Diagnostics, Prague, Czechoslovakia. Both reagents were diluted 1:16. For the complement-fixation test the antigen was prepared by freezing and thawing of a 20% suspension of the transformed cells. The antigen was tested against 4 units of antibody.

Tests for the presence of SV40. Materials tested for virus included the supernatants and the cell extracts from transformed cells. In cocultivation and fusion experiments, 5 samples of the supernatant were taken during the 3-week incubation period. At the end of this period, cultures were frozen and thawed 3 times and the cell extract was obtained. From all test preparations 0.2 ml of the undiluted material was inoculated on BS-C-1 cultures which were observed for 6 weeks.

Results. Two days after seeding, the primary cultures (Fig. 1) were inoculated with SV40. At that time the cultures consisted predominantly of spindle-shaped cells. Frequent observation of the inoculated cultures revealed no cytopathic changes. Four weeks after inoculation, foci of rapidly dividing epithelioid cells were seen in 2 of 3 inoculated cultures. These cells grew faster than the surrounding fibroblasts and had the appearance of morphological transormation (Fig. 2). No such cells were noted in the control cultures. The transformed cells could be subcultured at weekly intervals, while the control cells could be subcultured only once. At subcultures 6 and 14, the transformed cells were tested for the presence of T antigen, which was present in almost all nuclei as shown by the IF test (Fig. 3). At passage 31 the CF titer for T antigen was 32.

The transformed cells were investigated by various methods for the presence of infectious virus. Supernatant fluids from each of 27 sequential cultures (subcultures 3 to 30) were tested for SV40. No virus was found. Further attempts to demonstrate virus included cocultivation and cell fusion at subcultures 9, 18, and 24. Despite heterokaryon formation in the fusion experiments as shown by autoradiography, these attempts were unsuccessful.

Discussion. The production of virus before crisis is characteristic of SV40-transformed human cells. The amount of virus detectable in the supernatant usually decreases with the approach of crisis (8). Of the lines which survive some continue to release virus; recent experiments showed that in many others production of SV40 can be induced by fusion with appropriate indicator cells (15). Thus far, all our attempts to obtain virus from the transformed liver cells, including cocultivation and fusion, have been unsuccessful. As far as we know, this is the first example of

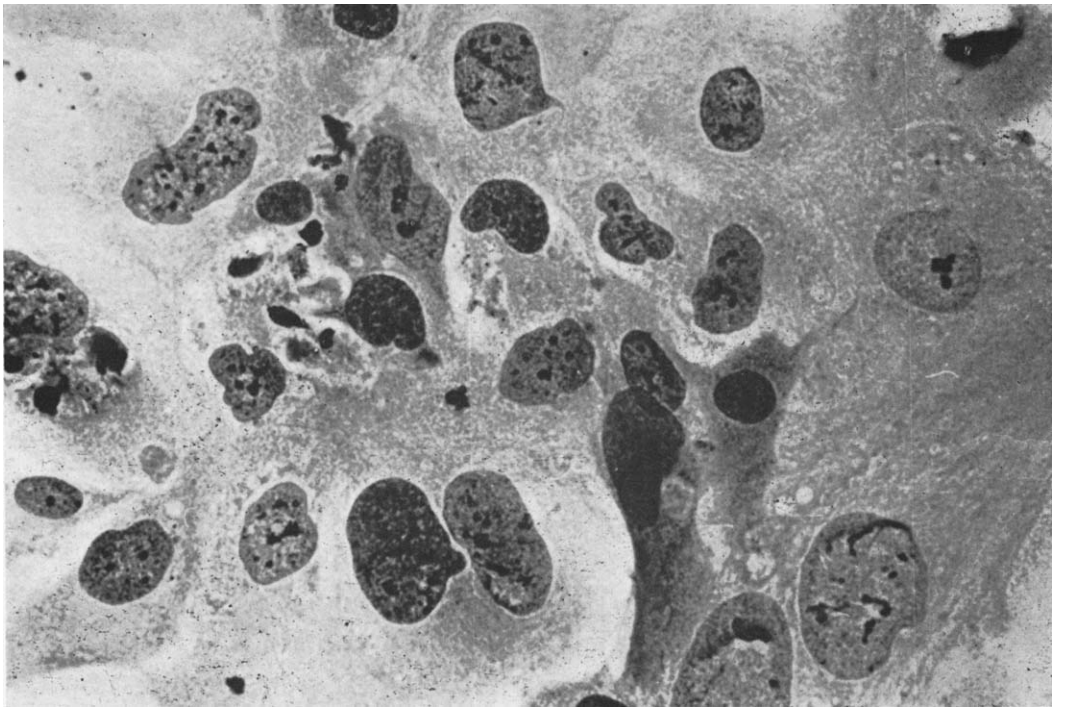

FIG. 1. Primary culture of human embryonic liver cells; hematoxylin–eosin, 330×.

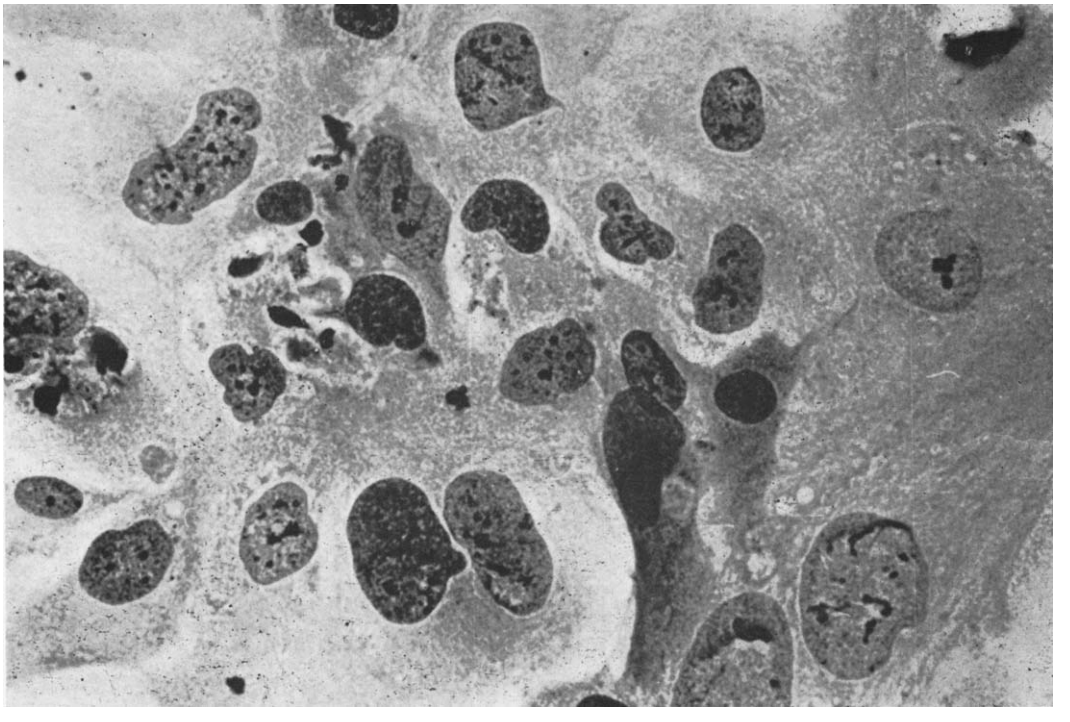

FIG. 2. Human embryonic liver cells transformed by SV40: fifth subculture; 330×.

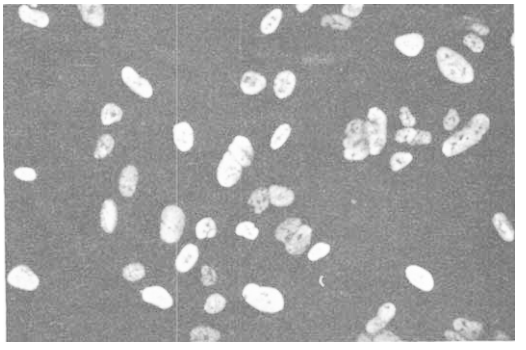

FIG. 3. Immunofluorescent staining for tumor antigen of SV40-transformed human embryonic liver cells: Eleventh subculture; 220×.

SV40-transformed human cells from which no virus has been recovered *before* crisis. Pontén and associates described several clones which did not release infectious virus detectable in the supernatant; nevertheless, SV40 was clearly demonstrable in the parent cultures (16).

Prior to the transformation of human cells, cytopathic effects have been observed by most investigators. Whether the absence of cytopathic effects in the present study is related to the absence of virus production cannot be ascertained. This hypothesis received some support, however, from observation in SV40-transformed hamster cell systems, where the absence of cytopathic effects and the difficulty in demonstrating infectious virus were more often the rule (17, 18).

Recent experiments showed that defective SV40 particles are capable of antigen production in green monkey kidney cells (19). Furthermore those defective particles were shown to contribute to tumorigenicity in newborn hamsters (20). In the light of these observations, it seems possible that defective viral particles may be involved in this SV40-transformed human cell system.

Summary. Cells derived from the liver of a human embryo were transformed after 4 weeks by SV40. In contrast to previous studies, this transformation was not preceded by recognizable cytopathic effects. All attempts to demonstrate infectious virus, including Sendai-induced cell fusion, were unsuccessful. It is suggested that the absence of CPE and of the production of infectious virus may be related; and furthermore, that a defective SV40 particle may be involved in this transformation system.

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