sorbed Co^{60} must have been still in the form of B_{12}.

The intestinal mucosa is such that the free ends of the villi desquamate while new cells regenerate in the crypt at a rapid rate(5). Complete separation of adherent mucus and mucosal debris from the absorptive mucosa seems infeasible even *in vitro* at present. The washing technic employed in this study for adsorption measurement was, admittedly, not ideal, but it was necessary to apply *in situ* and was good enough to demonstrate that in the intestine the bulk of a physiological dose of B_{12} is first adsorbed to the mucosal surface, whether closely or loosely, and that such adsorption is of complex nature. Cooper(6) recently observed, using everted guinea pig intestine and human gastric juice, that part of the radioactive B_{12} that was taken up by the intestine and not washed with saline could be removed further with EDTA. He called it S-E fraction. Whether his fraction is related to the demonstrated nonspecific adsorption remains to be seen.

Summary. Using the rat intestinal loop technic, it was demonstrated that a large part of a physiological dose of Co^{60}-B_{12} was adsorbed to the mucosal surface in the presence of rat intrinsic factor (IF) at a faster rate than it was without IF, and ethylenediaminetetraacetate markedly inhibited this *in vivo* adsorption. The early mucosal adherence of Co^{60}-B_{12} did not represent transmucosal absorption, and transserosal absorption was further delayed. It was concluded that there are at least 2 kinds of surface adsorption; one represents a phase of physiological absorption involving IF, and the other, a nonabsorptive process.

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Induction of Malignancy *in vitro* in Newborn Hamster Kidney Tissue Infected with Simian Vacuolating Virus (SV40). (27780)

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Studies of *in vitro* induction of malignancy by oncogenic viruses have been recently reviewed by Dawe and his associates, and they have described their own experiments with organ cultures of mouse salivary gland infected with polyoma virus(1). Morphologic "transformations" of hamster embryo cell cultures infected with polyoma virus have been reported by several groups(2,3,4) and transplantation of the transformed cells to hamsters has generally resulted in sarcomas at the inoculation sites. In the present studies, cells from monolayer cultures derived from explants of newborn hamster kidney tissue infected with SV40 have produced tumors when transplanted to newborn hamsters. Histologically, these tumors were composed predominantly of undifferentiated carcinoma with areas of adenocarcinoma, sarcoma and well-differentiated epidermoid carcinoma.

Materials and methods. Virus. SV40 virus, strain Vac777 L1 6/14/61, was obtained from Dr. Paul Gerber, Division of Biologics Standards, National Institutes of Health. It was passaged in our laboratory in tube cultures of primary African green monkey kidney cells (GMK) obtained from Microbiological Associates, Bethesda, Md. Preparation of virus pools, limiting dilution titrations, and virus isolations were carried out using methods described previously(5).

Newborn hamster tissues. Newborn Syrian

hamsters obtained from the Animal Production Unit, National Institutes of Health were killed on the day of birth, and the kidneys were removed with aseptic technic. The kidneys were minced into explants 1 mm in diameter for culture.

Culture methods. In the first experiment, 6 test tube cultures were initiated using explants from one kidney in each tube. Two ml of medium (composed of 20% fetal bovine serum and 80% NCTC 109 with 80 units of penicillin and 80 μg of streptomycin per ml) were added to each tube and tubes were incubated in an inclined rack at 37°C on a rotary shaker operated at 70 rotations per minute. In the second experiment, the same procedures were followed; however, mixture 199 was substituted for the NCTC 109 and the tubes were incubated in stationary racks. In the third experiment, the explants were grown in 2-ounce prescription bottles (Brockway Saniglas) using mixture 199 in the medium.

Monolayer cultures were trypsinized using 0.25% trypsin in Dulbecco's tris buffer free of calcium and magnesium. The cell sheets were exposed to the trypsin solution for 30 minutes at 37°C, centrifuged, resuspended in medium, and passed to 2-ounce prescription bottles.

Injection of cell suspensions in animals. Cell suspensions were prepared by scraping the monolayers with bent-tip Pasteur pipettes. The suspensions were agitated vigorously, but clumps of cells persisted and cell counts could not be carried out.

Newborn hamsters (less than 24 hours old) were injected subcutaneously with 0.05 ml of cell suspension and intracerebrally with 0.02 ml. Weanling male hamsters used for cheek pouch injections received 0.2 ml of cell suspensions.

Animals that developed tumors after injection of cells were killed with ether and autopsied. Tissues were fixed in Zenker-formol solution and sections stained with hematoxylin and eosin. Sections of tumors were also prepared with Wilder's reticulum method and Masson's trichrome stain.

Results. In the first experiment, the 6 cultures of newborn hamster kidney tissue were incubated on the rotary shaker for 3 days, then 3 of the cultures were infected with $10^{8.4}$ $TCID_{50}$ of SV40. Incubation and agitation of the cultures were continued and culture medium was changed twice weekly. Although the explants did not adhere to the glass surfaces and no cell growth was seen, pH of media was lowered in all cultures in the intervals between media changes.

After 23 days, the cultures were changed from the rotary shaker to a roller drum operated at one revolution every 5 minutes. One week later, explants had become adherent to the glass surfaces and outgrowths of cells were seen in all of the cultures. Initially, the outgrowths were composed of elongated and polygonal cells growing in sheets, and no significant difference between infected and non-infected cultures could be detected. Subsequently, the growth in the infected cultures was much greater than in the non-infected cultures, with more rapid decrease in pH of media in the infected cultures.

Six weeks after initiation of the cultures, there was a sparse growth of elongated cells in the non-infected tubes with very little change in pH in the intervals between media changes. The infected tubes, however, contained large sheets of elongated and polygonal cells with occasional islands of cuboidal cells. The infected cultures required change of media 3 times weekly because of rapid lowering of pH.

Eight weeks after initiation of the cultures, cellular material scraped from the wall of one of the infected cultures was injected into the cheek pouch of an adult Syrian hamster. The animal has been observed for 6 months and there has been no evidence of tumor in the cheek pouch. Ten weeks after initiation of the cultures, a cell suspension from an infected culture was injected into the left cheek pouch of an adult hamster and some of the sparse cellular growth from a non-infected culture was injected into the right cheek pouch of the same animal. There has been no evident tumor growth in either pouch during a 5½-month observation period.

Ten weeks after initiation of the cultures, a cell suspension from one of the infected cultures was injected intracerebrally into a lit-

ter (11 animals) of newborn hamsters. Only a few elongated cells remained in the non-infected cultures and therefore intracerebral injection of a control cell suspension could not be carried out. One week after inoculation, 4 of the animals had been eaten by the mother. One animal was killed one week after inoculation and 2 were killed 2 weeks after inoculation and the brains of these animals were examined grossly and microscopically; however, no evidence of the transplanted cells was found. Seventy-six days after inoculation, one of the animals had a subcutaneous nodule 0.3 cm in diameter on its head. This nodule grew slowly and one month later the animal was killed and autopsied.

Grossly, there were no visceral tumors. A mass, 1.5 cm in diameter, was present in the subcutaneous tissues of the head and it extended through the skull into the anterior portions of both cerebral hemispheres. Microscopically, the tumor consisted predominantly of sheets and groups of polygonal and elongated cells with pleomorphic hyperchromatic nuclei and moderate amounts of basophilic cytoplasm. Mitoses were numerous and hyperchromatic giant nuclei were present (Fig. 1). These areas had the histologic appearance of an undifferentiated carcinoma, and in sections prepared with Wilder's reticulum stain, groups of tumor cells were usually surrounded by, but not infiltrated with, reticulum.

In some areas, there were collections of tubule-like structures lined by columnar cells with nuclear pleomorphism and hyperchromatism (Fig. 2 and 3). These structures had a histologic appearance consistent with a diagnosis of adenocarcinoma. Some of the tubules were partially lined by stratified squamous epithelium with cellular atypism consistent with a diagnosis of well-differentiated epidermoid carcinoma (Fig. 2 and 3). Although the tumor appeared to be predominantly epithelial, there were sarcomatous areas composed of elongated tumor cells closely associated with abundant reticulum and collagen.

Twelve weeks after initiation of the cultures, 2 of the non-infected tubes had no cells while one had a few scattered elongated cells. The 3 infected tubes contained heavy growths of elongated, polygonal, and cuboidal cells. One infected tube was trypsinized and the cell suspension transferred to a 2-ounce prescription bottle. The cells grew rapidly forming a monolayer that was composed of sheets of polygonal cells with scattered islands of cuboidal cells with a distinct epithelial arrangement. Sixteen weeks after initiation of the cultures, one litter of newborn hamsters (13 animals) was injected subcutaneously with a cell suspension prepared from the prescription bottle culture. Seventeen days later, subcutaneous nodules appeared on the backs of all of the animals. One of the animals was killed and autopsied 25 days after injection and the subcutaneous tumor was histologically similar to the tumor in the intracerebrally inoculated animal. It consisted of large areas of undifferentiated carcinoma and adenocarcinoma with some sarcomatous areas; however, no epidermoid carcinoma was seen. The tumors in the remaining 12 animals grew slowly but became very large. Some of the hamsters have died with tumors 8 cm in diameter, and, at autopsy, pulmonary metastases composed of undifferentiated carcinoma and adenocarcinoma were present (Fig. 4). Five months after inoculation, only 3 animals remain alive and these all have very large subcutaneous tumors.

Virus isolation studies. The fluids removed from the infected cultures in the first experiment were pooled and frozen at –20°C each time medium was changed. Fluids obtained 6, 10, 15, 19, 24, and 67 days after initiation of the cultures were tested for SV40 and virus was recovered from each of them.

Although the 6, 10, 15, 19, and 24-day fluids were not titrated, the rapid appearance of the characteristic vacuolated cytopathic effect (CPE) in the GMK cultures suggested that large amounts of virus were present. The 67-day fluid, however, required 14 days for production of CPE and, in a limiting dilution titration, it contained $10^{2.0}$ $TCID_{50}$/ml.

A portion of the tumor from the animal inoculated intracerebrally with infected cells was minced into 1-mm fragments and these were placed in a culture tube with 2 ml of milk medium (20% autoclaved nonfat milk

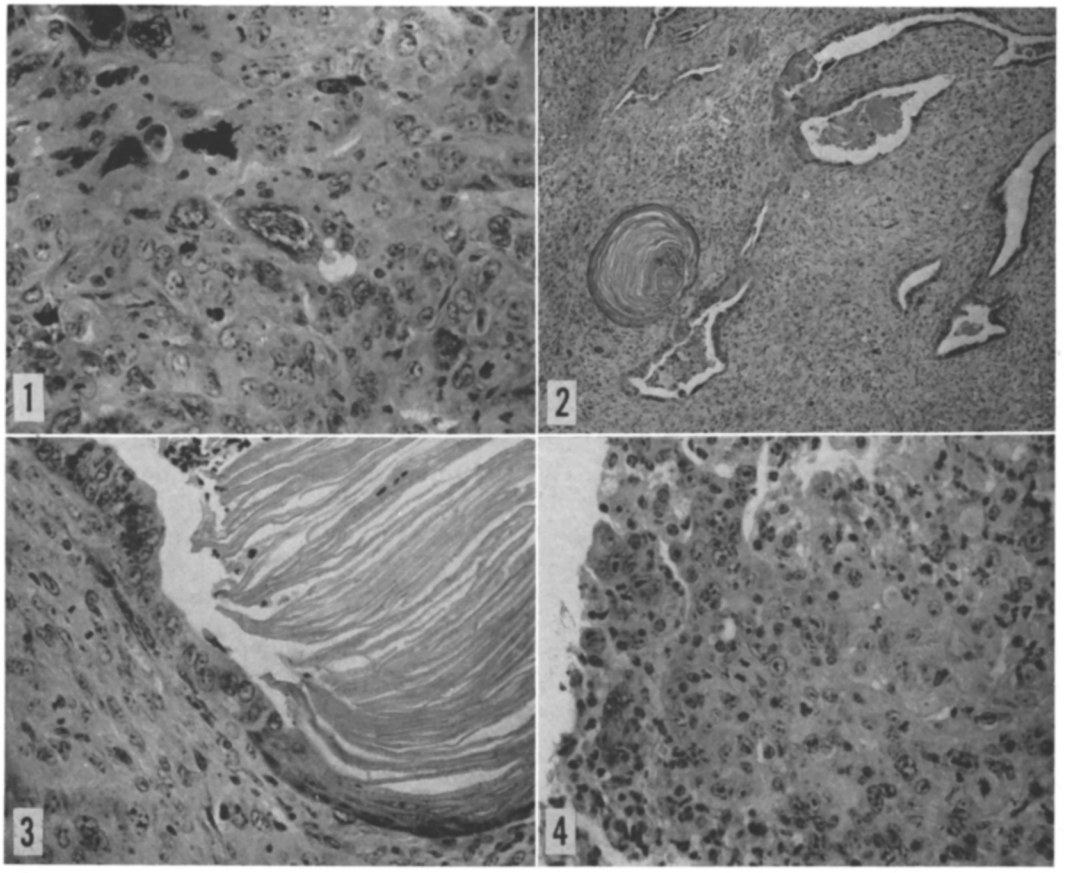

FIG. 1. Section of tumor in brain and subcutaneous tissue of head of hamster produced by intracerebral injection of cells from culture of newborn hamster kidney tissue infected with SV40. Histologically, there were large areas of undifferentiated carcinoma with giant cells containing bizarre hyperchromatic nuclei. Hematoxylin and eosin, 430 ×.

FIG. 2. Same tumor as in Fig. 1. An area of undifferentiated carcinoma with interspersed tubular structures lined by columnar and stratified squamous epithelium is seen. Hematoxylin and eosin, 100 ×.

FIG. 3. Same tumor as in Fig. 1. Tubule-like structure is lined by both columnar and keratinizing stratified squamous epithelium. Hematoxylin and eosin, 430 ×.

FIG. 4. Section of pulmonary metastasis from subcutaneous tumor in hamster produced by subcutaneous inoculation of cells from culture of newborn hamster kidney tissue infected with SV40. Histologically, the metastasis consists of undifferentiated carcinoma with many mitotic figures. Hematoxylin and eosin, 430 ×.

and 80% mixture 199). The tube was incubated at 37°C in a roller drum for 4 days and then frozen at −20°C. It was thawed and fluid from it was inoculated into 5 GMK tube cultures in an attempt to isolate SV40. The tubes were observed for 15 days with no evidence of CPE. They were frozen and thawed and the fluids passaged to new GMK tubes, but no SV40 was isolated.

A tumor from one of the animals injected subcutaneously with cells from an infected culture was also tested for SV40. Four months after injection, the animal had a subcutaneous tumor measuring 4 cm in greatest diameter. Explants, 1 mm in diameter, were prepared from a portion of the tumor and 2 to 4 of these were inoculated into each of 5 GMK tube cultures(6). Fourteen days after inoculation, characteristic vacuolated CPE appeared in the 5 tubes containing the fragments of tumor while 5 control GMK tubes remained free of CPE for 21 days. Fluids from tubes with CPE were passaged to new GMK tubes and vacuolated CPE typical of

SV40 was observed in 3 days.

Second and third experiments. In the second experiment, in which tube cultures were incubated in stationary racks and mixture 199 was substituted for NCTC 109, the results were similar to those described in the first experiment. During the first 2 weeks, elongated and polygonal cells grew from explants in both control and infected tubes, and the control cultures lowered pH more rapidly than the infected cultures. After one month, however, the cell growth was more abundant in the infected than in the control cultures with more rapid lowering of pH in the infected cultures. Ten weeks after initiation of the cultures, cell suspensions from infected and control tubes were injected subcutaneously into newborn hamsters; however, no tumors have developed during a one-month period of observation. Twelve weeks after initiation of the cultures, the infected tubes contained large sheets of polygonal and cuboidal cells while the non-infected tubes contained only small groups of elongated and polygonal cells.

The third experiment was carried out with cultures in stationary 2-ounce prescription bottles rather than test tubes. The results were similar to those in the first and second experiments with more rapid cell growth in infected cultures; however, there has been no evidence of growth of cells transplanted to newborn hamsters during a one-month period of observation.

Discussion. The possibility that the tumors in the hamsters injected with cell suspensions from infected cultures were produced by SV40 acting on the subcutaneous tissues of the recipient animals rather than by local growth of transformed hamster kidney cells cannot be completely eliminated, but it seems unlikely. The subcutaneous tumors which develop after subcutaneous injection of virus in newborn hamsters are sarcomas with no epithelial elements, and the latent period is usually several months(7,8). The tumors produced after inoculation of the kidney cell suspensions were predominantly epithelial and appeared as soon as 17 days after inoculation. Unfortunately, the precise immunogenetic technics used by Dawe in his studies of polyoma virus in inbred mouse tissues to prove that tumors were produced by transplanted tissue rather than virus acting on the recipient's tissue are not available in Syrian hamsters(1).

Although induction of malignancy *in vitro* by polyoma virus in hamster embryo and newborn hamster kidney cell cultures has been described by several investigators(2,3,4) the tumors produced by the transformed cells have been sarcomas with no evidence of malignant transformation of epithelial cells. The induction of malignancy *in vitro* in renal epithelium rather than connective tissue after infection with SV40 probably indicates a significant difference between polyoma virus and SV40; however, the culture methods we have employed which involve use of explants rather than monolayers prepared from trypsinized cell suspensions may also be of importance.

It is of interest in this regard that Koprowski and his associates have described transformation of human cells infected with SV40 using explant cultures rather than trypsinized monolayers(9). We have also studied a strain of cells derived from explants of adult human thyroid tissue infected with SV40 and have found that they grow more rapidly than cells from cultures derived from non-infected explants. It has not been possible to prove that the changes observed in the human cell strains infected with SV40 represent induction of malignancy *in vitro*; however, the findings with the infected hamster kidney tissues indicate that malignant transformation *in vitro* can be produced in cultures infected with SV40.

Summary. Cell cultures derived from explants of newborn hamster kidney infected with simian vacuolating virus (SV40) consisted of sheets of rapidly growing polygonal, cuboidal and elongated cells. Cultures derived from similar explants free of SV40 consisted initially of slowly growing polygonal and elongated cells, and, after 12 weeks *in vitro,* only a few scattered elongated cells remained. Cell suspensions from infected cultures injected into newborn hamsters produced tumors at the sites of injection, the tumors becoming apparent as early as 17 days after injection of cells. Histologically, in

contrast to the sarcomas produced after malignant transformation of hamster tissue *in vitro* by polyoma virus, the tumors were predominantly undifferentiated carcinomas with areas of adenocarcinoma, sarcoma and, in one animal, well-differentiated epidermoid carcinoma. SV40 was recovered from the infected cultures and from one of 2 tumors tested.

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Endotoxin Shock in the Primate.* (27781)

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Endotoxin shock is important as an experimental model for septic shock. It is characterized in most species by hypotension(1) which is sufficient by itself to limit the chances for survival. Studies in the dog have shown this to result largely from hepatosplanchnic pooling with a consequent fall in venous return and cardiac output(2,3). But in monkeys, as well as in cats and rabbits, there is no evidence of such hepatosplanchnic pooling(4) so that hypotension in these species has not been entirely explained.

Since the effects of endotoxin in the monkey are more likely to resemble those in man, an explanation for the monkey's response is of particular interest. The present study concerns the effect of endotoxin and subsequent artificial alterations in venous return on cardiac output and total peripheral resistance in the monkey.

Methods. Eight rhesus monkeys (*Macaca mulatta*) weighing 3.4 to 5.7 kg were anesthetized with intravenous sodium pentobarbital, 30 mg/kg. Pressures were measured by Statham transducers and recorded by a direct writing oscillograph. Arterial pressure (BP) was taken from a femoral artery. Central venous pressure (CVP) was measured with a polyethylene catheter passed through the internal jugular vein to the superior vena cava. This catheter was also used for injection of indocyanine green to obtain dye dilution curves for calculation of cardiac output (CO). Dye was injected in 1-ml volumes, containing 1.25 mg of indicator, and the curves were recorded from blood aspirated through a 150 mm long, 0.86 mm I.D. polyethylene catheter inserted through the left common carotid artery to the aortic arch. Optical density changes of this blood were measured with a Colson densitometer(5) and recorded by the oscillograph. Dye dilution curves were replotted on semilogarithmic paper, and the cardiac output was calculated by the method of Hamilton *et al.*(6). Calibration of the densitometer system was done by drawing

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