changes in conscious dogs. The chronic preparation was especially valuable for comparison of dose response in the same animal by both intravenous and intrajejunal injections in order to measure degree of absorption through the intestinal tract. The data presented in Table I offered certain interesting features. The ease of absorption of digitoxin as indicated by the small intrajejunal-intravenous ratio substantiated the conclusion in man(3). The poor absorption of ouabain from jejunum agreed with the clinical observation that by mouth its efficacy is negligible (11). Acetyl strophanthidin surprisingly had about the same intrajejunal-intravenous ratio as digitoxin; it deserves a clinical trial for verification. Bufalin and lanatoside E may require twice the intravenous dose for oral administration. The loss of 10 dogs after bovoside A, due to persistent anorexia, makes a clinical investigation of this steroid unjustifiable. The presence of convulsant action of bufalin similarly rules out any consideration of application to human beings.

Summary. 1. Six cardiotonic steroids—digitoxin, lanatoside E, acetyl strophanthidin, ouabain, bufalin and bovoside A—have been compared with reference to intestinal absorption in dogs with a chronic jejunal loop. 2. Because of the dominance of vomiting before electrocardiographic changes took place the median emetic dose was estimated after intravenous and intrajejunal injections in order to appraise the absorbability of each steroid from the intestine. 3. In the dog digitoxin and acetyl strophanthidin were more easily absorbed than lanatoside E and bufalin. Ouabain was least absorbed—less than 5%. Although bovoside easily crossed the intestinal membrane it produced persistent loss of appetite resulting in death.

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Development of Tumors in Hamsters Inoculated in the Neonatal Period with Vacuolating Virus, SV_{40}* (27298)

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Previous reports(1-2) from this laboratory described the recovery from cell cultures of rhesus and of cynomolgus monkey kidney of a previously undescribed virus. This agent was unique among simian viruses since it grew but did not cause a cytopathic effect in the rhesus or cynomolgus kidney cell cultures from which it was derived. Instead, it propagated and caused marked cytopathic changes in kidney cell cultures of a heterologous species, *i.e.,* the grivet monkey, *Cercopithecus aethiops,* of Equatorial East Africa. The new virus was called "vacuolating virus" by us because of the prominent cytoplasmic

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vacuolation seen in infected grivet cell cultures and was designated officially simian virus 40, SV_{40}. It was noted in the original publications that no clinically significant short-term effects of SV_{40} virus were observed in any animal species tested; the possibility, however, of an oncogenic quality of the virus was recognized.

More recently, Eddy *et al.*(3) recorded the appearance of undifferentiated sarcomas in subcutaneous tissue, kidneys and lungs of hamsters which had been injected as newborns with the cell extracts of frozen and ground primary cultures of rhesus monkey kidney. The nature of the oncogenic stimulant was not defined and attempts to recover virus from extracts of the tumors in primary vervet monkey kidney (*Cercopithecus aethiops*, S. Africa), primary mouse embryo and continuous line rhesus kidney cell cultures were negative. No studies to define the simian virus population of the rhesus cell cultures used to inoculate the hamsters were carried out. Eddy *et al.* labelled their tumor inducing material as a "substance".

Present controlled studies in our laboratory have shown that SV_{40} virus may induce fibrosarcomatous tumors in hamsters inoculated as newborns and that the virus may be demonstrated in the tumors by virus recovery and by specific immunofluorescent staining. These findings are the subject of the present report.

Materials and methods. Experimental design. Pregnant hamsters obtained from the Lakeview Hamster Colony, Newfield, N. J. were housed individually in isolation filter cages.† The newborn hamsters less than 24 hours of age were inoculated intracerebrally with 0.03 ml and subcutaneously with 0.2 ml into the area between the scapulae with one of the following undiluted inocula: (a) SV_{40}-infected primary grivet monkey kidney (GMK) cell culture extracts, (b) non-infected, SV_{40}-free GMK cell culture extracts (TCF), or (c) tissue culture maintenance medium (MM) which had not been in contact with cell cultures. In certain experiments, the subcutaneous route alone was employed. Uninoculated control animals were included in each experiment. The animals were observed for development of palpable masses and further appropriate procedures were carried out as described below. Hamsters which died were routinely autopsied to determine the cause of death but cannibalism sometimes made this impossible. *Tissue cultures.* Primary cell cultures of GMK were prepared from trypsinized cell suspensions and were grown in Melnick's lactalbumin-yeast extract medium(4) containing 5% heat-inactivated calf serum. During virus propagation, the cell cultures were maintained using medium 199(4) containing 2% heat-inactivated chicken serum and at initial pH 7.6. Mouse embryo cell cultures were prepared using Eagle's basal medium (BME)(4) containing 10% horse serum and were employed in first subculture for virus isolation purpose. For maintenance purpose, BME with 2 to 5% horse serum was utilized. All culture fluids contained 50 μg of penicillin and 50 units of streptomycin per ml. All cell cultures were held in a stationary position and were incubated at 36°C. *Preparation of SV_{40} virus and control materials.* Prototype SV_{40} strain VA 45-54 recovered from uninoculated GMK cell culture was used. The agent was passed in GMK cultures shown to be free, by cultural tests, of detectable extraneous simian viruses to prepare several standard SV_{40} pools designated GMK1 of 9/22/60, GMK2 of 2/10/61 and GMK2 of 6/27/61. Serum neutralization tests performed using SV_{40} antisera and other biological tests indicated that SV_{40} virus was the only detectable agent present. The infected cultures, at time of harvest, were frozen (–20°C) and thawed (37°C) several times and the extracts were employed crude or after clarification by low-speed centrifugation. The samples were stored frozen at –70°C in flame-sealed glass containers until injected into the animals. The control TCF was prepared in a similar fashion from uninoculated SV_{40}-free GMK cultures. For the first experiment, a pool of control material was prepared by combining the content of 2 culture flasks from each of 19 GMK culture lots. In all other experi-

† Modification of basic design by Dr. L. Kraft, Yale University, for work with gastroenteritis in newborn mice.

ments, the control material consisted of uninoculated GMK extract of the same lot of cells used to prepare SV_{40} virus and was processed in identical manner. The control MM in all but the first experiment was the same lot employed to prepare the SV_{40} virus and the corresponding control TCF. *Observation of animals.* The suckling hamsters were weaned at 18 days of age. At that time the mothers were bled for purpose of SV_{40} and polyoma serologic tests and were discarded. The animals were housed in filter cages until day 75 when they were transferred to open-top cages for holding. Some of the litters which received like material were pooled at that time. After the seventy-fifth day, the hamsters were observed twice weekly for palpable masses until the first tumor was observed; thereafter, they were palpated more frequently. *Pathology.* At the time of sacrifice of a tumor-bearing hamster for study, the animal was bled and the serum tested for SV_{40} neutralizing and mouse polyoma hemagglutination-inhibiting (HAI) antibody. The excised tumor was divided for histopathologic diagnosis, for fluorescent antibody studies, for virus isolation attempts, and for transplantation to hamsters. For pathology, the tissues were fixed in 10% formalin and the paraffin sections were stained with hematoxylin and eosin. For fluorescence microscopy, 5 to 7 μ thick sections were prepared in a cryostat set at –24°C, fixed in acetone at room temperature for 10 minutes, and stained by the indirect method employing normal, SV_{40}-immune and polyoma-immune rabbit antisera and goat anti-rabbit fluorescein conjugate. For transplantation purpose, the tissue was rapidly minced in Hanks' balanced salt solution (HBSS)(4) and implanted *via* trocar into the subcutaneous space on the backs of 1- to 3-month-old hamsters. *Virus isolation.* Tumor tissue was blended for 10 minutes at 16,000 rpm in an Omnimix cup held in an icebath employing sufficient HBSS to give a 15% suspension. The supernate obtained following centrifugation at 2,200 rpm for 20 minutes was stored frozen at –70°C in flame-sealed glass ampoules until tested for virus by passage to primary cell cultures of GMK, first subculture mouse embryo cells, or to newborn hamsters inoculated subcutaneously as described above. The cell cultures were inoculated with 0.2 ml of undiluted tumor suspension or with dilutions of the suspension prepared in HBSS or in medium 199 and were refed after 24 hours incubation. The cultures were observed for at least 21 days for development of cytopathology after which they were frozen and thawed and passed again to fresh cell cultures. Additionally, the fluids from mouse embryo cell cultures, following treatment with receptor destroying enzyme (RDE), were examined for hemagglutinins for guinea pig erythrocytes to rule out the presence of mouse polyoma virus. *Serology.* Virus isolates were identified by usual procedures(1,2) in GMK cultures employing standard SV_{40} rabbit antiserum. Sera from the mothers and from the tumor-bearing hamsters in the experiments were tested for SV_{40} antibody by the neutralization procedure and for mouse polyoma antibody by the HAI test following treatment with RDE. *Polyoma controls.* For control purpose, a line of fibrosarcoma tumors was derived from a tumor of a hamster inoculated when newborn with polyoma virus by Dr. B. Eddy, and routinely transplanted in our laboratory. These tumors were excised and tested in cell cultures and by fluorescent microscopy procedures as described above for the SV_{40} tumors.

Results. Tumor incidence. Table I presents results of experiments in which newborn hamsters were given SV_{40}-infected or uninfected GMK culture fluids, maintenance medium, or were held uninoculated. Tumors developed in all experiments in animals which received SV_{40} virus; by contrast, no tumors developed in animals of any of the control groups. In Experiment 1, 16 of 20 animals which survived 75 days and which did not die spontaneously thereafter developed tumors. The first tumor was palpated on day 132 post-inoculation and the last was observed on day 165. The average incubation period for tumor development in the 16 animals was 144 days. Control animals are still under observation and, although 10½ months old, have not developed tumors. Non-specific deaths were more numerous among the SV_{40} infected

TABLE I. Tumors Induced in Hamsters Infected with SV_{40} Virus When Less Than 24 Hours Old.

Inoculum			Results of tests in hamsters			
Code	Passage level in GMK	Infectivity titer in GMK (.2 ml)	No. weaned	No. non-specific deaths	No. developing tumors	No. survivors without tumors
Exp. 1 (1/26/61)						
SV_{40} 9/22/60 *F & T 2×, crude	1	$10^{-5.7}$	52	32	16	4
Normal TCF pool F & T 2×, crude	—	$<10^{0}$	51	10	0	41
Medium	—	—	50	5	0	45
Uninoc. controls	—	—	39	6	0	33
Exp. 2 (3/20/61)						
SV_{40} 2/10/61 F & T 4×, cent'd	2	$10^{-7.5}$	55	21	16	18
Normal TCF F & T 4×, cent'd	—	$<10^{0}$	59	20	0	39
Medium	—	—	43	16	0	27
Uninoc. controls	—	—	66	26	0	40
Exp. 3 (7/13/61)						
SV_{40} 6/27/61 F & T 3×, cent'd	2	$10^{-6.0}$	44	4	10	30
Normal TCF F & T 3×, cent'd	—	$<10^{0}$	62	9	0	53
Medium	—	—	49	5	0	44
Uninoc. controls	—	—	90	3	0	87

Hamsters were inoculated intracer. and subcut. in Exp. 1 and 2, subcut. only in Exp. 3.

* F & T designates number of times cultures were frozen and thawed and whether material was clarified by centrifugation.

animals in this experiment than among the controls. The cause for this is unknown other than that parasitic infestation with intestinal obstruction was a frequent cause of death. Detailed findings relating to the tumors in these animals are shown in Table II. Additionally, the findings relating to tumors in 3 animals which received transplants of tumor from animal C1 are shown.

Experiments 2 and 3 were initiated more recently and new tumors are still appearing in the SV_{40} group. In Experiment 2, the tumors are appearing more slowly than in Experiment 1. The range, in days, for appearance of tumors among the 16 animals was 130 to 327, the average was 230 days. In Experiment 3, tumor development was more rapid, the range for the 10 animals being 103 to 144 days and averaging 122 days. These variations may be within normal expectation for the phenomenon or may be the result of other variables such as freezing and thawing of the cultures, clarification, or virus passage history.

Tumor pathology. The tumors which developed were located in the subcutaneous area on the backs of the animals and were usually in close proximity to the site of deposition of the SV_{40} virus inoculum. Fig. 1 shows one animal (C2) in which a large tumor developed in the subcutaneous space of the scalp and another animal (C6) with bilateral masses. Single and multiple tumors have occurred in animals of both sexes and, once palpable, these developed rapidly to a size greater than that of the host. When allowed to develop further, these larger tumors became ulcerated at the surface of the skin and the hamsters died.

At autopsy, the tumors appeared grossly as non-hemorrhagic hard white masses attached either to the skin or to the superficial fascia of the carcass (Fig. 1). In a few cases, attachment to the paravertebral muscles or

TABLE II. Summary of Studies of Tumors of Hamsters Inoculated with SV_{40} Virus in Experiment 1.

Animal identification	Tumor occurrence: Day post-inoc. First palpable	Day post-inoc. Autopsy	Tumors No.	Tumors Wt, g	Virus isolation results: Tumor extract GMK infect. titer	Mouse embryo	Blood GMK	Other GMK†	Serology: Fluor. mx. SV_{40}	SV_{40} neut.	Polyoma HAI	Tumor transplant
Primary SV_{40} tumors												
A1-♀*	146	167	1	2.2	$<10^{0}$	$<10^{0}$	$<10^{0}$	$<10^{0}$	ND	<1:1	<1:10	ND
A2-♀	146	161	1	5.1	$10^{-1.0}$	"	"	"	Pos	"	"	"
B2-♀	137	211	>1	215.0	ND	ND	"	ND	"	"	"	"
B3-♀	144	147	1	2.0	$<10^{0}$	$<10^{0}$	ND	$<10^{0}$	"	"	"	"
B4-♀	155	195	>1	47.0	$>10^{-2.5}$	"	"	ND	"	"	"	Pos
C1-♀	132	133	1	15.0	$10^{-1.0}$	"	"	"	"	1:2	"	"
C2-♂	132	161	>1	12.4	$10^{-.75}$	"	$<10^{0}$	$<10^{0}$	"	1:1	"	ND
C3-♀	133	153	>1	5.5	Trace	"	ND	"	Weak pos	<1:1	"	"
C4-♂	137	138	1	2.0	ND	ND	"	ND	ND	"	"	"
C5-♀	137	161	1	6.1	Trace	$<10^{0}$	$<10^{0}$	$<10^{0}$	Pos	"	"	"
C6-♂	137	161	>1	23.2	$10^{-1.25}$	"	"	ND	"	"	"	"
D1-♀	133(?)	167	1	1.2	$<10^{0}$	"	"	"	ND	"	"	"
D2-♂	165	186	1	10.3	$>10^{-2.5}$	"	ND	"	"	"	"	"
Transplant tumors												
C1-1A-♂	22	57	1	8.2	$10^{-1.0}$	"	ND	ND	Pos	<1:1	<1:10	ND
C1-1B-♂	35	62	1	5.0	$10^{-1.5}$	"	"	"	"	"	"	Pos
C1-1C-♂	39	70	>1	13.0	$10^{-1.5}$	"	"	"	ND	"	"	"

* Sex, male or female.
† Urine, feces, mouth washing.

ND = not done.

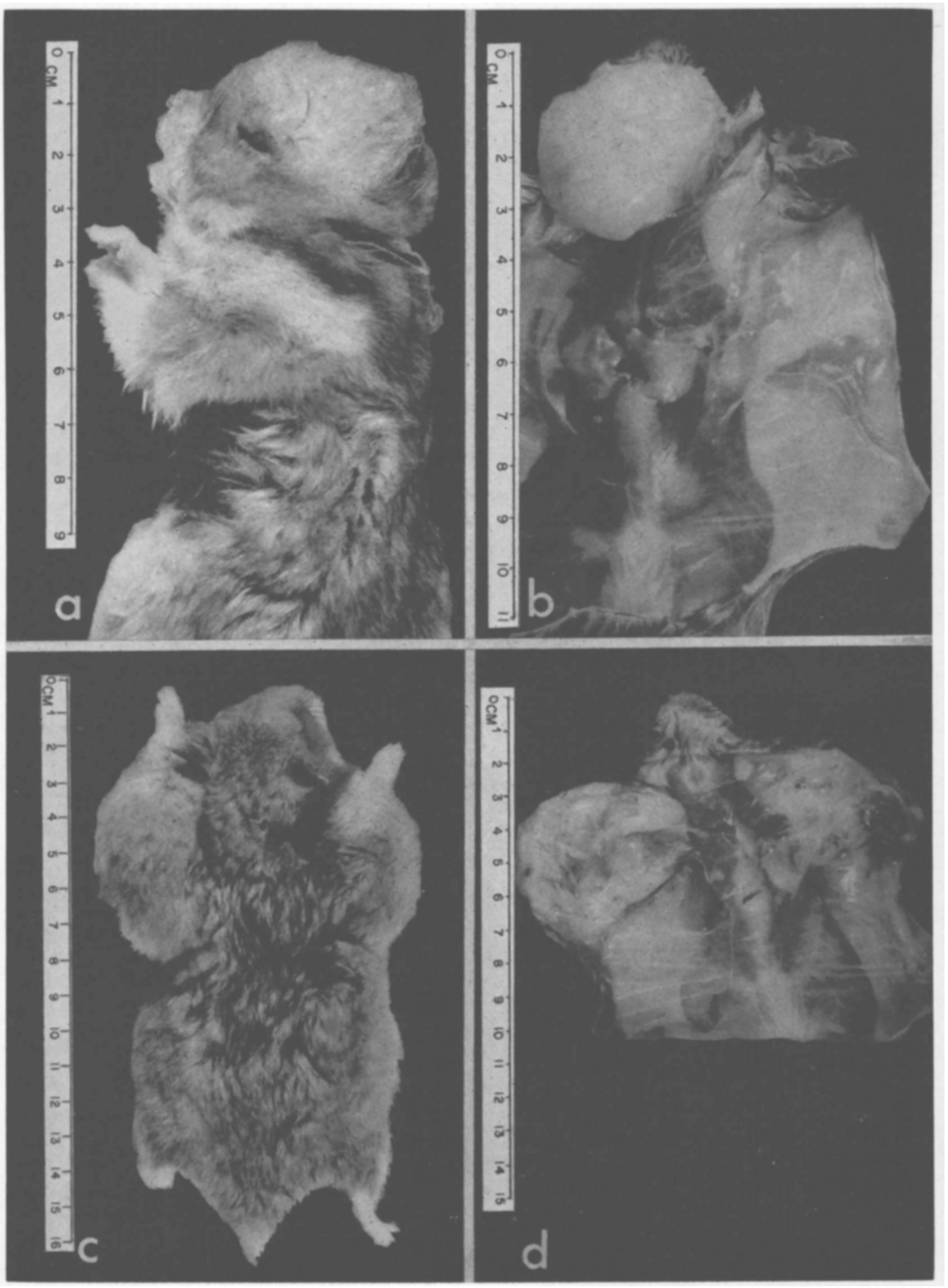

FIG. 1. Tumors in 161-day-old hamsters inoculated when newborn with SV_{40} a. and b. Single subcut. fibrosarcoma located on crown of head. c. and d. Bilateral subcut. fibrosarcomas.

deep fascia was firm and there was extension of the tumor although metastases were not evident grossly. In one case, C4 of Table II, there was a malignant mesothelioma in the peritoneum with metastases to the lung but its relationship to the SV_{40} virus is not known. Except for the presence of tumors, the animals were in apparent good health and other tissues appeared normal grossly.

Histopathologic examination of the tumors revealed wide histomorphologic variation (Fig. 2). Interlacing strands composed of spindle-shaped cells blended with areas of densely packed anaplastic cells having pleomorphic nuclei, little cytoplasm, and indistinct cell borders. Multinucleated, bizarre, tumor giant cells with hyperchromatic nuclei were scattered throughout the sections, being more numerous in some areas than in others. Mitoses were not uncommon. Invasion of adjacent muscle and adipose tissue was apparent in the edges of some of the sections. Histologically, the tumors were considered to be fibrosarcomas of varying degree of malignancy.

Virus isolation findings. SV_{40} virus was recovered on first passage in GMK (Table II) from primary tumors of 8 of 11 animals tested and from all of 3 transplant tumors which derived originally from animal C1. The SV_{40} infectivity titers of the crude tumor extracts varied from trace amounts, *e.g.*, 1 of 4 tubes positive at undiluted, to $>10^{-2.5}$. Only 3 tumor extracts, A1, B3, and D1, yielded no virus even on blind passage and with repeated isolation attempts. It is of interest that these 3 tumors were smaller in size (1.2 to 2.2 g) than the others which were examined and found positive. In all instances, the SV_{40} virus isolate was specifically neutralized by SV_{40} antiserum and not by normal hamster or rabbit sera.

For control purpose, each lot of GMK cells employed for virus isolation purpose was inoculated with tissue extract of mouse polyoma fibrosarcoma of hamsters to rule out the possibility that the neoplastic tissue was acting merely as an activator of SV_{40} already present in latent form in the GMK cells. Results were negative with all cell lots. For additional control purpose and to rule out mouse polyoma as a factor, each SV_{40} tumor extract was inoculated into mouse embryo cell cultures and blind passage was performed after 3 to 4 weeks to fresh cultures. Cytopathic effects were absent and hemagglutinins for guinea pig erythrocytes were not detected in frozen, thawed, and RDE-treated culture fluids at either passage level.

Attempts to demonstrate an SV_{40} viremia in 9 tumor bearing animals, were unsuccessful when individual heparinized bloods were tested. Likewise, no virus was recovered from urine, feces, or mouth swabs of 6 tumor-bearing animals.

SV_{40} fluorescence microscopy. Tests were made of frozen sections of 9 primary and 2 transplant tumors (Table II). All 11 tumors were found to contain antigen capable of causing fluorescence with SV_{40} immune serum as shown in Fig. 3 but not with polyoma immune serum or with control pre-immunization sera. The fluorescence was predominantly in the perinuclear zone and in the cytoplasm of the cells. Frozen control sections prepared from mouse polyoma fibrosarcomas of hamsters did not show antibody localization with SV_{40} immune serum (Fig. 3D) indicating that the reaction of the serum with SV_{40} tumors was not due to non-specific fixation to hamster neoplastic tissue. Such control tissue preparations were examined each time an SV_{40} tumor was studied. Tumor C3 caused only weak immunofluorescence and it is of interest that only a trace of infective virus was recovered from this tumor. Tumor B3 gave specific immunofluorescence even though it yielded no virus on repeated isolation attempts.

Serology. Only 2 of 16 SV_{40} tumor-bearing hamsters developed SV_{40} antibody, both at the low titer of 1:1 or 1:2 (Table II). All other sera were negative even when collected 52 days after the tumors became palpable. Further, the sera of tumor-bearing animals did not neutralize the autologous viruses recovered. The mothers of the inoculated hamsters were found to be free of SV_{40} or mouse polyoma antibody and none of the tumor-bearing animals had polyoma HAI antibody when tested at a dilution of 1:10.

The possibility was considered that ham-

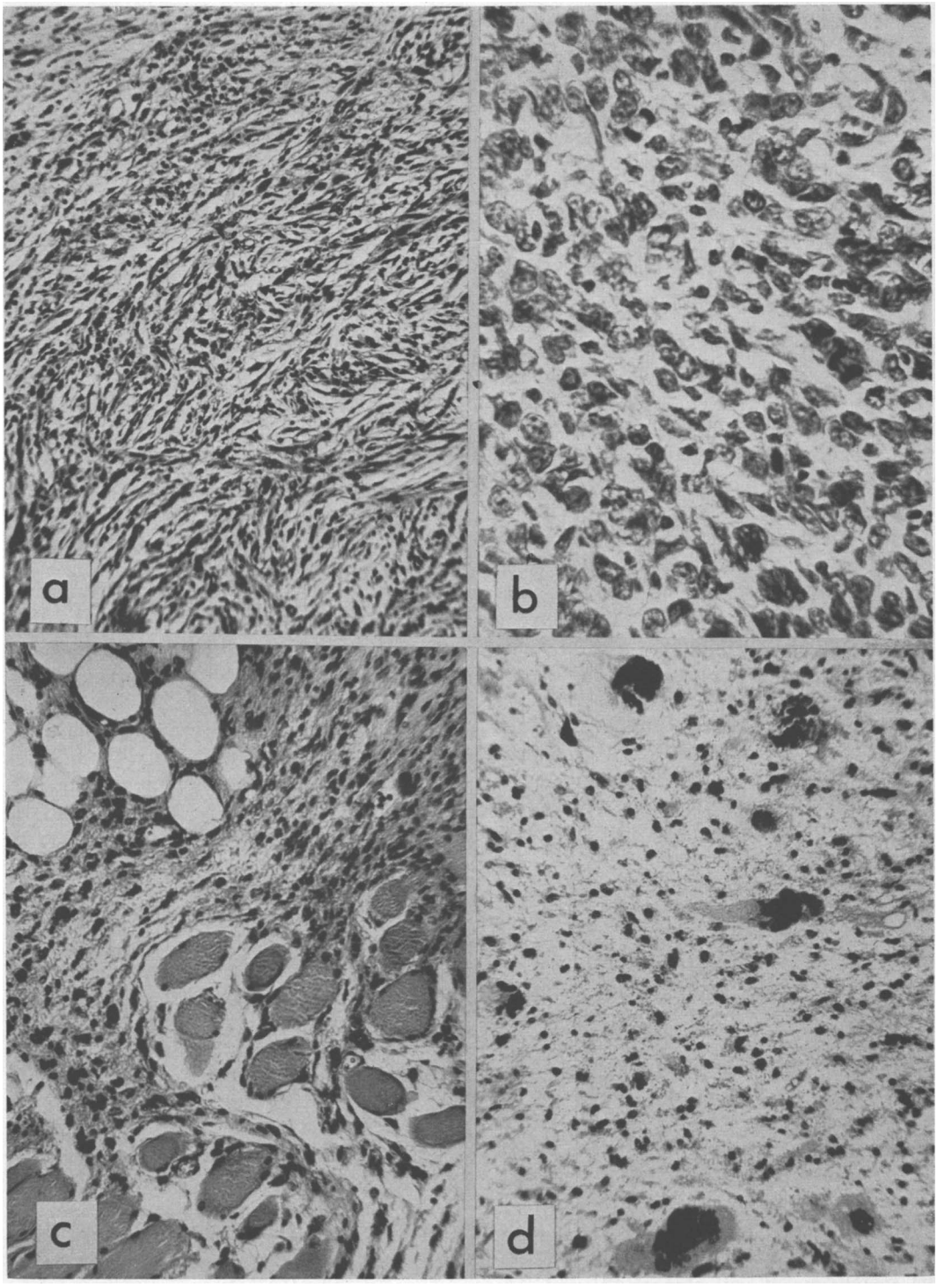

FIG. 2. Sections of a subcut. tumor in a hamster sacrificed when 133 days of age. a. Area of differentiation in the fibrosarcoma. H & E. Mag. 100×. b. Undifferentiated sarcomatous cells. H & E. Mag. 312×. c. Invasion of muscle and fatty tissue of the host by sarcoma cells. H & E. Mag. 100×. d. Giant cells in the sarcoma. H & E. Mag. 100×.

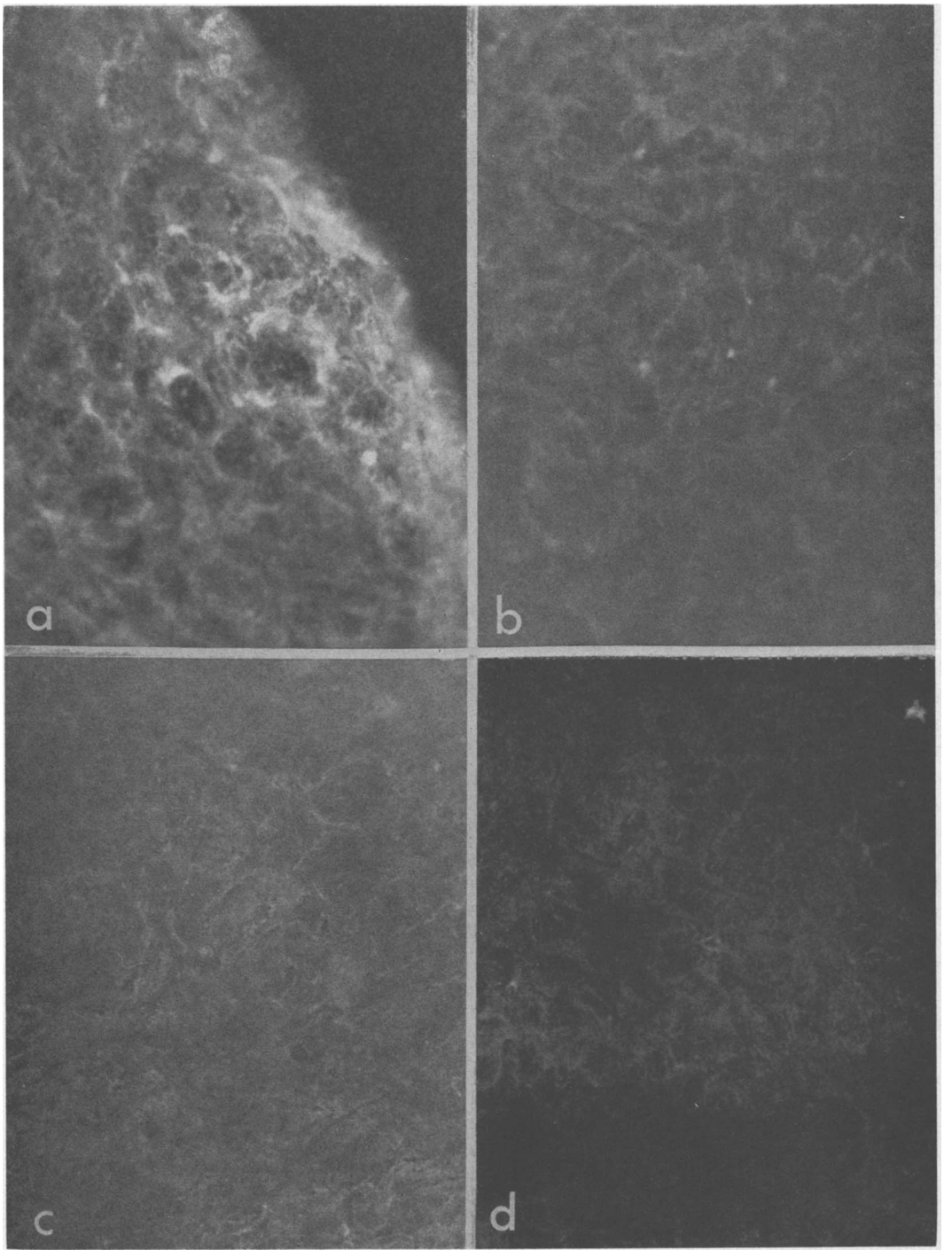

FIG. 3. a.-c. Cryostat sections of a subcut. tumor in a hamster sacrificed when 186 days of age and examined by fluorescent antibody technics. Mag. 115×.

a. SV_{40} tumor with rabbit anti-SV_{40} immune serum.
b. " " " " normal serum.
c. " " " " anti-polyoma immune serum.

3d. Cryostat section of a polyoma hamster transplant tumor examined after treatment with rabbit anti-SV_{40} immune serum.

TABLE III. Transplantation in Hamsters of SV_{40}-Induced Tumors.

Transplantation			Results	
Code*	Transplant No.	Age of recipients	No. with tumors/Total No. inoc.	Time of appearance of tumors, days
C1-1	1st	4-6 wk	8/8	22-46
B4-1	"	2-3 mo	2/5	19
C1B-2	2nd	2-3 mo	3/4	19-53
C1C-2	"	4-6 wk	20/20	11-41
B4A-2	"	3 mo	13/15	12-41
C1-3	3rd	2.5 mo	14/15	14-24
C1-4	4th	2.5 mo	5/5	16-21

* C1 and B4 are numbers of original animals with tumors, Table II.

sters inoculated in the early neonatal period might have developed immunologic tolerance to the SV_{40} virus and failed to respond with antibody production, even though virus was present in the tumors. To test this hypothesis, 3 groups of animals from Experiment 1 were selected for further study: (a) 3 seronegative SV_{40} tumor bearing animals, (b) 4 animals which had received SV_{40} virus but had not developed either tumors or SV_{40} antibody, and (c) 4 animals which had received the normal tissue culture control material. Each animal was bled, injected intraperitoneally with 1.0 ml (10^6 TCD_{50}) of standard SV_{40} virus, and rebled 3 weeks later. The pre-injection bleedings were all seronegative at 1:2 for SV_{40} whereas all the post-bleeding samples were positive for SV_{40} neutralizing antibody, indicating immunologic competence rather than immunologic tolerance in the animals.

Tests were also made for possible antigenic alteration of SV_{40} virus in the tumor. For this purpose, antisera were prepared in rabbits using SV_{40} virus recovered from 2 of the tumors. Both of these antisera neutralized the standard SV_{40} virus as well as the homologous isolate.

Transplantation studies. Serial transplantation of primary SV_{40} tumors C1 and B4 was accomplished with ease as shown in Table III. Tissue minces from the outer and the inner portions of the C1 tumor were transplanted separately into the subcutaneous space of the right and left flanks, respectively, of each of eight 4- to 6-week-old hamsters. Tumors developed between days 22 and 46 in all 8 animals. Tissue from the outer portion of the primary tumor was more active than the inner in that the former developed sooner and grew more rapidly. Additionally, in 4 animals, tumors developed only on the right flank where the outer tumor tissue was given. Transplants of the C1 tumor have been passed 4 times to date without difficulty. The B4 primary tumor has been passed twice. The transplant tumors of C1 (Table II) gave similar virus recovery and immunofluorescent findings as did the primary tumors. Thus, SV_{40} virus was recovered from the tumors and SV_{40} antigen was demonstrated in sections of the tissue by immunofluorescence. The tumors, histopathologically, were fibrosarcomas and were generally similar to the primary tumors. Virus has been recovered even after 2 transplant passages of the C1 tumor (10^{-2} titer).

Extracts of 7 primary SV_{40} tumors (Table II) have been passed to newborn hamsters by the subcutaneous route, as previously described, using 0.2 ml of the undiluted 15% tumor extracts. None have responded to date, *i.e.*, 150 days, with tumors.

Discussion. A wide variety of presumably spontaneous tumors including fibromas have been observed by Fortner *et al.*(5,6) in hamsters from the Lakeview Hamster Colony employed in the present work. Sarcomas have also been observed in hamsters following inoculation with extracts of cell cultures of rhesus monkey kidney(3), mouse polyoma virus(7), bile from a patient with benign biliary disease(5), sodium cholate(6), stainless steel powder(8), polyethylene(9), and a variety of the ordinary chemical carcinogens (6). The literature relating to sarcomas of hamsters has been reviewed by Fortner *et al.* (6).

The findings of the present study give evidence for the role of SV_{40} virus in induction of fibrosarcomatous tumors of hamsters. In 3 separate experiments, and in more recent experiments not presented herein, tumors developed with high incidence among hamsters given SV_{40} virus while the groups of control animals which were given uninfected cell cul-

ture or medium or which were held as uninoculated controls failed completely to develop tumors during the time period of the observations. Although spontaneous fibrosarcomas may occur among Lakeview Hamsters(5), this must be a rare event. In studies in our laboratories in which more than 1000 newborn hamsters were inoculated with human malignant tissue extracts or in which 150 newborns received a multinucleating simian virus, no fibrosarcomas were noted in any animal during the 1.5- to 2-year observation period.

Recovery of the SV_{40} virus from the tumor extracts was not the result of activation of a latent SV_{40} contaminant in the GMK cultures employed for isolation purpose since polyoma "control tumor" did not induce such reaction and since SV_{40} virus was clearly shown to be present in the cells by means of fluorescent antibody studies. The SV_{40} immune serum employed in the fluorescent antibody work did not fix to hamster neoplastic tissue in a non-specific way since polyoma tumor sections, similarly treated with the serum, were negative. Polyoma virus was not recovered from SV_{40} tumors, polyoma antibody did not appear in the hamster sera, and fluorescent antibody studies failed to detect polyoma antigen in the SV_{40} tumors.

Although related in the causal sense to tumorigenesis, the possible role of SV_{40} virus as a primary oncogenic agent, as a co-oncogenic agent or perhaps simply as an evoking agent for an already present oncogenic principle is unknown. The fact, however, that the virus was present in the tumor following a very long latent period favors a primary role of the virus in the oncogenic process. The SV_{40} virus in the tumor appeared to be strongly localized as evidenced by lack of viremia, by lack of secretion into the urine, feces or oral cavity, by failure to develop metastatic processes, and by the lack of or very poor antibody response. The failure to develop antibody was clearly not due to immunologic tolerance since the animals remained immunologically competent and was not due to antigenic alteration since the virus recovered from the tumor was indistinguishable antigenically from the original SV_{40} agent.

The tumors which developed in hamsters inoculated with SV_{40} virus were similar in many respects to those found by Eddy *et al.* (3) in hamsters injected with uninoculated primary rhesus monkey renal cell culture material. Generally, the time of appearance of the tumors, the predominant occurrence of tumors at the inoculation site, the transplantation findings, and the histopathologic appearances are similar. By contrast, our SV_{40} studies did not indicate gross lesions of malignancy in other than subcutaneous tissue, with one possible exception, whereas Eddy *et al.* (3) recorded tumor occurrence in the lungs and kidneys of several animals. In addition, SV_{40} virus was recovered from all but the smallest of our tumors by inoculation of extracts into GMK whereas Eddy *et al.*(3) did not detect virus in their tumors. SV_{40} is an essentially ubiquitous contaminant of rhesus monkey kidney cell cultures and it is not unreasonable to expect that SV_{40} virus was present in the Eddy *et al.* preparations. The negative findings might have been due, therefore, to inadequate utilization of the vervet monkey kidney cell culture, which is a medium known to be sensitive to SV_{40} virus. No gross lesions were noted in the brains of the hamsters inoculated intracerebrally with SV_{40} virus in our studies and histopathologic observations were not carried out. Such indeed might have occurred unnoticed and might have been responsible for certain of the early deaths in the animals given SV_{40} virus by the cerebral route.

The findings reported here relate to the oncogenic activity of SV_{40} virus in hamsters only and do not warrant extrapolation relating to oncogenesis in other mammalian species. As such, they represent the first definitive evidence implicating a virus of primate origin as a malignant oncogenic agent in an experimental animal.

These investigations have provided new avenues for experimental studies in viral oncogenesis which deserve concerted effort during the next decade. Age susceptibility, virus dose response, influence of tissue culture passage history, effect of filtration, communicability, uniqueness of SV_{40} virus as an oncogenic agent for hamsters, and definition of

the whereabouts of the virus during the latent period in the SV_{40}-hamster system are all questions for the future.

Summary. Intracerebral and subcutaneous injection into newborn hamsters of vacuolating virus, SV_{40}, grown in renal cell cultures of grivet monkey resulted in single or multiple fibrosarcomas at site of injection which were histologically of varying degree of malignancy. These occurred 3½ to 8 months post-inoculation and in both sexes. Animals injected with appropriate control materials or held uninoculated failed to develop tumors. Tests to exclude mouse polyoma virus as a factor were clearly negative. Evidence for the role of SV_{40} virus as a primary oncogenic agent was provided by recovery of the virus from the tumor and by demonstration of SV_{40} antigen in tumors by fluorescent antibody staining. The agent appeared to be localized in the tumors since the virus was not detected in blood or excretions, since antibody response was minimal or lacking, and since gross metastases were lacking. Transplantation and serial passage of SV_{40}-induced tumors were accomplished with ease. The data represent the first definitive evidence implicating a virus of primate origin as a malignant oncogenic agent in experimental animals. The findings relate to observations of oncogenesis in hamsters and do not warrant extrapolation to oncogenesis in other mammalian species.

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A New Model of Skin Injury.* (27299)

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The increased vascular permeability at inflamed skin sites is invariably preceded and accompanied by vasodilation(1). This observation suggests a causative relationship, even though it has been demonstrated that capillary permeability is a function of the intracapillary blood pressure rather than the capillary diameter(2).

According to one view(3), the passage of proteins across vascular walls occurs at cellular junctions due to changes in the properties of a cement substance which was thought to be present in this location. This hypothesis was later modified in that protein extravasation was attributed to changes in the geometry and dimensions of the intercellular spaces due to the swelling of the endothelial cells which accompanies the inflammatory process(4). More recently, a hypothesis was presented, according to which the transendothelial passage of macromolecules occurs by a process akin to pinocytosis(5,6). This last view dissociates vasodilation from the transfer of protein molecules across endothelial membranes, since changes in vascular

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