TABLE II. Passive Transfer of EAE with Spleen Cells: Acceleration by Adrenalectomy.

Spleen cell pool	No. of cells to each recipient	Recipients Type	No.	EAE in recipients: Clinical, day of onset*	EAE in recipients: Histologic scores†
1	4.7×10^8	Adrenex	2	2 3	4 3
		Intact	2	3 –	4 1
2	1.6×10^8	Adrenex	5	2 2 3 3 3	4 4 3 3 2
		Intact	5	3 – – – –	2 1 1 0 0
3	1.6×10^8	Adrenex	9	2 2 2 3 3 3 3 3 3	3 3 3 3 2 2 2 2 1
		Intact	9	– – – – – – – – –	2 1 1 1 1 1 1 1 0

* Each figure represents a single rat with clinical signs that appeared on the indicated day; each hyphen represents a single rat without signs.

† Each figure is score of a single rat, graded from 0 to 4 according to number and intensity of lesions on randomized slides read without knowledge of the experimental group.

and mitotic inhibition(6).

Although our experiments have demonstrated only an accelerating influence of adrenalectomy, there may be situations in which the number or potency of lymphoid cells is so close to a threshold value, that passive transfer is successful *only* with the aid of adrenalectomy of recipients. This true enhancement has not yet been observed in our experiments.

Summary. Passive transfer of EAE has been accomplished with spleen as well as lymph node cells from actively sensitized donors. Adrenalectomy of recipients accelerated passive transfer of EAE. Adrenalectomy also accelerated active induction of EAE, especially when surgery was performed at least 2 days before injection of the antigen-adjuvant mixture.

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Neutralizing Antibodies to Simian Virus 40 (SV40) in Human Sera From India.* (30765)

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In some regions of north India, especially in the state of Uttar Pradesh (U.P.), man and the rhesus monkey live in close ecological association. In these areas, the contact of man with rhesus varies from maximal as with rhesus dwelling in crowded bazaars, to little as with rural roadside groups of rhesus. An educated guess of the number of rhesus in U.P. in 1960 was 800,000, and it was estimated that more than 80% of these lived in association with human communities(1): the human population of U.P. was 73.7 millions

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in 1961. Southwick *et al,* in a survey in U.P. (1) found one or more resident rhesus groups in 14 to 22% of the villages (population up to 2,000 people) and in 58 to 91% of the towns (2,000 to 15,000 population).

In an earlier investigation(2), sera of free-living rhesus in U.P., with moderate to maximal human contact, were tested for antibodies to certain viruses. Antibodies to measles virus were detected in five of 10 adults (5 to 15 years) and one of 40 juveniles (10 months-4 years); to parainfluenza 3 virus in nine of 10 adults and 5 or 42 juveniles; and to simian virus 40 (SV40) in all of 5 adults and 7 of 35 juveniles.

The presence of antibodies to measles and parainfluenza 3 viruses in free-living rhesus suggested that the rhesus may naturally contract some respiratory virus infections from man. The present investigation was designed to enquire if natural human infection with SV40 occurred. Sera were collected from people living in areas of high rhesus prevalence and from employees of firms which handled large numbers of rhesus for export. In addition, specimens were obtained from some cancer patients in Calcutta, West Bengal and Agra, U.P. These sera were tested for presence of neutralizing antibodies to SV40.

Materials and methods. A total of 247 specimens was collected. The donors were largely adults and had no history of vaccination with inactivated or attenuated poliovirus vaccine. They were: (a) 37 cancer patients, of whom all except 2 were residents of West Bengal, where rhesus are uncommon(1); (b) 12 cancer patients, from different parts of U.P.; (c) 161 donors drawn from general hospitals, out-patient clinics and healthy population in 2 areas of U.P. with known high rhesus prevalence, *viz.,* Aligarh city and nearby rural areas, and the Mathura-Brindavan region; and (d) 37 employees from 2 monkey-export firms. The employees cared for several thousand juvenile rhesus exported each month, and were the group most heavily exposed to rhesus.

Even in areas of high rhesus prevalence, the human population is not exposed uniformly to rhesus as each rhesus group has a characteristic pattern of movement(1). For a crude estimate of their own judgment of their contact with rhesus, the donors were asked if they saw rhesus in their neighborhood (a) daily (b) less often or (c) seldom.

Neutralization tests. These were performed in tubes of continuous *Cercopithecus* kidney cell line BS-C-1(3). Sera were inactivated at 56°C for 30 minutes and were serially diluted in 2-fold steps. The strain of SV40 used was obtained from Dr. B. Eddy. Initially, sera were tested at a single dilution of 1:2 against 300 TCD_{50} of virus; those that prevented or delayed virus cytopathic effect were retested undiluted and in dilutions up to 1:16 against 50-100 TCD_{50} of virus. Serum-virus mixtures were incubated at 35°C for 45 minutes and then overnight at +4°C. 0.2 ml of the serum-virus mixture was inoculated into each of two tubes of BS-C-1 cells. After adsorption for one hour at room temperature, 2.0 ml of maintenance medium (199 with 2% fetal calf serum and antibiotics) was added to each tube. Medium was replaced approximately every 5 days.

The tubes were incubated at 35°C in a stationary position and, after 7 days, were examined every day or on alternate days for cytopathic effect. Tests were terminated on day 21. A serum dilution was interpreted as positive if it completely prevented virus cytopathic effect when all of 4 control tubes inoculated at the same virus dilution showed cytopathic effect of at least 2 to 3+ and the effective virus dose was at least 10-30 TCD_{50}. These conditions were realized on the 9th or 10th day with the test dose of 300 TCD_{50} and between the 11th and 13th day with a test dose of 50-100 TCD_{50}.

Results. The results of neutralization tests of the sera from cancer patients and from the general population are given in Table I. None of 49 sera from cancer patients (37 from West Bengal and 12 from U.P.) was protective. Antibodies were detected in 14 of 161 (8.7%) sera from the areas of U.P. with known high rhesus prevalence: in 5 of 82 (6.1%) from Aligarh and in 9 of 79 (11.3%) from Mathura-Brindavan. Antibodies were detected in both sexes and in all age groups, and there were no consistent

TABLE I. Prevalence of Neutralizing Antibodies to SV40 in Human Sera.

Donors	Region	Rhesus prevalence in region	No. positive/No. tested Age in years 8–20	20–39	40+	Total	% positive
Cancer patients	West Bengal	Low		0/14	0/23	0/37	
	Uttar Pradesh (U.P.)	Variable		0/4	0/8*	0/12	
Total				0/18	0/31	0/49	
Healthy, or non-cancer patients	Aligarh	High	0/13	2/32**	3/37	5/82	6.1
	Mathura-Brindavan (U.P.)	"	4/14*	3/32	2/33**	9/79	11.3
Total			4/27	5/64	5/70	14/161†	8.7

* Each asterisk represents a doubtfully positive serum in the denominator.
† Sex distribution: Males 7/104; Females 7/57.

TABLE II. Relationship Between Donors' Judgment of Rhesus Prevalence in Their Neighborhood and Prevalence of SV40 Neutralizing Antibodies.

	No. positive/No. tested Rhesus seen in neighborhood by donor Daily	Less often	Seldom	Total
Aligarh–rural	2/17	0/12	1/13	3/42
" –city	0/9			0/9
Mathura-Brindavan	1/29	5/25	3/25	9/79
Total	3/55	5/37	4/38	12/130

differences in the antibody prevalence in the various groups.

In Table II, the data are analyzed by the donors' estimate of their contact with rhesus. There was no significant difference in antibody prevalence in the 3 groups which saw rhesus in the neighborhood (a) daily (b) less often or (c) seldom.

The titers of the positive sera† were low. Ten of 14 sera had titers of 1:1 or 1:2 and a single serum a titer of 1:16. Only 2 sera prevented virus cytopathic effect for the full observation period of 21 days; the others delayed the onset of cytopathic effect from 2 to 10 days. Six other sera showed, on the day of the interpretation of the test, marked reduction of the cytopathic effect but not complete prevention. This effect was reproducible and probably represented low antibody titers.

† Five of these sera were kindly tested by Drs. S. S. Tevethia and F. Rapp (Dept. of Virology and Epidemiology, Baylor Univ. College of Med., Houston) in neutralization tests by the plaque reduction technique; their results were generally confirmatory.

The 6 donors were: 2 from Aligarh, 3 from Mathura-Brindavan and one from the 12 cancer patients in U.P. Each of these donors is indicated by an asterisk in Table I.

The results of tests of sera of the employees in the monkey-export firms are given in Table III. Four of 22 sera from employees of one firm and 6 of 15 from the other were protective, giving a combined prevalence of 10 out of 37 (27%). The presence of antibodies was related to length of service. Antibodies were detected in one of 17 who had served for less than 5 years, in 4 of 13 who had served between 5 and 10 years, and 5 of 7 who had served for more than 10 years.

The titers were higher than those of the protective sera from the U.P. residents (Table IV). Six of the 10 positive sera protected tissue cultures for the full observation period of 21 days and 5 had titers of 1:16 or greater.

Discussion. The neutralization of SV40 by 14 of 161 sera from areas of high rhesus prevalence in U.P. and by 10 of 37 sera of monkey handlers probably indicate naturally acquired previous infection of the donors with SV40, and provide an instance of natural infection of man with a tumor virus of non-human origin. The donors had not received any vaccine of monkey kidney origin and as far as is known, no other human infection produces antibodies that will cross-react with SV40. The higher proportion of positives in the monkey handlers and the higher titers of their sera probably reflect repeated exposure to virus.

The titers of the protective sera were low, particularly those of the positive sera from

TABLE III. Relationship Between Length of Service in Monkey Export Firms and Prevalence of SV40 Neutralizing Antibodies.

Years of service	No. positive/No. tested Export firm I	II	Total
<1 to 5	1/15	0/2	1/17
6 to 10	3/7	1/6	4/13
11 to 13		5/7	5/7
Total	4/22	6/15	10/37

U.P. In studies of Morris *et al*(4) where 35 volunteers were infected by the respiratory route with 10,000 TCD_{50} of SV40, the antibody titers were not high; of the 22 who developed antibodies, titers at 3 weeks post-infection were 1:10 or less in 13. Further, in the 2 volunteers that were followed, antibody titers dropped from 1:80 at 3 weeks to 1:20 and 1:5 at 34-38 weeks after infection. After oral administration of SV40, there was apparently no antibody response(5,6) even though virus was occasionally detected in stools as late as 5 weeks after ingestion(6). Thus, SV40 produces a low-grade infection in man with a low antibody response. It is possible that the actual prevalence of SV40 antibodies in the U.P. sample was higher than the estimated 8.7%, because the neutralization test in tubes, employed in this study is not sensitive enough to detect very low levels of antibodies(7).

There is little information on the course of SV40 infection in rhesus, but infection in the *Cercopithecus* monkey has been studied (8,9). Virus circulated in the blood of infected animals for a period of 2 to 14 days, was detectable in pharyngeal secretions for about a week beginning 8-10 days after infection and was excreted in urine for 4 weeks post-infection. It established a latent infection in the kidney and was recoverable as late as 8 months after infection from outgrowths of kidney tissue but not from ruptured cells of the kidney. If the course of infection in the rhesus is comparable, then some of the possible ways in which infection could be transmitted from rhesus to man are by droplet infection, through an arthropod, by inhalation of dust contaminated by virus-containing urine, or by contamination of food and water. SV40 is remarkably stable to heat, and to chemical and physical agents (10).

SV40 produces malignant tumors in hamsters and transforms human and hamster cells *in vitro*(11-13). The transformed human cells are believed to have, at least in some instances, neoplastic properties for man(14). The virus multiplies readily and to high titers in cells of human origin(10-13). Several investigators have raised the question of the possible role of SV40 in the etiology of human cancer(10,14). It will be of great interest to investigate how the virus is transmitted from rhesus to man and if infection with this virus is associated with malignant human tumors in areas of high rhesus prevalence in north India. Some of the conditions which facilitate detection of virus-caused tumors in experimental systems may be present in the Indian population, *viz.*, infection in early life (in infancy or early childhood), simultaneous infection with other viruses, and individuals infected sufficiently long ago to allow for an incubation period of many years.

Summary. Neutralizing antibodies to SV40 were detected in 14 of 161 human sera from residents of Uttar Pradesh in North India. Antibody titers were low. The donors gave no history of immunization with any vaccine of monkey kidney origin, but lived in areas of high rhesus prevalence. Neutralizing antibodies were also detected in 10 of 37 individuals who cared for large numbers of juvenile rhesus collected for export. The prevalence of antibodies was related to length of service and titers were higher than in the

TABLE IV. Comparison of Antibody Titers of Positive Sera from Residents of U.P. and from Monkey Handlers.

	No. of sera with antibody titers						
	Doubtful	1:1	1:2	1:4	1:8	1:16	>1:16
Residents of U.P.	6	6	4	1	2		1
Monkey handlers	0	1	2		2	1	4

positive sera from the residents of Uttar Pradesh.

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Gel Precipitation with ECHO 4 and Other Enteroviruses.* (30766)

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The method of double diffusion gel precipitation has been used in recent years for investigations on human enteroviruses(1,2,3). Studies on ECHO viruses have been reported by Middleton *et al*(4) and Balayan *et al*(5). In the present investigation, this procedure was used for the study of serologic variation occurring in ECHO virus type 4. Other ECHO serotypes as well as poliovirus and the Coxsackie virus were included for comparative purposes.

Materials and methods. Preparation for viral antigens. The following strains of viruses were used: ECHO 4—Pesascek strain (prototype), Shropshire strain, isolated in 1956 during an epidemic of aseptic meningitis in Buffalo, N. Y., and found by neutralization tests to be a serologic variant of ECHO 4(6); ECHO 14—Tow; Coxsackie B6—Schmitt; poliovirus II—MEF-1. Viruses were propagated on primary cultures of rhesus monkey kidney. The cultures were grown at 36°C in Blake bottles using a medium consisting of 0.5% lactalbumin hydrolysate in a base of Hanks' balanced salt solution (BSS) with 2% monkey serum. After 3 days of growth, the medium was exchanged with medium 199 plus 2% monkey serum.

Monolayers were usually complete after 6 days of incubation. At this time the cultures were inoculated with 1.0 ml of virus and the inoculum was allowed to adsorb for 90 minutes at 36°C. The fluid was then removed from each bottle and the monolayers were rinsed 3 times. The cultures were then maintained on medium 199 without serum. This procedure was employed because the viruses used for inoculation were grown in a medium containing bovine serum components. The presence of bovine serum antigens in viral preparations interfered with

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