were staphylococcus, proteus, a coliform organism or streptococcus. 3. The incidence of bladder calculi was higher in instances of more radical cystectomy (⅔ of the bladder), and followed closely the incidence of infection. Urinary calculi were always present when proteus was the infecting agent.

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Hamster Ascitic Fluids Containing Complement-Fixing Antibody Against Virus-Induced Tumor Antigens. (30464)

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Virus-specific, complement-fixing (CF) antigens have been demonstrated in tumors produced by adenoviruses(1), SV 40(2), Schmitt-Ruppin strain of Rous virus(3) and polyoma(4). Animals bearing these tumors develop the corresponding CF antibodies. Since the CF antigens appear to be highly specific and are present in both *in vitro* and *in vivo* transformed cells, they have provided an additional parameter in virus-tumor studies. Up to the present the only source of antibody to these antigens has been the serum of tumor-bearing animals. Because the animals involved are generally small rodents (hamsters and mice) there is a limited volume of usable antiserum readily available. Investigators studying other types of antibody produced in mice were confronted with similar problems of small volumes and were able to resolve them by techniques developed by Munoz(5) and Lieberman *et al*(6,7). These workers were able to obtain high titer antibody to various antigens in the ascitic fluids of mice which had been given intraperitoneal injections of adjuvant. By similar procedures, we have been able to produce relatively large quantities of ascitic fluids containing CF antibody to antigens in tumors induced by SV 40, adeno-18 and polyoma viruses as reported here.

Materials and methods. Tumors. A transplantable SV 40 virus-induced hamster tumor obtained from Dr. Bernice Eddy was used during initial studies, and a similar tumor produced by inoculation of suckling hamsters with a large-plaque strain of SV 40 virus derived in this laboratory was subsequently studied. This latter tumor has been designated as the SV 40-LP-T. A polyoma tumor was similarly produced after inoculation of suckling hamsters with polyoma virus. The adeno-18 hamster tumor was obtained from Dr. Wallace Rowe. All of these tumors were readily transplantable by subcutaneous (s.c.) inoculation of adult hamsters and were free of demonstrable infectious virus.

CF test. The technique of the test has previously been described(4). Four to eight units of antigen in the form of whole tumor emulsions or suspensions of washed cells grown in tissue culture were used for titration of antibody. End-points represent the highest dilution of serum or ascitic fluid giving 3 or 4 plus fixation. Positive control sera were included in every test. Titrations given in Table I were obtained in tests using SV 40 transformed human diploid culture cells (W1 18 Va 2)(8) as antigen.

Results and discussion. Preliminary experiments were conducted with the SV 40 tumor obtained from Dr. Eddy. Hamsters were inoculated s.c. with 10^6 trypsin-dispersed cells. After 2-3 weeks when the tumors had attained a size approximately 5 to 10 mm, they

TABLE I. Determination of Optimal Conditions for Production of SV 40 CF Antibody in Hamster Ascitic Fluid.

Group and treatment	Hamster No.	CF titer Serum	CF titer Ascitic fluid
I			
Tumor excised; adjuvant + tumor homogenate I.P.	104	8	16
	110	32	32
	118	64	64
	102	32	64
	111	<4	4
II*			
Tumor not excised; adjuvant + tumor homogenate I.P.	113	32	32
	141	32	32
	121	4	16
	118	64	64
III			
Tumor not excised; adjuvant only I.P.	108	<2	<2
	105	<4	<0
	103	4	4
	125	<2	0
	47	<4	16

* One hamster died.

were partially excised and a 20% tumor suspension in phosphate buffered saline, pH 7.2, was prepared by homogenization in a Virtis homogenizer in a chilled ice bath for 10 minutes at 10,000 rpm. The tumor homogenate was then frozen and thawed 3 times to destroy any viable cells. Hamsters were given 0.5 ml of an emulsion of equal volumes of adjuvant (Freund's complete adjuvant, Difco) and tumor homogenate intraperitoneally (i.p.) every fifth day for a total of 5 injections. The development of ascites usually occurred within a few days after the fifth and sometimes after the fourth injection.

The ascitic fluid was withdrawn with a 20 ml syringe and was generally clear after centrifugation to remove small amounts of red blood cells. The fluids were inactivated at 56°C for 30 minutes. The CF antibody titers to SV 40 tumor in the ascitic fluids proved to be as high as those of the serum from the same animals, titering between 1:32 to 1:64. Up to 30 ml of fluid was obtained from individual animals compared to 1.5 to 2.0 ml of serum. Moreover, the animals which survived frequently developed ascites again within a week and could be "re-tapped" for more antibody-containing fluid.

By similar procedures, ascitic fluids were obtained from animals with adeno-18 tumors and levels of CF antibody as high as 1:256 were found.

These preliminary experiments indicated that tumor-bearing hamsters could indeed be induced to develop ascites and that such fluids contained high levels of CF antibody. A more detailed study with the SV 40 tumor was therefore undertaken to determine the best procedure for obtaining optimal titers of antibody. Fifteen adult, female hamsters were inoculated subcutaneously with 10^6 SV 40-LP-T cells. Two weeks later, the tumor-bearing animals were randomly divided into 3 groups of 5 animals each. Tumors in animals of Group 1 were partially excised and these animals received tumor homogenate mixed with adjuvant. Animals in Group 2 also received tumor mixed with adjuvant but their tumors were not removed. Animals in Group 3 were inoculated with adjuvant only and their tumors were not excised.

All animals developed ascites after the 5th inoculation. Serum and ascitic fluids were collected separately from each animal and tested for CF antibody. These results are given in Table I. It is apparent that most animals in Groups 1 and 2 developed levels of antibody in the ascitic fluids equally as high as those in their sera. Although it was previously thought that removal and subsequent regrowth of tumor stimulated antibody production, this experiment showed that partial excision of tumors did not influence antibody levels found in the 2 groups. The critical factor appeared to be the inclusion of tumor homogenate with the adjuvant used to produce the ascitic fluid. Animals in Group 3 which received adjuvant without tumor homogenate responded poorly and CF antibody was not detected in either serum or ascitic fluid of 3 of the 5 animals. Antibody was found at low titer (1:4) in the ascitic fluid and serum of one hamster in this group, and was present only in the ascitic fluid of the other animal. It should be noted, however, that good serum antibody titers can be obtained in animals that have been carrying tumors for a period of some weeks.

It therefore appears that the antigenic stimulus provided by the tumor homogenate mixed with adjuvant is necessary for produc-

tion of high levels of antibody in ascitic fluid. This strongly suggests that the antigen present in the adjuvant-tumor homogenate is stimulating local antibody production. In other experiments with a polyoma tumor, it was found that hamsters *without* tumors given a mixture of adjuvant and tumor homogenate responded very poorly, most of the animals failing to develop any CF antibody. This suggests that a primary antigenic stimulus provided by a previously growing tumor is important for maximum response to the tumor homogenate-adjuvant booster dose of antigen.

Production of highly specific tumor CF antibody in ascitic fluid of hamsters with primary tumors induced by SV 40 and polyoma viruses. One-day-old hamsters were inoculated s.c. with a large-plaque mutant of SV 40 virus. Polyoma tumors were produced by inoculating suckling hamsters with polyoma virus. Tumors subsequently developing in these animals were partially excised and homogenized separately, and each animal received the adjuvant-tumor homogenate prepared from its own tumor. Pooled ascitic fluids from 2 of the hamsters with polyoma tumors titered 1:32 while the serum end-point was 1:64. The CF titer of the ascitic fluids from 2 hamsters with SV 40 tumors was 1:64, the same as that found in serum. Since these animals were inoculated with tumor homogenate from their own tumors, the CF antibody titers presumably represent antibody to only the virus-specific tumor antigen. This is an important consideration since it has been demonstrated by Sabin *et al*(9) and confirmed by us that isoantigens exist in some hamsters to which others lacking the antigen can produce CF antibodies. By producing the ascitic fluid antibody in the individual hamster with its own original virus-induced tumor as antigen such "non-specific" antibodies can be avoided.

Although the use of these ascitic fluids in the immuno-fluorescent technique to demonstrate SV 40 or adeno-18 tumor antigens has not been tested as yet, it is likely that they can be used for such studies. In the immuno-fluorescent studies of Pope and Rowe(10,11) with SV 40 and adeno-12 tumors, specific staining was obtained only with CF-positive sera, thus indicating that both types of tests measure the same antigen-antibody reaction.

These studies have been confined to virus-induced tumors of hamsters, but it is expected that the procedures will apply equally well to mice since other workers have reported antibody production in ascitic fluids of these animals using a variety of different antigens (5,6,7). However, mouse strains vary in their ability to produce ascitic fluid(12), and it may be necessary to alter the procedures with mouse tumors.

That the CF tumor antibody found in ascitic fluid is specific has been shown by the absence of cross-reactions when they were tested with antigens from unrelated tumors. As further evidence for specificity, the antibody to SV 40 tumor obtained in ascitic fluid has been used for detecting tumor antigens in several lines of human diploid cells transformed by SV 40 virus, a tissue culture line of SV 40 hamster tumor cells, and an SV 40 transformed mouse cell line obtained from Dr. George Todaro.

Summary. A procedure has been described for the production of large volumes of CF antibody to antigens in hamster tumors induced by SV 40, polyoma, and adeno-18 viruses. Antibody titers in ascitic fluids were as high as those found in serum. Optimal titers of SV 40 antibody were obtained in animals which had growing tumors and were given a series of intraperitoneal inoculations of a combination of complete Freund's adjuvant mixed with tumor homogenate.

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Simian Enterovirus SV2 Hemagglutination Studies. II. Effect of Various Treatments on the Hemagglutinin and Its Cell Receptors.* (30465)

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As a part of a continuing study of the characteristics of the simian enteroviruses, we have demonstrated that hemagglutinating activity (HA) is closely associated with the infectious virus particle of SV2(1) and appears to have the properties of hemagglutinins found associated with human enteroviruses(2). Since considerable work has been done on the cell receptors for the human enteroviruses, both hemagglutinating(3,4) and non-hemagglutinating(4-7), it was decided to extend our comparison by studying the receptors for SV2 HA on RBC and primary rhesus monkey kidney cells (PMK). This has been done by determining the effect of various treatments on the ability of SV2 HA to adsorb and elute from these cells.

Materials and methods. The virus and methods for its growth and assay have been described(1). All HA tests were done at 4°C.

Adsorption of HA and infectivity to PMK. Monolayers of PMK were washed 1× with PBS and inoculated at an approximate multiplicity of 1. At intervals the monolayers were washed 4× with PBS and overlaid with 0.25 ml of 1% α-chymotrypsin for 15 minutes at 37°C. HA and infectivity were then determined.

Treatment of cell receptors. Chemical. One-tenth milliliter amounts of packed RBC or 50% PMK in PBS were treated with 0.9 ml of the chemicals at the concentration indicated in Table I. After ½ hour at room temperature the mixtures were dialyzed overnight against cold PBS. In the case of chloroform, ether, butanol or genetron, 0.9 ml of PBS was added to the cells followed by 2 ml of solvent. After homogenization on a vortex mixer, the mixture was centrifuged to separate the phases and the solvent phase discarded.

Physical. Cells suspended in PBS were rapidly frozen and thawed 3× or disintegrated in a 10 Kc Raytheon sonic oscillator.

Enzymatic. A mixture of 0.9 ml of enzyme solution at the concentration indicated in Table II and 0.1 ml cell suspension was incubated at 37°C for 1 hour. The trypsin was obtained from Difco (1:250) and receptor destroying enzyme (RDE) from Microbiological Associates, Inc. All other enzymes came from Worthington Biochemical Corp. The pH of the reaction mixtures was 7.2-7.4 except for elastase (pH 8.0) and pepsin (pH 4.0).

Test for adsorption of HA. After treatment of the cells, 0.1 ml of virus was added and the mixture incubated in an ice bath for ½ hour. The cell debris was then sedimented at 1000 × *g* for 10 minutes and HA determined on the supernatant fluid.

Release of cell bound HA. Equal volumes of virus and packed RBC or a suspension of PMK containing approximately 5 × 10^6 cells per milliliter were mixed and incubated ½ hour at 2°C. The cells were then sedimented and washed 3× with PBS. One-tenth milliliter aliquots of the washed RBC or a

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