STUDIES OF MOUSE POLYOMA VIRUS INFECTION

I. Procedures for Quantitation and Detection of Virus

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Precise information on the natural history of an infectious disease process depends primarily on the epidemiological method, and this in turn depends for significance on the accuracy of the tests available for detection of microbial activity. Equally important is the simplicity of the survey method employed; if studies cannot be carried out with sufficient scope, information of high significance cannot be acquired.

The work of Stewart and Eddy (1–5), and more recent findings from this laboratory (5, 6) show that mouse polyoma virus can be studied by standard virologic techniques, including growth in tissue cultures, hemagglutination inhibition (HI) tests, and complement fixation tests. These techniques provide an exceptional opportunity to study the natural history and properties of a mammalian tumor virus.

With these procedures several endpoints can be used for detection of viral infectivity. Production of tumors in mice inoculated as newborns (1, 2, 7), while yielding direct and indispensable information on infectious particles with oncogenic activity, is too cumbersome for epizootiologic surveys of viral infection. The shorter incubation period for tumor production in suckling hamsters (8) provides a more technically feasible procedure for detection of infectivity based on oncogenic activity. Eddy *et al.* (3) also showed that tissue culture cytopathogenicity could be used to titrate infectivity. More recently, as shown here, it was found that adult mice develop HI antibody following intraperitoneal inoculation of high dilutions of virus-containing material making possible indirect tests for infectious virus. This report presents an evaluation of detection and quantitation procedures using tissue culture cytopathic effects (CPE), production of antibody in weanling mice (mouse antibody production test, or MAP test), and production of tumors in suckling hamsters as endpoints. Data on recovery of virus from tissues of spontaneously infected mice are also presented.

Materials and Methods

Tissue Cultures.—Trypsinized whole mouse embryo cultures were used throughout. Cultures were grown in a medium consisting of 10 per cent human serum in Eagle's basal

medium (BME) (9); when confluent cell sheets had developed, the cultures were rinsed twice with BME and given a maintenance medium consisting of 5 per cent inactivated (56°C. for 30 minutes) horse serum in BME. All media contained penicillin, 250 u/ml., and streptomycin, 250 μg./ml. For production of virus pools, 32 ounce flask cultures were used, with 40 ml. of medium; stationary tube cultures, used for virus isolations and titrations, contained 1 ml. of medium. Fluids were changed once weekly.

Virus.—The strain of polyoma virus used as a prototype in this laboratory is the LID-1 strain, which was isolated in mouse embryo tissue culture from a mouse parotid gland tumor supplied by Dr. Lloyd W. Law and Dr. Clyde J. Dawe. This tumor had developed following inoculation of newborn C3Hf/Bi mice with filtered culture fluid from tissue cultures of a transplantable C3Hf/Bi leukemia (No. 388) which had originally been induced by Dr. Ludwik Gross through inoculation of a filtrate of AKn leukemic tissues (10). In the studies reported here the virus was in the 5th to 9th serial passage in mouse embryo tissue culture.

Virus stocks were prepared by inoculation of flask cultures of mouse embryo tissue with 0.4 ml. of 10^{-1} or 10^{-2} dilution of infectious tissue culture fluid. Supernatant fluids were saved at each fluid change, and when cytopathic changes were essentially complete, the cells were saved with the fluid. Fluids were stored in a mechanical deep freeze at −60°C.

Hemagglutination (HA) Tests.—Serial twofold dilutions of virus were made in pH 7.2 phosphate buffered saline in Kahn tubes; 0.2 ml. of 0.4 per cent guinea pig erythrocytes was added to 0.2 ml. of the virus dilution, and the tubes placed in the cold room at 2–4°C. Readings were made in the cold, by the pattern method.

Tissue Culture Titrations.—Serial tenfold dilutions of virus were prepared in tissue culture maintenance medium, and 0.1 ml. inoculated per mouse embryo tissue culture tube. In most tests two tubes were inoculated per dilution, but in several instances three or four tubes were used. The cultures were observed for CPE at 2 to 4 day intervals and were maintained as long as viable tissue remained. Near the titration endpoint the specificity of the CPE observed was confirmed by testing for hemagglutinins in the culture fluid; in the rare instance when the HA test was negative, the fluid was passed to fresh mouse embryo culture tubes. In most tests, culture fluids from each dilution were checked for HA at about 2 weeks and again when the test was discarded. Titers were calculated by the Reed-Muench method (11), and titers expressed as the number of tissue culture infectious doses ($TCID_{50}$) per 0.1 ml.

Mouse Antibody Production (MAP) Tests.—This test of viable polyoma virus is based on tests for polyoma antibody in serums of mice given test material intraperitoneally at specified time periods prior to bleeding.

Weanling mice of the "general purpose" non-inbred Swiss strain were obtained from the Animal Production Unit of the National Institutes of Health, under Dr. George E. Jay, Jr., and Mr. Samuel M. Poiley. This colony is free of polyoma infection as indicated by the absence of antibody in more than 1100 adult mice of various strains tested (12, 13). In virus titrations, serial tenfold dilutions were inoculated intraperitoneally in volumes of 0.2 ml. per mouse; usually, three mice were used per dilution. For detection of virus in organ suspensions inocula up to 1.0 ml. per mouse were used, depending on the volume of test material available. In routine procedures, mice were bled 21 days after inoculation in tests of materials not containing antibody, and at 35 days in tests of materials such as organ suspensions when neutralizing antibodies were expected to delay viral activity. The evidence on which these arbitrary routines were based will be discussed in the text. Mice were usually bled by decapitation; when serial bleedings were required, heart punctures were used.

Buffered saline solution was added to the whole blood in a volume of 4.5 times that of blood, making an approximate serum dilution of 1:10. The clot was stirred into the saline with an applicator stick, and the prediluted serum was removed after standing at refrigerator temperature overnight. Serums were heated at 56°C. for 30 minutes and tested for HI anti-

bodies at dilutions of 1:100 and 1:200; occasional serums were tested for CF antibodies as indicated. In HI tests, 0.2 ml. serum dilution plus 0.2 ml. of a dilution of stock LID-1 virus containing 16 HA units were held at room temperature for 30 minutes, at which time 0.1 ml. of 1.0 per cent guinea pig erythrocytes was added. The tubes were placed in the cold room and read in the cold. Serums giving complete inhibition of hemagglutination at 1:200 were considered positive for antibody, while serums positive at 1:100 and not at 1:200 were repeated after treatment with receptor-destroying enzyme (RDE); if the repeat test was positive at 1:100 or greater, the serum was considered to contain polyoma antibody.

Two groups of controls were included with each MAP test; some were mice given 0.2 or 1.0 ml. of saline solution or uninfected tissue culture fluid intraperitoneally, and some were uninoculated. Generally the number of controls was 20 to 30 per cent of the total number of mice receiving test material. Of 846 controls bled after 3 to 6 weeks in the laboratory, 2 (0.25 per cent) developed antibody, as did 5 (9 per cent) of 55 bled after 14 to 15 weeks. The control mice developing antibody at 14 to 15 weeks had been test-bled one to three times previously, and had been negative for antibody. In addition, three of the controls bled at 3 to 6 weeks had RDE-sensitive inhibitor at 1:100 or 1:200.

Tests of Mouse Organ Suspensions for Virus—Individual mice were exsanguinated, and a pool made of the liver, spleen, kidneys, lungs, pancreas, gonads, thymus, submaxillary lymph nodes, and submaxillary and parotid salivary glands; in a number of instances the salivary glands were tested separately. The pooled organs were ground in a mortar with sterile alundum, and made to a 10 per cent suspension in physiologic saline. The suspension was clarified by centrifugation at 2000 R.P.M. for 10 minutes. One mouse embryo tissue culture tube was inoculated with 0.1 ml. of the supernate, and another with 0.05 ml. Isolation tests in tissue culture were not considered satisfactory unless the tubes remained in observable condition for at least 28 days; many of the tests were followed for 45 to 60 days. When CPE were seen the isolation was confirmed by passage of the virus and by HA and HI tests against standard LID-1 strain mouse antiserum.

One ml. of the clarified organ suspension was inoculated intraperitoneally into each of 3 to 5 weanling mice; salivary gland suspensions were inoculated in volumes of 0.2 ml. per mouse, because of the smaller volume of suspension. The mice were bled after 35 days and the serums tested for HI antibody as described above.

EXPERIMENTAL

Virus Titrations by CPE Endpoints in Tissue Culture.—Titrations of polyoma virus in mouse embryo tissue cultures using cytopathic effects (CPE) as the endpoint (3) presented problems similar to those encountered in the quantitation of adenoviruses in HeLa cell cultures (14). Within a single batch of cultures, there was a straight line relationship between virus dilution and time of appearance of 1-plus CPE, with a 2 to 3 day delay per tenfold dilution of virus; near the end dilutions the relationship became irregular. Limiting dilutions of virus produced CPE after incubation periods which ranged from 14 to 32 days. Occasionally a culture tube inoculated with the limiting dilution or a tenfold further dilution showed no observable effects on prolonged observation, yet yielded virus on blind passage or gave positive results in HA tests.

Although standard pools exhibited a linear dose-response on titration, when the same virus pool was titrated in different sets of mouse embryo cultures there was considerable variation in the slope and displacement of the curve;

this rendered a well defined standard procedure for quantitation virtually impossible. As a result of this variation, titrations with a reasonable arbitrary termination gave an inadequate reflection of the actual amount of infectious virus. Table I shows the results of four titrations of a pool of LID-1 strain polyoma virus, performed in three batches of mouse embryo cultures; the apparent ID_{50} titers are given for readings made on the 12th and 14th day, as well as the final reading made after 21 to 35 days and the titers obtained when the same dilution fluids were tested by the adult mouse antibody production test, as described below. The final titers agreed within tenfold, which is within chance expectation for titrations using two or three tubes per dilution. However, it is apparent that if the titrations had been terminated at 12 or 14 days,

TABLE I

Apparent Infectivity Titer as a Function of the Time of Reading Tissue Culture Titrations

Four titrations of Pool 339 in three batches of mouse embryo tissue cultures.

Titration no.	Cell lot	CPE titer* as read on:			Simultaneous MAP titration* (21 days)
		12th day	14th day	> 21 days	
1	A	4.3	4.7	5.8	6.8
2	A	5.3	5.3	6.3	7.0
3	B	3.5	6.5	6.5	
4	C	2.3	4.0	6.8	7.2

* Titers expressed as the $\log_{10}$ of the number of ID_{50} per 0.1 ml.

the apparent endpoints would have varied by 3 and 2.5 logs, respectively, and would have borne little relationship to the final titers obtained by prolonged observation of tissue cultures and by the mouse antibody production test.

Infectivity Titrations by Antibody Production in Weanling Mice (MAP Test).—When it was observed that adult mice responded with CF and HI antibody following intraperitoneal inoculation of low dilutions of virus, it appeared desirable to evaluate this system for its sensitivity and reproducibility in detecting and quantitating virus. Pilot experiments established that high level HI antibody did not develop regularly by 14 days after inoculation of approximately 100 ID_{50} given intraperitoneally or intravenously but did so by the 21st day. Mice which did not develop antibody within 21 days after receiving limiting dilutions, rarely became positive when bled 5 and 8 weeks after injection. Consequently, for titration of infectious virus in tissue culture fluids, a routine procedure based on intraperitoneal inoculation of 3 mice per dilution and bleeding at 21 days was adopted. In general, titration endpoints were clear cut; for example, in a titration of pool 339, all 19 mice given 10^{-5} and 10^{-6} dilutions developed antibody, 3 of 10 at 10^{-7}, and none of 10 at 10^{-8}. HI antibody titers

in mice given limiting dilutions were generally slightly lower than in mice given larger doses.

Table II presents a comparison of tissue culture and MAP titrations of tissue culture fluids; in all instances the two titrations were made simultaneously, the

TABLE II

Comparison of Infectivity Titers of Polyoma Virus Infectious Tissue Culture Fluids Using CPE in Mouse Embryo Tissue Culture and Development of HI Antibody in Mice as Endpoints

Fluid tested	HA titer	CF antigen titer	Infectivity titer (log_{10} ID_{50}/0.1 ml.)	
			Tissue culture	MAP test
Pool 349 (10 × conc.)	20480	1:16	6.5*	6.5*
Pool 359 (5 × conc.)	20480		6.8	6.7
R-1	2560	1:2	7.6	6.9
			5.5	6.2
90012	2560	1:2	6.2	6.2
Pool 339	1280	1:2	6.0	6.7
			5.5	6.5
			6.5	7.2
Pool 358	640	<1:2	6.5	5.2
			7.0	6.9
			6.2	5.5
			6.0	6.5
			4.2‡	3.9‡
74556	320	<1:2	5.5	7.2
			5.5	5.2
74491	80	<1:1	6.5	5.2
			4.2	5.2

* Figures on same line are simultaneous tests.

‡ Test of fluid stored at 37°C. for 8 weeks.

tissue cultures and mice being inoculated with fluid from the same dilution tube. The titers obtained by the two procedures were generally comparable, neither procedure having any apparent advantage in sensitivity. The reproducibility of titers was also approximately the same. There were two instances in which titrations of aliquots of tissue culture fluids by the MAP test gave endpoints differing by more than 1 log, and two such instances in the tissue culture titrations. It is significant that pool 339 has been titrated 12 times by the MAP test,

and all titers were between $10^{6.4}$ and $10^{7.2}$ ID_{50} per 0.1 ml., which is regarded as an acceptable degree of variation for titrations. It is possible that different passage lines of polyoma virus differ in relative infectivity for tissue culture and mice.

Titrations of Tumor-Producing Activity in Suckling Hamsters.—For certain purposes it is desirable to quantitate the virus directly in terms of tumor-producing ability. The short incubation period of tumor development in suckling hamsters (8) indicated the possibility of such a titration procedure with an endpoint in less than 2 months. Serial tenfold dilutions were inoculated sub-

TABLE III

Representative Titration Results in Suckling Hamsters

Dilution Inoculated	Pool 339 (total of 13 titrations)			Fluid 74307	
	Tumors, sacrifice at 20 to 24 days	Tumors, sacrifice at 25 to 35 days	CF and/or HI antibody, 25 to 35 days	Tumors, 28th day	CF antibody, 28th day
10^0	10/10* (100%)	15/15* (100%)	14/14‡		
10^{-1}	53/62 (86%)	34/37 (92%)		4/6*	3/3‡
10^{-2}	24/53 (45%)	24/36 (67%)		0/11	4/4
10^{-3}	16/63 (25%)	10/34 (29%)	9/9	1/8	4/4
10^{-4}	7/47 (15%)	10/63 (16%)	6/8	1/2	2/2
10^{-5}	1/17 (6%)	1/35 (3%)	8/13	0/3	3/3
10^{-6}		1/14 (7%)	3/9	1/8	3/4
10^{-7}		0/15	1/8		
10^{-8}		0/9	0/5		
10^{-9}		0/12	0/8		
Control TC fluid (10^0)	0/29	0/57	0/36	0/5	0/4

* No. of hamsters with tumors/No. sacrificed.

‡ No. of hamsters with antibody/No. tested.

cutaneously in volumes of 0.2 ml. per animal into suckling hamsters 3 to 4 days of age, and after 20 to 35 days the surviving animals were sacrificed and the abdominal and thoracic organs examined in the gross for the characteristic hemangiomas and sarcomas. Animals which died before the time of sacrifice were autopsied if found in suitable condition, and were included as positive only if characteristic tumors were observed; animals dying but not demonstrating tumors or which were eaten by cage mates were not included in the tabulations. The results of such titrations were generally highly unsatisfactory. One virus pool, pool 339, was highly pathogenic for hamsters at low dilutions and served as seed for neutralization tests. Table III shows the total tumor incidence in hamsters receiving various dilutions of the pool, in 13 consecutive titrations; also shown is a titration of another fluid which is representative of the usual results of short observation titrations. In neither instance was the

tumor-endpoint titration at all satisfactory, in that there was a spread of 6 log dilutions in which there was production of tumors in some, but not all, of the recipient animals. As shown by the production of antibody in the same hamsters receiving the various dilutions, infection rates were considerably higher and more consistent than the morbidity rates. These results indicate marked differences in susceptibility of individual hamsters to the early oncogenic effects of polyoma virus. It is possible that prolonged observation titrations in hamsters, which would utilize primarily the incidence of the subcutaneous sarcomas characteristic of later stages of infection, or detailed histologic study, would give results suitable for quantitative comparisons. Of course, the practical considerations involved would limit the usefulness of such titrations.

Detection of Virus in the Presence of Antibody.—Another need for virus detection procedures is in the isolation or detection of virus in tissues of infected animals. Since infected mice and hamsters generally have high levels of HI and neutralizing antibody, the conditions for virus detection are different from assays of virus in tissue culture fluids. Consequently, experiments were conducted to compare the usefulness of tissue culture and mouse antibody production procedures for detection of virus in artificial virus-antibody mixtures and in tissues of potentially infected mice.

Serial tenfold dilutions of pool 339 were made in three diluents: saline, 1:10 dilution of pooled serum of mice inoculated with control tissue culture fluid, and 1:10 dilution of pooled serum of mice inoculated with polyoma virus infectious tissue culture fluid. The latter serum had HI antibody titer of 1:6400, hamster tumor-neutralizing titer of 1:1000 to 1:10,000, and tissue culture CPE neutralizing antibody titer of 1:1000. The mixtures were allowed to stand at room temperature for 2 hours, and aliquots were inoculated into two mouse embryo tissue culture tubes, 0.1 ml. per tube, and into three weanling mice, 0.2 ml. intraperitoneally per mouse. The lower dilutions of the virus plus immune serum were inoculated into ten mice each. The tissue cultures were observed for 35 days, and the mice were bled at 21 days and again at 35 days. Table IV shows the results of this experiment as well of an essentially identical test which was assayed only by the MAP test. The results of the two tests indicate that antibody at this concentration reduced, by 3 to 4 logs, the amount of virus that could be detected by either procedure, and delayed the onset of effects of the limiting dilutions. In tissue culture, the virus dilutions made in saline or control mouse serum either produced CPE within 21 days or did not become positive throughout the remainder of the experiment, while in the immune serum mixtures, virus effects were produced by two additional tenfold dilutions on the 24th to 31st day. Similarly, the presence of antibody in the inoculum delayed the appearence of antibody in mice. Experiment 2 in Table IV shows that bleeding at 8 weeks did not increase the sensitivity of the mouse test for the detection of virus in the antibody mixtures.

A comparison of the effectiveness of tissue culture and mouse antibody production tests for detection of virus in organs of mice is shown in Table V. The mice whose organs were tested were uninoculated adult mice from two colonies with high rates of spontaneous polyoma virus infection.

The data in Table V show that by the procedures employed, the two techniques were generally comparable in sensitivity, with the MAP test perhaps slightly more effective.

Although the positive organ suspensions have not been titrated, there are several indications that the quantity of detectable virus was relatively low. Thus, many suspensions did not immunize all the recipient mice, several were positive in only one of the two tissue culture tubes, and generally the incubation period of the cytopathic effects was prolonged: 18 to 27 days. Also, sample bleedings at 21 days in many of the mouse tests indicated that the 35 day test was necessary for maximal sensitivity, as was characteristic of dilutions near

TABLE IV

Infectivity for Tissue Cultures and Adult Mice of Serial Dilutions of Polyoma Virus with and without Antiserum, as Determined at Different Times after Inoculation

Experiment No.	Diluent	Infectivity titers* in different assay systems				
		CPE in tissue culture		Mouse antibody production (MAP) test		
		21 day endpoint	35 day endpoint	21 day bloods	35 day bloods	56 day bloods
1	Saline	6.0	6.0	6.7	6.7	
	Normal mouse serum (1:10)	5.5	5.5	6.5	6.9	
	Immune mouse serum (1:10)	1.5	3.5	2.1	2.8	
2	Normal mouse serum (1:10)			7.2	7.2	
	Immune mouse serum (1:10)			2.0	3.2	3.2

* Titers expressed as $\log_{10} ID_{50}$/0.1 ml.

TABLE V

Comparison of Tissue Culture and Mouse Antibody Production Tests for Detection of Virus in Organ Suspensions of Uninoculated Mice from Colonies with Spontaneous Infection

65 specimens from 46 mice, tested by both procedures.

Isolation in mouse embryo tissue culture	Detection in MAP test		
	Positive	Negative	Total
Positive	12	3	15
Negative	7	43	50
Total	19	46	65

the endpoint in the artificial virus-antiserum mixture tests described above. It can also be inferred that the actual amount of virus present in the organ suspensions may have been considerably greater, but masked by antibody in the suspensions.

The findings in Table V indicate that tissue culture is a highly sensitive method of detecting virus in mouse organ suspensions, particularly in view of

TABLE VI

Detection of Polyoma Virus in Tissues of Normal Mice from Colonies with Spontaneous Infection, in Relation to Strain, Age, and Antibody Status

Strain	HI antibody status	Frequency* of virus demonstration by age of mice tested				
		1 to 2 mos.	5 to 7 mos.	8 to 11 mos.	15 mos.	Total
AKR	+		9/10	0/1		9/11
	−	0/2		0/2		0/4
C3H	+		3/4		1/2	4/6
	−	0/6	1/7	0/1		1/14
C58	−		1/4			1/4
DBA	+		0/1		0/1	0/2
RFM	+			2/4		2/4
STOLI	+			0/3		0/3
	−	0/2				0/2
Total...	+		12/15	2/8	1/3	15/26 (58%)
Total...	−	0/10	2/11	0/3		2/24 (8%)

* Numerator, No. of mice positive by tissue culture and/or MAP tests. Denominator, No. of mice tested.

the much larger total mass of mouse tissue actually tested in the MAP test. Because of toxicity of the organ suspensions, it was not possible to test more than 0.1 ml. of suspension per tube; to obtain maximal effectiveness from the tissue culture procedure, it would be necessary to use large numbers of tubes or to inoculate flask cultures. It was concluded, however, that the MAP test was much simpler to carry out and a practical method for demonstrating virus.

Table VI summarizes our over-all experience with virus detection tests on organs of uninoculated mice from infected colonies; the results are given in relation to the strain, age, and HI antibody status of the donor mice. All specimens were tested by the MAP procedure, and as described above, many were also tested in mouse embryo tissue culture. Virus was recovered from 15 (58

per cent) of 26 mice with HI antibody, and from only 2 (8 per cent) of 24 mice without antibody. One of these two mice was positive for only one of five recipient mice in the MAP test; the other was positive for the majority of recipients in two MAP tests, but was negative in tissue culture. In view of the high conversion rate in the Law colony, from which these two mice came (13), it is possible that they were in the early stages of infection. It should be noted that two of the virus-positive mice were known to have antibody 14 days, and two others, 58 days, before sacrifice.

Studies of the organ localization of virus in naturally infected mice have not been completed except for 21 of the above mentioned mice whose salivary glands were tested separately from the remainder of the organs. For these tests the parotid and submaxillary salivary glands were pooled. In 7 mice both the salivary glands and thoracoabdominal viscera were positive, in 2 the viscera were positive and the salivary glands negative, in 1 the salivary glands were positive and the viscera negative, and in 11 both specimens were negative.

DISCUSSION

Since the procedures used for detecting and quantitating polyoma virus are primarily based on viral attributes other than tumor induction, it is necessary to review the evidence that hemagglutination, cytopathogenicity, and tumor induction are functions of a single agent and not the result of a mixture of agents. Eddy *et al.* (3) presented evidence for the identity of the tumor agent and the agent producing cytopathic effects. The evidence obtained in this laboratory, chiefly unpublished, can be summarized as follows.

1. In tests with tissue culture preparations of LID-1 strain, it has not been possible to separate hemagglutinating ability, cytopathogenicity, and ability to produce tumors in hamsters or mice by ether treatment; heating at 60°C. for 30 minutes; testing limiting dilution fluids from tissue culture titrations; carrying the agent through 11 serial passages in suckling hamsters; or by carrying out five successive cycles of adsorption onto fresh guinea pig erythrocytes and elution into fresh saline solution.

2. Thirteen strains of polyoma virus isolated in tissue culture from mice from various sources have been studied; all produced characteristic cytopathic effects, hemagglutinated guinea pig erythrocytes, and produced tumors in suckling hamsters.

3. In sera of mice from colonies with spontaneous polyoma virus infection, there was close correlation between presence of absence of hemagglutination-inhibiting antibody and presence or absence or neutralizing antibody using inhibition of tumor development in suckling hamsters as the endpoint (13).

The methods described here were developed primarily for use in epizootiologic studies of the natural history of polyoma virus and its behavior in labo-

ratory study systems as well. For many purposes the higher degree of accuracy provided by plaque-counting procedures is of course required; Eddy (5), has reported plaques with polyoma virus in mouse embryo tissue culture. However, the method has not yet been developed as a standard procedure. It seems probable that the long incubation period necessary for production of CPE by small doses of virus in presently available tissue culture systems will limit the practicality and usefulness of plaque techniques for polyoma virus.

While for the present purposes, assays of polyoma infectivity by tissue culture CPE and the MAP test had comparable sensitivity and reproducibility, each procedure had its advantages and disadvantages. The MAP test had the advantage that tests were not lost for technical reasons, in contrast to the considerable number of tissue culture tests discarded because of non-specific degeneration of tissue or bacterial or fungal contamination. The risk of a false positive result appears minimal with the MAP test in view of the rarity of antibody in control mice held 3 to 6 weeks. With tissue culture tests, the number of fluid changes in long observation tests and the extraordinary stability of polyoma virus make cross-contaminations a distinct risk. Also, MAP tests require less use of highly trained personnel than do tissue culture tests. The MAP procedure requires that the stock of test mice be shown to be free of spontaneous infection. An advantage of tissue culture tests over MAP tests is that they provide some indication of results during the course of the test, although for final results there is no difference in time required. Furthermore, the tissue culture procedures yield strains of virus for study; thus genetic and serologic variants of polyoma virus would presumably be missed in mouse antibody production tests. Another situation in which the MAP test could not replace tissue culture tests is in detection of residual virus in inactivation experiments, since the mice might be immunized by non-infectious virus in low dilutions.

For maximal sensitivity in virus isolation tests, it is apparent that both procedures should be used.

The recovery of virus from the organs of normal mice confirms the findings of Gross (15, 16), who induced parotid tumors and subcutaneous sarcomas in mice inoculated as newborns with filtrates or centrifugates of organ suspensions of normal C3H and C57BR/cd mice. However, the ability to detect virus within 3 to 5 weeks, by either tissue culture cytopathogenicity or the MAP test, greatly simplifies and shortens the time necessary for such studies. As reported elsewhere (12, 13), antibody was infrequent in mice less than 3 months of age in heavily infected colonies. The failure to recover virus from such mice (Table VI) suggests that the late appearance of antibody is due to the failure of the mice to become infected rather than to a delayed response to virus acquired in utero or neonatally, presuming, of course, that such virus would exist as completely formed virus and not in a lysogenic state.

SUMMARY

Three procedures have been compared for usefulness in titration and detection of polyoma virus: production of cytopathic effect (CPE) in mouse embryo tissue culture, production of HI antibody after inoculation into weanling mice (MAP test), and production of tumors in suckling hamsters during a 3 to 5 week observation period. The tissue culture and mouse antibody production tests were generally comparable in sensitivity, reproducibility, and time required to obtain results. Titration by tumor production in suckling hamsters was not suitable for quantitation because of marked variation in susceptibility among animals.

Virus was detected in tissues of normal mice from spontaneously infected colonies by either production of CPE in mouse embryo tissue culture or by the MAP test; virus was found in organs of 15 (58 per cent) of 26 mice with antibody, and 2 (8 per cent) of 24 mice without antibody.

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