STUDIES OF MOUSE POLYOMA VIRUS INFECTION

III. Distribution of Antibodies in Laboratory Mouse Colonies

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Tissue culture propagation (1–3) of mouse parotid tumor virus (4, 5), more recently referred to as "S.E. polyoma virus" (3), and the ability of the agent to produce multiple tumors in mice (1, 2) and hamsters (6), stimulated interest in the virus and greatly facilitated its laboratory study. Hemagglutination inhibition (HI) and complement fixation (CF) procedures (7, 8) for demonstrating antibodies to polyoma virus provided simple, reliable tools for the study of the natural history of polyoma virus infection. This report describes the results of antibody surveys as a measure of the prevalence and incidence of polyoma infection in various mouse stocks; preliminary observations have been presented elsewhere (9).

Materials and Methods

Animals.—The mice were uninoculated animals which had never been in cage contact with animals inoculated with any material. Eight colonies were studied:—

1. The animal production colony of the National Institutes of Health, under Dr. George E. Jay, Jr., and Mr. Samuel M. Poiley. The animals are raised in a building separate from the experimental laboratories, with separate personnel. Strict isolation procedures are employed.

2. Mice from the Jackson Memorial Laboratories, Bar Harbor, consisting of two groups: mice held for observation and breeding in an experimental animal room, and mice obtained through their commercial supply operation. The mice in the experimental animal room are of a wide variety of inbred and hybrid strains reared in a room with mice bearing a variety of transplanted tumors.

3. Colony of Dr. Lloyd W. Law, of the National Institutes of Health. Extensive work with polyoma virus in newborn mice is conducted in this laboratory, and as described below, many breeding mice are housed in the rooms with experimental animals, and are cared for by the same personnel.

4. Colony of Dr. Ludwik Gross, Veterans Hospital, Bronx, New York. Intensive work with inoculation of leukemic filtrates, many of which result in the production of parotid tumors

(4, 10), is conducted in this laboratory, and breeder mice are housed in the same and adjoining rooms to inoculated animals and experimental procedures.

5. Colony of Dr. Walter E. Heston, of the National Institutes of Health. These mice are raised in a single room, which does not house experimentally inoculated animals. This animal room is located one floor above and at the opposite end of the corridor from animal rooms in which are large numbers of mice experimentally inoculated with polyoma virus. The personnel in the two areas are distinct, and there is no transfer of air or equipment between the areas, except for possible exchange of rubbish cans which have been washed but not sterilized.

6. Colony of Dr. Margaret Deringer, of the National Institutes of Health. The animal room is adjacent to that of Dr. Heston, and does not house experimentally inoculated mice.

7. Colony of Dr. Howard Andervont, of the National Institutes of Health. The mice are housed in rooms approximately above Dr. Heston's and which do not house experimentally inoculated mice.

8. The colony of Microbiological Associates, Inc., Bethesda. Mice are housed in a separate building, and are cared for by separate personnel, not in contact with polyoma or other tumor research.

Collection of Serum.—Mice were bled on arrival in the laboratory or in the original colony. Blood was obtained by heart puncture, inner canthus puncture, or decapitation. When a small volume of blood was taken (0.2 or 0.3 ml.), sterile 0.85 per cent NaCl solution was added to the clotted blood, in a volume of 4.5 times the quantity of blood, making an approximate serum dilution of 1:10. Sera were stored at −20°C. until tested, and were heated at 56°C. for 30 minutes immediately before use in CF or HI tests. A number of animals were bled repeatedly to test for serologic conversions and for persistence of antibody, but the tabulations of antibody prevalence reported here include only the original bleeding.

Serologic Tests.—

Production of antigens: Tissue culture grown LID-1 strain (11) of polyoma virus was used as antigen for both HI and CF tests. "General purpose" Swiss mice approximately 14 days pregnant from the Jay-Poiley colony, were sacrificed with ether, and the embryos removed aseptically. The whole embryos were minced with scissors, and the mince subjected to trypsinization with 0.25 per cent trypsin (Difco) in a flask equipped with a magnetic stirrer and held at 36°C. After 15 minutes the supernate was decanted and fresh trypsin solution added. After an additional 15 minutes treatment, the second supernate was pooled with the first, and the cells sedimented by centrifugation at 1000 R.P.M. for 5 minutes. The supernate was then discarded, and the cell pack diluted 1:200 in a medium consisting of 10 per cent pooled human serum in Eagle's basal medium (12), with penicillin and streptomycin. Forty ml. of the cell suspension was transferred to each 32 ounce prescription bottle, and the bottles placed at 36°C. The medium was changed at 24 hours, and again at 3 days. When a confluent cell sheet had developed, the growth medium was replaced by a maintenance medium of 5 per cent horse serum in Eagle's basal medium, with penicillin and streptomycin.

The flask cultures were inoculated with virus by addition of 0.4 ml. of 10^0, 10^{-1}, or 10^{-2} dilution of a tissue culture fluid pool of LID-1 strain. Fluids were changed twice weekly, and after early cytopathic changes (3) were seen, the fluids were collected individually in screw-capped vials and stored at −60° or −20°C. When cytopathic changes were almost complete, the cells were shaken into the fluid, and the suspension saved as before. Each fluid was assayed for content of hemagglutinin and of CF antigen, using a standard pool of mouse antiserum. Fluids with 1:4 CF antigen titer were pooled for use in CF tests. Fluids with HA titers of 1:640 or higher were pooled for HI tests and centrifuged at 2000 R.P.M. for 15 minutes; aliquots of the supernatant fluid were stored at −20°C.

Hemagglutination inhibition tests: Tests were done in Kahn tubes, at refrigerator temperature, and were read by the pattern method. Dilutions of serum, antigen, and cells were

made in pH 7.2 phosphate buffered saline; 0 2 ml. of serum dilution was mixed with 0.2 ml. of a virus dilution containing 8 to 16 hemagglutinating units as determined by preliminary titration, in duplicate, on the morning of the test. The serum-virus mixture was held at room temperature for 30 minutes, when 0.1 ml. of 1.0 per cent guinea pig erythrocytes was added. The tubes were shaken and placed in the cold room (2–4°C.). Readings were made several times during the settling of the cells, since slippage of the agglutinated cells frequently occurred; readings were done in the cold room. During much of the study, sera were screened at a dilution of 1:40, expressed as the dilution prior to addition of virus and cells, and sera positive for HI were titrated in a subsequent test. Later, when extensive experience indicated that positive serums essentially always had titers of 1:160 or greater, the screening was done at two dilutions, 1:100 and 1:200. Sera giving inhibition at 1:100 only were retested after overnight treatment with receptor-destroying enzyme (RDE) of *Vibrio cholerae*. Sera with HI antibody titers of 1:200, or 1:100 after RDE treatment, were considered positive for antibody. In support of the specificity and sensitivity of the HI test at this level of testing are the following findings. RDE sensitive inhibitor at a level of 1:200 or over was found only twice in a series of more than 800 sera from adult Swiss mice. Secondly, of 26 normal mouse sera with HI antibody at 1:100 or greater, 24 were positive for neutralizing antibody in a test using development of tumors in suckling hamsters during a 25 to 30 day period as the index of infection; of 21 HI-negative sera of mice from the same colony, none was positive for hamster tumor-neutralizing antibody (13). Controls for the HI test consisted of a cell control, duplicate titration of virus, a serum control for each serum, testing at the lowest dilution used in the test, a test of a standard negative mouse serum, and titration of a standard positive mouse antiserum.

Complement fixation tests: The modified Bengtson procedure described elsewhere (14) was employed, using overnight icebox fixation with 2 full units of complement. Sera were tested at a dilution of 1:10 against 2 units of antigen.

EXPERIMENTAL

Incidence of Infection in the Colonies Surveyed.—Table I summarizes the frequency with which HI antibody was detected in mice of different strains and ages in the colonies studied.

Two factors appeared to have a marked influence on the incidence of polyoma virus antibody: colony and age. In four colonies, no mice of any strain showed antibody, while in four other colonies, antibody was present in the majority of strains. There was no apparent tendency for infection to be mouse strain-specific; thus, AK and C3H mice showed antibody when reared in colonies in which other mice were positive, while mice of the same strains, and in some instances, of the same sublines, did not develop antibody when reared in colonies devoid of antibody. Also, the frequency of infection of a given mouse strain differed greatly between different infected colonies, but reflected that of the remainder of the colony. There was no difference in antibody incidence by sex.

In the colonies with the highest prevalence of antibody (Gross and Law), positive results were almost exclusively found in mice over 3 or 4 months of age. These two colonies are also those in which the breeding animals were in the greatest proximity to experimental work and inoculated animals.

TABLE I

Frequency of HI Antibody in Uninoculated Mice by Colony, Strain, and Age (in months)

Mouse strain	Gross		Law					Jackson Laboratory				Heston			Jay-Poiley			Andervont		Deringer	Microbiological Associates
								Experimental colony	Commercial mice												
	1–3	4–6	1–2	3–5	6–8	9–11	≥12	≥10	1–3	7–9	≥10	3–5	6–11	≥12	1–3	6–9	≥10	4–9	≥10	≥12	≥13
A								5/18				0/24	0/16		0/20	0/2	0/29				
AK	0/30	33/33		9/28	18/45	0/11			1/53	1/21					0/10	0/131					
BALB															0/5		0/15	0/28	0/15		
C3H	1/31	14/23	0/42	6/15	7/35	16/26	20/29	6/18		3/20		0/35	3/41	2/30	0/21	0/7	0/89	0/32	0/20	0/27	
C57BL					0/5	2/7	10/30	3/16			0/21			2/24	0/19	0/8	0/25				0/21
C57BR		2/2		0/2	2/10	1/6	1/1														
C57L													0/3	0/15							
C58				0/15	0/12	3/14	0/1	1/5													
DBA			4/33	0/1	0/3	5/13	12/16	7/15			0/21				0/31	0/10	0/10				0/21
GP															0/325		0/180				0/20
NIH															0/46		0/60				
NH			0/6	0/2	0/3	1/5															
RF			0/12	0/8	15/25	7/7		4/6													
STOLI			0/2	3/19	0/1	3/6	3/5														
Miscellaneous inbred strains								20/51							0/62	0/10	0/31				
Miscellaneous hybrids					1/1	4/15		64/110													
Total..........	1/61	49/58	4/95	18/90	43/140	42/110	46/82	110/239	1/53	4/41	0/42	0/59	3/60	4/69	0/539	0/168	0/439	0/60	0/35	0/27	0/62
Per cent positive	2	84	4	20	31	38	56	46	2	10	0	0	5	6	0	0	0	0	0	0	0
Total by colony.	50/119		153/517					110/239	5/136			7/188			0/1146			0/95		0/27	0/62

Numerator: No. of mice with HI antibody (≥1:100).
Denominator: No. of mice tested.

There was a striking contrast in frequency of infection between the two populations of mice from the Jackson Memorial Laboratories, the highest incidence of antibody being in the group in room contact with experimental mice. Of the mice obtained commercially, the AKR/Jax mice 7 to 9 months of age had been born and raised in the experimental animal room mentioned previously, but the C3H, C57BL, and DBA mice had been raised in outbuildings, completely separate from experimental animals. It is significant that 3 of the 20 C3H mice were positive for HI antibody; a possible contact with infected mice would be their parents or other animals of breeding age brought in from a pedigreed expansion stock located in one of the experimental laboratories.

TABLE II

HI Antibody Titers in Mice of Two Infected Colonies (Law and Gross) by Age and Presence or Absence of CF Antibody

CF antibody (1:10)	No. of mice with HI antibody titer in given range, by age															
	1-5 mos.				6-11 mos.				≥12 mos.				Total			
	<100	160-320	640-1280	≥2560	<100	160-320	640-1280	≥2560	<100	160-320	640-1280	≥2560	<100	160-320	640-1280	≥2560
Positive	0	0	2	9	0	3	21	44	(1)*	0	7	7	(1)*	3	30	60
Negative	212	0	1	0	131	4	5	1	22	4	0	1	365	8	6	2
No test (anticomplementary)	0	0	0	0	5	0	1	0	1	2	8	5	6	2	9	5
Total	212	0	3	9	136	7	27	45	24	6	15	13	372	13	45	67
Per cent in each titer range	95	0	1	4	63	3	13	21	41	10	26	22	75	3	9	13

* Serum gave incomplete fixation of complement (3+); serum from the same mouse, taken 5 weeks later, was negative in CF and HI tests.

Polyoma antibodies were present in pooled sera taken from mice in the Jackson Memorial Laboratories as early as 1955. A pool of sera from C57BL/Ks mice taken in February, 1955, was positive for HI antibody, as was a pool from C3H/Ks mice taken in June, 1957.

Antibody Titers in Infected Animals.—A number of sera from mice in the Gross and Law colonies were screened in both HI and CF tests, and positive sera were titrated for HI antibody. Table II shows the distribution of HI titers obtained in relation to the age of the mice and the presence or absence of CF antibody. Of 109 sera which were positive for HI antibody and satisfactorily tested in the CF test, 93 (85 per cent) were also positive for CF antibody; 366 HI negative sera were satisfactorily tested in CF, and there was only one questionable CF-positive.

The titers of HI antibody in the spontaneously infected mice were generally

high; of 125 HI antibody positive sera shown in Table II, 112 (90 per cent) had titers of 1:640 or greater, while 67 (54 per cent) had titers of 1:2560 or greater. These titers are generally comparable to those observed in artificially immunized mice. There was some tendency for sera from older mice to have lower HI antibody titers and to be negative in the CF test.

Persistence of Antibody and Serologic Conversion in Mice in an Infected Colony.—In order to interpret results of serologic surveys, it is not only necessary to have information concerning the relative specificity and sensitivity of the tests used, but information about the persistence of antibody is also of great importance. If, as is observed with the majority of viral infections, HI antibody remains detectable for prolonged periods, the incidence of antibody reflects the accumulated experience of the population. The absence of antibody in a given individual then suggests that that individual has not been infected with the agent in question. Contrariwise, when an agent stimulates formation of antibody which persists for a short period, presence of antibody reflects for the most part recent experience only, and a negative finding is of little value in ruling out past infection.

Our experience with mice inoculated with polyoma virus indicates that HI and CF antibodies are of the former type; of 21 mice inoculated intraperitoneally or intranasally as newborns, all were positive for HI and CF antibody when bled 7 to 9 months later (13). Serial bleedings of mice in Dr. Law's colony provided an opportunity to confirm this finding in spontaneously infected mice, as well as to observe the frequency with which his mice convert from negative to positive serology.

Table III summarizes the results of serial bleedings on 159 mice, according to HI antibody status in the initial serum. All mice with antibody on first bleeding were still positive when rebled 2 to 17 weeks later, while approximately 30 per cent of initially negative mice had converted to positive within 2 to 3 months. The conversion rate in mice rebled after 7 to 17 weeks was somewhat higher in mice 1 to 5 months of age when bled initially (19/48, or 40 per cent) than in mice 6 months or older (12/47, or 26 per cent).

Antibody Distribution by Litter in an Infected Colony.—It was of interest for epizootiologic analysis to examine an infected colony to see if antibody-positive mice tended to distribute by cage. Table IV shows the incidence of HI antibody by cage, as well as by strain and age, for breeder mice in the Law colony. Since these mice are brother-sister mated, cage mates here are also litter mates, and have been in lifelong contact. The impression gained from the data as a whole is that litters generally showed one of three patterns: no mice infected, one mouse infected, or all mice infected. The number of litters in individual mouse strains were insufficient to indicate whether there were significant differences in infection patterns between strains.

For statistical analysis of the observed distribution, the observed number of litters in each age group having various percentages of animals positive was

compared with the distribution expected by random distribution, as determined by the formula $\Sigma N_i(p_i + q_i)^n$, in which N_i is the number of litters of n mice, and p_i and q_i are the proportion of mice with and without antibody, respec-

TABLE III

Persistence of HI Antibody and Serologic Conversion in Mice of an Infected Colony (Law)

Interval between bleedings	HI antibody status of initial serum	No. of 2nd sera positive / No. tested
wks.		
2–6	Positive	14/14
	Negative	0/20
7–12	Positive	8/8
	Negative	5/19 (26 per cent)
13–17	Positive	22/22
	Negative	26/76 (34 per cent)

TABLE IV

Incidence of HI Antibody among Litter Mates in an Infected Mouse Colony (Law), by Strain and Age

Strain	No. of mice with antibody / No. of mice in litter tested, by age of mice				
	1–2 mos.	3–5 mos.	6–8 mos.	9–11 mos.	>12 mos.
AKR/Lw		2/3	1/5; 1/3; 0/5		
C3Hf/Lw	0/2	0/5	0/3	1/3; 1/4	4/4; 1/3
C3Hf/BiLw	0/3; 0/5; 0/4	4/4; 2/4; 0/2	1/4; 1/3	4/4; 4/4; 3/3; 0/4	
C3Hf/Fg	0/4		1/3	0/2	
C3Hf/BiGs					2/2; 0/4
C57Br/cd		0/4	1/4; 1/4	1/4; 1/2	
C58/Lw			0/2; 0/4	2/5; 1/2; 0/3	
DBA/2Lw	0/2; 0/3		0/3	2/3; 1/3; 1/3; 1/2	3/3; 3/3; 2/3; 2/3; 2/4
RFM/Lw	0/5; 0/4; 0/3; 0/2	0/4	4/4; 5/5; 1/2; 1/3; 0/3; 0/3; 0/4	4/4; 2/2	
STOLI/Lw	0/2	2/2; 1/3; 0/3; 0/4; 0/2; 0/2		3/3; 0/3; 0/2	2/2

tively, in the age group. The observed distribution differed significantly from random distribution ($p < 0.001$); the number of litters with all mice infected, adjusted for age, also differed significantly from chance expectation ($p < 0.001$).

It is noteworthy that nine of the litters with some positive and some negative mice were rebled 7 to 17 weeks later, and in only two had all mice become positive.

TABLE V

Incidence of HI Antibody in Mouse Strains According to Animal Room in an Infected Colony (Law)

Room no.	Type of experimental animal in room	Strain	Incidence of HI antibody in uninoculated mice, by age			
			6–11 mos.	≥12 mos.	Total	
						Per cent
1	Newborn mice inoculated with polyoma virus	AKR/J	7/9		7/9	(78)
		C3H × AKR	5/16		5/16	31
		C3Hf/Fg	2/4	2/5	4/9	(44)
		RFM/Lw	22/32		22/32	69
		Total........	36/61 (59 per cent)	2/5	38/66	58
2	Adult mice given polyoma virus as newborns, and transplanted leukemias	AKR/Lw	5/6		5/6	(83)
		C3Hf/BiLw	17/28	11/11	28/39	72
		C3H/BiGs		2/6	2/6	(33)
		C3Hf/Lw	2/11	5/7	7/18	39
		C57Br/cd	3/16	1/1	4/17	24
		DBAf/2Lw	5/16	12/16	17/32	53
		NH/Lw	1/8		1/8	(12)
		STOLI/Lw	3/7	3/5	6/12	50
		Total........	36/92 (39 per cent)	34/46 (74 per cent)	70/138	51
3	Transplanted leukemias only	AKR/Lw	6/41		6/41	15
		C3Hf/He	2/18		2/18	11
		C57BL/KaLw	2/12	10/30	12/42	29
		C58/Lw	3/26	0/1	3/27	11
		ST/Lw	0/3	0/1	0/4	
		Total........	13/100 (13 per cent)	10/32 (31 per cent)	23/132	17

The findings on litter distribution of infection in this colony are interpreted as indicating that while there is a significant tendency for polyoma virus to infect cage mates, the agent is not extremely contagious among adults.

Antibody Prevalence in an Infected Stock According to Animal Room.—As described above (Table I), the mouse colonies with the highest incidence of infection were those in closest contact with experimentally inoculated animals.

The Law colony provided an additional opportunity to investigate the role of the extra-cage environment on the acquisition of polyoma virus infection. Breeding mice are housed, generally by strain, in three non-communicating animal rooms in which are also housed various experimental animals according to type of experiment. Table V shows the incidence of infection in the various strains in the three rooms, as well as the type of experimental animals in the room. There was a distinct tendency for the strains housed in rooms 1 and 2, which also housed mice experimentally infected with polyoma virus, to have higher rates of infection than those in room 3, which did not house virus-inoculated mice.

TABLE VI

Cross Infection in Swiss Mice from a Negative Colony Held in Animal Rooms with Inoculated Mice and Hamsters

Age when brought into laboratory	Type of contact with infected mice	Frequency of antibody after various lengths of time in laboratory						
		3 wks.	4 wks.	5–6 wks.	8–9 wks.	14–15 wks.	5–6 mos.	8 mos.
Weanling or adult	Mothers of mice inoculated when <12 hrs. old		21/21					
	Males mated with females inoculated with virus as weanlings 53 days previously				4/10			
	In cage with weanlings inoculated with virus	1/276		0/29	1/35	0/1		
	Room	0/284	0/30	0/205	0/42	5/80		
Newborn	Room				0/6		21/66	3/3

In addition to the difference in incidence of antibody in the initial sera, conversion rates were also higher in rooms 1 and 2 than in room 3. When rebled after 7 to 17 weeks, 4 (36 per cent) of 11 mice in room 1, 25 (38 per cent) of 65 mice in room 2, and 1 (8 per cent) of 13 mice in room 3 converted from negative to positive serology.

Serologic Conversion in Mice from a Negative Colony in Contact with Infected Mice.—Experience with mice brought into our experimental laboratory from the negative Jay-Poiley colony has demonstrated that mice can become infected when held in a contaminated environment. Table VI shows the frequency with which antibody was found in mice held in the laboratory under various conditions of contact with infected animals. The polyoma-infected animals in the

rooms in which these mice were held consisted primarily of mice inoculated as adults, with smaller numbers of mice and hamsters inoculated as newborn or 2 to 4 day old sucklings, respectively. The mice in line 3 of the table were primarily controls for mouse antibody production (MAP) tests for polyoma virus (11), and consisted of mice of the same sex as the experimentally inoculated mice, held with the latter from the time of inoculation; many of these controls had been inoculated with saline solution or control tissue culture fluids. The mice in line 4 were also controls, but were in separate cages or cages in which none of the experimental mice developed antibody. The newborn mice, in line 5, had been inoculated intraperitoneally or intranasally with tissue culture fluids which did not contain polyoma virus. The mice in lines 1 and 2 were uninoculated and had not been bled prior to the bleedings listed on the table.

The data indicate that adult Swiss mice in cage contact with inoculated adults or in room contact with infected animals acquired polyoma infection, but at an extremely low rate during a 2 month period. On the other hand, mother mice nursing inoculated newborns were uniformly infected within 4 weeks of contact. This observation is in agreement with the epidemiologic evidence presented above and the results of virus excretion tests (15) which indicated that inoculated newborn mice are a source of intense environmental contamination.

Lack of Association of Polyoma Antibody with Leukemia in AK Mice.—Because of the repeated association of parotid tumor virus with AK leukemia, as observed in experiments with inoculation of leukemic filtrates into newborn mice (4, 5, 10), it was of interest to examine the available data on AK mice with respect to the occurrence of leukemia. First, the finding of antibody in 100 per cent of AK mice over 3 months of age in one colony (Gross), and in no AKR mice in another colony (Jay-Poiley) (Table I), suggests that polyoma virus does not play an etiologic role in the development of leukemia in AKR mice, the high rate of leukemia being a characteristic feature of the strain, independent of the colony. The Jay-Poiley AKR/LwN mice have the characteristic high rate of leukemia (16). Second, the AKR/Lw mice in Dr. Law's room 3 which were over 6 months of age at the time of original testing for polyoma antibody consisted of 24 leukemic and 17 clinically non-leukemic mice; 4 (17 per cent) of the leukemic mice and 2 (12 per cent) of the non-leukemic mice were positive. Also, of the 17 initially non-leukemic mice, 11 developed leukemia during the following 2 months, including the 2 antibody-positive animals, and there was no change in antibody status in any of the mice when rebled after the development of leukemia.

DISCUSSION

Various types of evidence presented here point to the environment as a major factor in determining the prevalence of spontaneous polyoma virus infection in captive laboratory mouse colonies; these may be itemized as follows:

1. Antibody was distributed by colony and not by mouse strain.

2. Antibody was found primarily and with greatest frequency in colonies in which breeding mice were housed in proximity to mice experimentally inoculated with polyoma virus or passage tumors.

3. The infection rate was highest in animal rooms housing mice experimentally inoculated with polyoma virus.

4. Antibody was seldom found in mice under 3 months of age, and increased in frequency with increasing age. It should be pointed out that newborn mice inoculated subcutaneously with small amounts of polyoma virus developed antibody within two weeks (13).

5. Mice from a colony free of antibody became infected when held in cages or animal rooms with artificially infected mice.

Although some of the above points require confirmation by long term controlled experiments with larger numbers of animals, the available evidence supports the hypothesis that the majority of polyoma infections in laboratory mice are acquired after the neonatal period, their frequency depending on the intensity of the environmental contamination. This concept also explains the rarity of the characteristic parotid tumor as a spontaneous neoplasm even in infected colonies (10, 17), since only suckling mice are susceptible to the oncogenic effect of the virus (18) during the usual periods of observation.

The mouse colonies surveyed for prevalence of polyoma virus infection can be divided into several categories: high prevalence (Gross, Law, and Jackson Memorial Laboratory experimental animal room), colonies with exposure to mice with artificial infections with polyoma virus; low prevalence (Jackson Memorial Laboratory commercial mice and Heston) with questionable exposure to laboratory work with polyoma virus; antibody-free in the small numbers tested (Andervont, Deringer, and Microbiological Associates) with little or no exposure; and antibody-free (Jay-Poiley), a colony with no known exposure. While the data presented on the importance of environmental contamination provide an explanation for the high prevalence observed in several colonies, it is not clear at present whether external artifactual sources of virus were also responsible for the infections in the low incidence colonies studied. It remains to be determined if the contagiousness of the agent is sufficient to maintain infection in laboratory colonies without external contamination.

Evidence to be presented in another paper (15) shows that saliva, urine, and feces of virus inoculated mice, particularly those infected as newborns, provide a significant degree of environmental contamination. Also, mice can be infected readily by intranasal instillation of virus, and occasionally by ingestion of large doses of virus (15). These observations are in accord with the findings reported here that antibody was most prevalent in animal rooms housing mice inoculated with virus. In addition to work with polyoma virus, transplanted mouse tumors may also provide a source of infection. Both Stewart *et al.* (2) and we (19) have observed spontaneous infection of transplanted mouse leukemias, the virus

being carried in high titer in the tumor tissues, at least during the 1st week of tumor growth and polyoma infection.

Conversion rates were significantly higher in the Law colony than in mice of the Jay-Poiley colony held in our animal rooms with experimentally infected mice and hamsters. Whether this difference was due to the larger number of inoculated newborns in the Law colony, some difference in animal management practices that facilitated cross-infection, or to greater susceptibility of the inbred strains in comparison to the "general purpose" Swiss mice is not known. While the serological surveys reported here did not demonstrate any marked differences in infection patterns between different mouse strains, it is quite possible that there exist significant variations between mouse strains in susceptibility to infection, intensity of excretion of virus, or even of mechanisms of virus transmission.

The data on distribution of antibody and serologic conversion according to litter mates indicate a highly significant association of positive mice by litter. The data on litter distribution of antibody in the initial bleeding (Table IV) as well as the pattern of conversions by litter, not presented, suggest a tendency for infected litters to have either one mouse or all mice infected. A possible explanation of this finding would be that mice are infected randomly at low frequency from external sources of virus, and differ in the amount of virus which they excrete; thus, a mouse excreting little or no virus would seldom infect cage mates, while a mouse excreting large amounts would frequently infect the remaining mice in the cage. This hypothesis would better explain the findings than to postulate that the intensity of environmental contamination determines the number of mice in a cage which become infected; this mechanism should produce a more continuous distribution of percentages of infected mice in different litters, while the former mechanism would produce the observed discontinuity. In view of the evidence of the importance of environmental contamination and the fact that these studies were done on highly inbred mice, it seems very unlikely that the observed litter distribution could be explained on a genetic basis.

The lack of correlation of polyoma antibody with occurrence of leukemia in AK mice suggests that polyoma virus has little or no etiologic relation to spontaneous AK leukemias. This is in agreement with the findings of Gross, who showed that the leukemogenic activity of AK leukemic filtrates was destroyed by ether treatment, while parotid tumor induction was not affected (20). Polyoma virus in tissue culture fluid is also resistant to overnight treatment with diethyl ether (7).

The finding that certain mouse colonies are essentially free of polyoma virus carries several practical implications. Availability of mice known to be free of infection is obviously of great value to the laboratory studying the virus. Also, a study of spontaneous tumors in a polyoma-free colony could simply and con-

clusively rule out certain types of neoplasm as being caused by natural polyoma virus infection.

In view of the wide pathogenic potentialities of the agent, knowledge of the level of infection in colonies of mice used for experimental cancer work would appear to be of great importance.

SUMMARY

Eight mouse colonies were surveyed for prevalence of antibody to mouse polyoma virus. Frequency of HI antibody varied from 0 to 84 per cent in adult mice in different colonies. Antibody was infrequent in mice less than 3 months of age, and increased in frequency with age. There was no evidence that infection was specific for particular mouse strains. The highest frequency of infection was found in colonies in which breeding mice are housed in proximity to mice inoculated with polyoma virus or passage tumors, and within an infected colony, the incidence of infection was greatest in rooms housing mice inoculated with polyoma virus. Mice from a colony free of antibody became infected when held in room or cage contact with virus-inoculated mice, but at very low rates except in mothers of inoculated litters. These results were interpreted as indicating that artificial contamination of the environment is an important factor in determining the prevalence of infection in the colonies observed.

There was no correlation between polyoma infection and spontaneous leukemia in AK mice.

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