THE EPIDEMIOLOGY OF MOUSE POLYOMA VIRUS INFECTION

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An infectious disease process must be understood at four levels: the level of the agent itself, the level of the interaction of the agent with the individual cell, the level of infection of individual animals, or the pathogenesis of the infection, and at the level of the whole population, or the epidemiology and ecology of the infection. In this period of intense interest in the nature of the cell-virus interactions, it must be remembered that elucidation of the ecology of an infectious agent remains indispensable for determining the importance of the agent as a cause of disease, for devising control procedures, and even for working rationally with the agent in the laboratory. This truism applies as much to tumorigenic viruses as to any other agent. This report will discuss the results of ecologic and pathogenicity studies with the polyoma virus.

The polyoma virus was discovered independently by Gross (13) and by Stewart (34), during attempts to transmit AKR mouse leukemia to newborn mice by inoculation of filtrates of leukemic tissues. Subsequently, Stewart and Eddy (6, 36) made the major discoveries that the virus could be propagated in tissue culture, with production of cytopathic effects in mouse embryo cultures, and that infected tissue culture fluids inoculated into newborn mice induced multiple tumors, both of multicentric origin and of multiple histologic types (35). The most frequent tumors produced were mixed tumors of the salivary glands and respiratory tract mucous glands, subcutaneous sarcomas, mammary tumors, osteogenic sarcomas, and thymic epithelio- mas, but many other cell types were also involved. Even more remarkably, the virus was capable of inducing tumors in other species, producing hemangioendotheliomas and sarcomas in the hamster, sarcomas in the rat, and fibromas in rabbits, as also shown by Eddy, Stewart, and their co-workers (8-10).

The existence of an agent capable of inducing many tumor types in multiple species made it imperative to attempt to elucidate its natural history, and in particular to answer such questions as these: “Is this exclusively a mouse virus, or are other species naturally infected?” “What is the distribution of infection among mice, how is it transferred and maintained, and is it an infection of particular genetic strains of mice?” “What is the relation of polyoma virus to spontaneous neoplasms in the mouse and possibly in other species?”

These, of course, are questions which are amenable to approach by epidemiologic procedures, provided suitable techniques could be developed for detection of infection. Since the tissue culture cytopathogenicity reported by Eddy offered a possible tool for epidemiologic application, our laboratory undertook a program to develop procedures for detection of infection and to attempt to answer some of the above questions.

Fortunately, and unexpectedly, the development of diagnostic procedures turned out to be a straightforward virologic exercise when it was discovered, jointly with Dr. Eddy, that the virus hemagglutinates erythrocytes of many species (5). This procedure provided an invaluable tool for rapid identification of virus isolates and for detection of antibody. It was, of course, essential to prove that the hemagglutinating and cytopathogenic properties of infected tissue culture fluids were indeed attributes of the tumorigenic agent, and not a result of contamination with an extraneous mouse agent. This was conclusively demonstrated by the finding that there was excellent correlation of the tumorigenic and hemagglutinating activities in their distribution in individual tissue culture tubes at limiting titration dilutions, their adsorption and elution from red blood cells, their resistance to ether and heating at 60°C for 30 minutes, their sediments-
tion in the ultracentrifuge, and their inhibition or lack of inhibition by sera of individual uninoculated mice from infected colonies (28). Thus it was possible to apply the simple test in vitro with the utmost confidence that it was actually indicative of infection with the tumorigenic agent.

The virus was found to be spherical, about 44 μm in diameter in dried preparations, and in size and appearance very similar to the Shope rabbit papilloma virus (19). Like the Shope virus, it was highly resistant to environmental influences, including heat, ultraviolet light, and many disinfectants (2, 7).

The polyoma virus attaches to red cells by adsorbing to the same mucoprotein receptor sites as do the influenza viruses; either Vibrio comma receptor-destroying enzyme (RDE) or influenza viruses will destroy the red cell receptors for polyoma virus (17). However, polyoma virus itself is almost totally devoid of enzymatic activity, the only evidence for enzymatic activity being combination of virus with inhibitor after elution from erythrocytes (15). The mucoprotein receptors are apparently involved in entry of this virus into cells, since treatment of tissue culture cells with receptor-destroying enzyme significantly reduces their susceptibility to polyoma infection (unpublished data).

The development of hemagglutination procedures made possible the development of a hemagglutination inhibition test for detecting antibody responses. In addition, it was possible to develop complement fixation (27), tissue culture neutralization, and tumor neutralization tests, which were of particular value for establishing the degree of specificity, sensitivity, and reliability of the hemagglutination inhibition test (28). With the use of these antibody tests, it was established that the virus is antigenic, both newborn and adult mice responding regularly to inoculation of small doses of live virus with development of antibody. Development of antibody in weanling mice following intraperitoneal injection of virus-containing material is a highly sensitive method of detecting polyoma infectivity, having the same or somewhat greater sensitivity than prolonged observation of mouse embryo tissue cultures; this indirect immunization test, or mouse antibody production test, has been used as our standard method of detecting and titrating polyoma infectivity (28). One 50 per cent infectious dose for weanling mice or tissue culture corresponds to approximately 300 physical particles of virus in most preparations (19, 29).

Also, it was established that antibody response to polyoma virus is highly specific; no cross serologic reactions have been observed in tests against a wide variety of mouse viruses, human viruses, and tumor viruses of various species (27; unpublished data). Antibody in mice is highly durable, apparently persisting for life in the vast majority of infected animals.

With the development of well-evaluated, sensitive, and specific procedures for detecting virus and antibody, it became possible to determine the extent of spontaneous infection in various populations of laboratory and wild mice.

Figure 1 shows the patterns of antibody prevalence observed in various laboratory and commercial mouse breeding colonies (31). The total height of each bar represents the frequency of antibody in uninoculated mice over 3 months of age in a given colony, and the shaded portion, the frequency in mice less than 3 months of age; NT indicates that the younger mice were not tested. The number at the top of each bar is the number of mice tested. The important features to note are the extreme variation in prevalence of infection between the different colonies and the consistently higher incidence of antibody in the older mice than in young mice.

Infection in many of the laboratory colonies was readily explained by close exposure to experimental animal work with polyoma virus or to mice bearing transplanted neoplasms, many of which are carriers of the virus (30). By comparing infection rates in individual rooms of a single colony, evidence was obtained that exposure to mice which had received potentially infected material as newborns was associated with highest risk of infection (31). The importance of environmental exposure is further indicated by the findings in the three colonies listed in figure 1 as “derived colonies.” These are recently derived sublines of AKR or C3H mice originating from heavily infected colonies and reared for several generations in areas with either minimal environmental exposure, as in the case of the colony with 2 per cent infection, or with no environmental exposure, as in the case of the other two sublines, which were free from infection.

However, environmental exposure to artificial procedures is not necessary for maintenance of infection, as shown by its prevalence in certain
commercial mouse colonies which have no contact with experimental work. Among the various colonies tested there was no evidence of specificity of infection for particular genetic strains of mice. This is exemplified in table 1, which shows the frequency of antibody in four different colonies of AKR mice as related to the frequency of antibody in mice of other strains housed in the same environment. The AKR mouse was particularly important to study in this respect because of the frequency of the association of polyoma virus with spontaneous leukemia in this strain. The findings here show that there was no tendency for these inbred mice to have a uniform level of infection, but that the infection level was the same as that in other mice in the same colony.

It was necessary, of course, to confirm that the finding of hemagglutination-inhibiting (HI) antibody in these normal mice really reflected infection. Consequently, virus isolation studies were carried out on mice with and without antibody from two heavily infected laboratory colonies (23, 28), with results as shown in table 2. Virus was recovered from the organs of half of the mice who were positive for HI antibody, and from only 10 per cent of the antibody-negative mice. It should be emphasized that these antibody-negative mice were from the positive colonies, and some mice may have been converting or have developed subdetectable levels of antibody. No definite virus isolation has been obtained from antibody-negative colonies. It is noteworthy that there was no difference in frequency in virus recovery between normal mice and AKR mice with leukemia.

The quantity of virus detected in these normal mice was small; to date, no “high titer” carrier state, such as seen with mouse lymphocytic choriomeningitis infection, has been encountered.

The pattern of spread observed in experimental colonies is shown in table 3, which shows the

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**TABLE 1**

Incidence of HI antibody to polyoma virus in AKR mice in various colonies, as compared with incidence of antibody in other mice in the same environment.

<table>
<thead>
<tr>
<th>Colony</th>
<th>Antibody in AKR Mice*</th>
<th>Antibody in Other Strains*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33/33 (100%)</td>
<td>31/52 (60%)</td>
</tr>
<tr>
<td>2</td>
<td>27/84 (32%)</td>
<td>122/338 (33%)</td>
</tr>
<tr>
<td>3</td>
<td>1/21 (5%)</td>
<td>3/61 (5%)</td>
</tr>
<tr>
<td>4</td>
<td>0/131 (0%)</td>
<td>0/1608 (0%)</td>
</tr>
</tbody>
</table>

* More than 3 months old.

**TABLE 2**

Recovery of polyoma virus from organs of mice from laboratory colonies with spontaneous infection*.

<table>
<thead>
<tr>
<th>Mice</th>
<th>Virus Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HI-positive mice</td>
</tr>
<tr>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Normal . . . . .</td>
<td>15/26 (56)</td>
</tr>
<tr>
<td>Leukemic AKR . .</td>
<td>6/11 (55)</td>
</tr>
</tbody>
</table>

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1961

MOUSE POLYOMA VIRUS INFECTION

TABLE 3
Cross infection in Swiss mice from a negative colony held in animal rooms with inoculated mice and hamsters*

<table>
<thead>
<tr>
<th>Age when Brought</th>
<th>Type of Contact with Infected Mice</th>
<th>Frequency of Antibody after Various Lengths of Time in Laboratory</th>
</tr>
</thead>
</table>
| 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
|                  | Room                                             | 0/284
| Newborn          | Room                                             | 0/29

* Reprinted with permission from the Journal of Experimental Medicine (31).

frequency with which mice from a negative colony developed antibody after being held in our experimental animal rooms (31). Mother mice which nursed newborns that had been inoculated with virus rapidly acquired the infection. However, infection did not spread readily among weanling mice; a prolonged period of contact was necessary before a significant number of serologic conversions occurred. Comparisons of the ability of weanling mice of different genetic strains to infect cage mates indicated that there was no significant difference between the genetic lines (30).

To determine whether polyoma infection also occurred in wild mice, trapping studies were performed in several areas. Infection of wild Mus musculus has been detected in both New York City and in rural areas of Maryland (R. J. Huebner et al., unpublished data; 30). Table 4 shows the distribution of infection in New York by city block. Antibody was not found in the mice trapped in Queens, the Bronx, or lower Manhattan, but several areas in Harlem had high levels of infection. The contrast between the different blocks is very striking and indicates that the infection is focal in distribution. These focal infections are apparently stable patterns, as shown in table 5. When the same tenement apartment areas were re-trapped 3 to 6 months after the initial survey, the same frequency of infection was found as previously. In the first trapping of one block during June to September, 1959, there was 28 per cent incidence of antibody, and in

TABLE 4
Distribution of HI antibody to polyoma virus in wild house mice in New York City, by block.*†

<table>
<thead>
<tr>
<th>AREA</th>
<th>STREET</th>
<th>WEST SIDE BLOCKS</th>
<th>EAST SIDE BLOCKS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200-500</td>
<td>1-100</td>
<td>1-100</td>
</tr>
<tr>
<td>HARLEM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0/16</td>
<td>0/17</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0/4</td>
<td>0/3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0/12</td>
<td>0/13</td>
<td></td>
</tr>
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<td>4</td>
<td>0/27</td>
<td>0/26</td>
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<tr>
<td>5</td>
<td>0/13</td>
<td>0/12</td>
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<tr>
<td>6</td>
<td>0/17</td>
<td>0/16</td>
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<tr>
<td>7</td>
<td>0/26</td>
<td>0/22</td>
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<td>8</td>
<td>0/11</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0/10</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0/13</td>
<td>0/10</td>
<td></td>
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<td>11</td>
<td>0/17</td>
<td>0/14</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0/27</td>
<td>0/16</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0/12</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0/10</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>0/11</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0/12</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>0/11</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>0/16</td>
<td>0/16</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>0/12</td>
<td>0/11</td>
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<td>20</td>
<td>0/13</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>0/14</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>0/15</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>0/16</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0/17</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>0/18</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>0/19</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>0/20</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>0/21</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>0/22</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0/23</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>0/24</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>0/25</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>0/26</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>0/27</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>0/28</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>0/29</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>0/30</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>0/31</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>0/32</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>0/33</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>0/34</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>0/35</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>0/36</td>
<td>0/11</td>
<td></td>
</tr>
</tbody>
</table>

* Initial survey, June to September, 1959.
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January of 1960 there was 25 per cent frequency. In the other focus, many blocks away, infected mice were also found during the later trapping. As in the infected laboratory colonies, it was possible to isolate virus from the tissues of mice with antibody; 25 per cent of such mice were positive for virus, again in low titer.

It was possible to recover virus not only from the organs of the wild mice, but also from excreta and environment, as shown in table 6.
Persistence of virology in bedding antibody-positive mice was held in Maryland mice, rural and wild areas of polyoma was recovered in the virus from urine, saliva, glands, salivary glands, and excreta of infected mice. As shown in table 7, virus was recovered once from urine of an antibody-positive mouse and six times from the sawdust bedding of captive mice which had been held for 1 or 2 weeks in Mason jars. Also, virus was recovered four times from eleven samples of floor sweepings of closets and corners of apartments harboring infected mice.

Focal distribution of infection was also seen in rural mice, as shown in table 7. Seven barns in Maryland were sampled, each of which was at least 2 miles distant from the others. No antibody was found in a total of 99 mice from six barns, but in the seventh barn, 21 per cent of the mice had antibody.

It is interesting to contrast the focal distribution of polyoma virus infection with that of another virus which has a high tropism for the salivary glands, the mouse salivary gland virus, which was encountered incidentally during the polyoma studies. As shown in table 8, this virus was extremely widely disseminated among the wild mice, being found in every area that was adequately tested, as well as in many that were not.

As yet, antibody to polyoma virus has not been encountered in any nonlaboratory animal other than Mus musculus. No antibody was found in 444 sera from fourteen genera of American wild rodents, nor in 61 sera from eight genera of wild and domestic American higher mammals (30). Opossum sera have uniformly given inhibition of hemagglutination, but there is no corroborative evidence from the other serologic tests to indicate that this represents antibody (30). It should be pointed out that the majority of the animals tested were from areas in which it is not known whether polyoma virus is present in the wild mouse population. Consequently, whether the tested animals were really at risk of the infection is not known. However, we have tested sera of rats, cats, and humans in the infected areas of New York City, and found no antibody to polyoma virus. Many human sera from other areas have been tested as well, and no significant evidence of antibody has been obtained, although many humans have RDE-resistant hemagglutination inhibitors in low level (unpublished data).

Studies of the pathogenesis of infection in laboratory mice have contributed much to clarification of the epidemiology of polyoma virus (29, 30). It was found that mice are readily infected by intranasal instillation of virus, either as weanlings or newborns; approximately 1000 infectious doses, as measured by tissue culture or

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**TABLE 5**
Persistence of polyoma infection in positive foci in New York City

<table>
<thead>
<tr>
<th>Date of Trapping</th>
<th>100 Block W. St. 3</th>
<th>200 Block E. St. 43</th>
</tr>
</thead>
<tbody>
<tr>
<td>June–Sept. 1959</td>
<td>115/418 (28%)</td>
<td>15/62 (24%)</td>
</tr>
<tr>
<td>Jan. 1960</td>
<td>32/126 (25%)</td>
<td>4/8</td>
</tr>
</tbody>
</table>

**TABLE 6**
Recovery of polyoma virus from excreta and environment of infected mice in New York City*

<table>
<thead>
<tr>
<th>MATERIAL TESTED</th>
<th>VIRUS RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>URINE</td>
<td>0/9</td>
</tr>
<tr>
<td>HI POS. MICE</td>
<td>1/23</td>
</tr>
<tr>
<td>HI NEG. MICE</td>
<td></td>
</tr>
<tr>
<td>MOUTH SWAB</td>
<td>0/3</td>
</tr>
<tr>
<td>HI POS. MICE</td>
<td>0/3</td>
</tr>
<tr>
<td>HI NEG. MICE</td>
<td>0/3</td>
</tr>
<tr>
<td>BEDDING OF CAPTURED MICE (ALL HI POS.)</td>
<td>6/21</td>
</tr>
<tr>
<td>MITES (A. SANGUEUS)</td>
<td>0/5</td>
</tr>
<tr>
<td>COCKROACHES</td>
<td>0/5</td>
</tr>
<tr>
<td>FLOOR SWEEPINGS</td>
<td>4/11</td>
</tr>
</tbody>
</table>

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**TABLE 7**
Occurrence of HI antibody to polyoma virus in farm mice in Maryland

<table>
<thead>
<tr>
<th>Location</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barn H</td>
<td>14/67 (21%)</td>
</tr>
<tr>
<td>6 other barns</td>
<td>0/99</td>
</tr>
</tbody>
</table>

**TABLE 8**
Recovery of mouse salivary gland virus from wild mice

<table>
<thead>
<tr>
<th>NEW YORK (TISSUE SUSPENSIONS)</th>
<th>MARYLAND (MOUTH SWABS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BROOKS</td>
<td>BARN S 4/7</td>
</tr>
<tr>
<td>QUEENS</td>
<td>BARN H 18/22</td>
</tr>
<tr>
<td>MANHATTAN</td>
<td>BARN C 5/7</td>
</tr>
<tr>
<td>QUEENS Block E. St. 43</td>
<td>0/3 BLOCK B.</td>
</tr>
<tr>
<td>100 BLOCK E. St. 42</td>
<td>0/1 BLOCK W.</td>
</tr>
<tr>
<td>100 BLOCK E. St. 41</td>
<td>1/1 BLOCK K.</td>
</tr>
<tr>
<td>100 BLOCK W. ST. 20</td>
<td>0/2 BLOCK K.</td>
</tr>
<tr>
<td>500 BLOCK W. ST. 14</td>
<td>0/3 BLOCK K.</td>
</tr>
<tr>
<td>0 BLOCK W. ST. 9</td>
<td>0/1 BLOCK K.</td>
</tr>
<tr>
<td>0 BLOCK W. ST. 4</td>
<td>0/5 BLOCK K.</td>
</tr>
<tr>
<td>100 BLOCK W. ST. 3</td>
<td>1/1 BLOCK K.</td>
</tr>
</tbody>
</table>

As yet, antibody to polyoma virus has not been encountered in any nonlaboratory animal other than Mus musculus. No antibody was found in 444 sera from fourteen genera of American wild rodents, nor in 61 sera from eight genera of wild and domestic American higher mammals (30). Opossum sera have uniformly given inhibition of hemagglutination, but there is no corroborative evidence from the other serologic tests to indicate that this represents antibody (30). It should be pointed out that the majority of the animals tested were from areas in which it is not known whether polyoma virus is present in the wild mouse population. Consequently, whether the tested animals were really at risk of the infection is not known. However, we have tested sera of rats, cats, and humans in the infected areas of New York City, and found no antibody to polyoma virus. Many human sera from other areas have been tested as well, and no significant evidence of antibody has been obtained, although many humans have RDE-resistant hemagglutination inhibitors in low level (unpublished data).

Studies of the pathogenesis of infection in laboratory mice have contributed much to clarification of the epidemiology of polyoma virus (29, 30). It was found that mice are readily infected by intranasal instillation of virus, either as weanlings or newborns; approximately 1000 infectious doses, as measured by tissue culture or
antibody production following parenteral inoculation, sufficed to stimulate antibody formation when given intranasally. In contrast, about 1,000,000 infectious doses were needed to produce infection by feeding or drinking (30).

Figure 2 shows the course of virus growth after inoculation of newborn mice. The points represent the median infectivity titers, expressed per gram of tissue, of the pooled viscera determined at various days after inoculation. The course of virus multiplication was quite rapid, with the tissues attaining maximal infectivity titers within 7 to 10 days. During this stage, highest titers were attained in the kidneys and salivary glands, but high titers were found in almost all organs. After the initial peak, the virus titer declined slightly and after the 30th day underwent a marked decline. Antibodies appeared at 10 days, and reached maximal levels at 20 to 30 days. The lower curve shows the amount of virus in the urine of the same mice. Again, this is the median titer. The virus was found in urine in large quantity during the 1st month of infection. It is quite significant that grossly visible tumor did not appear until long after the peak of virus titer.

Virus is uniformly recoverable from the parotid tumors, but its detection may be hampered by the high levels of antibody. Cultivation of the tumor in tissue culture appears to be the most sensitive method for detection of virus (29, 33) as was observed previously with adenoviruses (32).

Figure 3 shows in greater detail the degree of viral excretion by mice which were artificially infected as newborns; this is a composite of many different animals in different experiments. The total height of the bar represents the number of tests, the doubly hatched portion is the number in which virus was definitely isolated, and the singly hatched portion represents recovery of only a trace amount of virus. Superimposed on the bar graph are individual points showing the titers found in certain selected specimens on which end points were determined. It is seen that urine was infectious in the majority of animals for periods of at least 120 days, and that titers in individual mice ranged as high as $10^6$ ID₉₀ per 0.2 ml of urine. Virus was isolated frequently from saliva as well, and again in high titers.

The epidemiologic studies of antibody prevalence presented earlier indicated that the weanling mice are not as effective in infecting environmental contacts as are the newborn mice, although they are highly susceptible to infection. Figure 4 shows some of the reasons for this. This chart shows results of an experiment comparable to those just described, in which the course of virus growth was followed at intervals after inoculation of weanling mice (29). For comparison, the curve obtained with the newborn mice is shown by the dotted line. The maximal infectivity titers in the tissues of the weanling mice were $10^{-4}$ X those in the newborns. However, despite the lower virus growth, the virus persisted for very long periods; small amounts were recovered from salivary glands as long as 210 days after inoculation. The excretion of virus by weanling mice is shown in figure 5, in which the results are plotted in the same way as previously shown for the newborns. Virus excretion was
much less frequent, but it is probably significant that the few mice which did excrete virus in urine had relatively high titers, several animals having $10^4$ ID$_{50}$ per 0.2 ml of urine. A similar pattern was seen with the saliva, but the feces were a rare source of virus.

The epidemiologic patterns in mice can be summarized as shown in figure 6. Mice which become infected as newborns or young sucklings constitute the major source of spread through a colony. They readily infect their mothers and cage mates, and the cage mates in turn may serve to infect other mice. However, the mice which become infected as adults are much less frequently sources of infection. The introduction of experimental procedures in which potentially virus-containing materials are inoculated into young mice constitutes a very efficient amplification procedure to maintain and disseminate the virus throughout the colony.

To explain the maintenance of infection in colonies such as the commercial mouse colonies...
and the wild mouse populations in which there is no exposure to experimental procedures, it appears probable that two mechanisms are active. In the commercial colonies, such large numbers of mice are housed in close contact that the inefficient excretion by the weanling mice may be sufficient to maintain the infection. However, in the smaller laboratory colonies, insufficient high titer excreters are available to maintain the infection. Newborn mice will not often be infected because the statistical chance of acquiring infection during the first few days of life is, of course, quite small, and in a colony with a high level of infection many newborns would be protected for a period of time by the maternally transmitted antibodies (22).

In the wild mouse populations, a tempting hypothesis is that the nesting areas become contaminated with urine of infected babies, and because of its high stability, the virus could remain in the nests and repeatedly infect subsequent litters, which in turn re-seed the nests with
TABLE 9

Epidemiology of polyoma virus in life-long observation mice at R.B. Jackson Laboratory; occurrence of antibody in mice with various tumors, as compared with nontumorous mice*

<table>
<thead>
<tr>
<th>POPULATION</th>
<th>TUMOR</th>
<th>ANTI Body in Mice with Tumor</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>INC. WITH TUMOR</td>
<td>OBSERVED</td>
<td></td>
</tr>
<tr>
<td>INBRED MALES FOR STRAIN &amp; SEX</td>
<td>ANY TUMOR</td>
<td>190</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>MAMMARY</td>
<td>66</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>LUNG</td>
<td>81</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>LYMPHATIC LEUKEMIA</td>
<td>30</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>RETICULLAE SARCOMA</td>
<td>39</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>SARCOMA</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>OTHERS</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>INBRED FEMALES FOR STRAIN &amp; SEX</td>
<td>ANY TUMOR</td>
<td>152</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>MAMMARY</td>
<td>53</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>LUNG</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>LYMPHATIC LEUKEMIA</td>
<td>35</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>RETICULLAE SARCOMA</td>
<td>25</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>SARCOMA</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>OTHERS</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>MULTIPLE TUMORS</td>
<td>27</td>
<td>23</td>
</tr>
</tbody>
</table>

* Reprinted with permission from Perspectives in Virology, II (30).

infected urine. This mechanism, of course, would not be highly effective in the laboratory and commercial colonies, in which the bedding and containers are frequently changed and sterilized.

Another aspect of the epidemiology of this virus is most important, i.e., the relationship of these spontaneous polyoma infections to the occurrence of mouse neoplasms. Table 9 shows results of a study performed in collaboration with the Roscoe B. Jackson Laboratories in Bar Harbor, Maine (Rowe, W. P., and Murphy, E., unpublished data). Mice which were under observation for tumor development were bled when moribund from any cause, and comparisons were made of the incidence of antibody in mice with particular tumors as compared with mice without tumors. The data were examined separately for the inbred and the hybrid strain mice because of certain population attributes which required adjustment. The over-all incidence of antibody in the colony was 38 per cent. When the incidence of antibody in the tumorous mice of the categories shown here was compared with that of the mice dying without tumor, no significant excess was observed in any category. Also, in none of these mice nor in any of the wild mice was a salivary gland tumor ever observed of the histologic type which is characteristic of polyoma virus infection in experimentally inoculated mice. Thus, these data suggest that even in a highly infected colony, spontaneous polyoma virus infection neither induced the characteristic polyoma tumors, nor contributed to the occurrence of other well recognized mouse tumors. The lack of association of infection with spontaneous leukemia is important to note, since it is increasingly clear from many types of evidence that the early association of polyoma virus isolation with AKR leukemia was entirely fortuitous (23).

The finding that polyoma infection in a large laboratory colony did not contribute to the occurrence of spontaneous tumors can be explained by several of the known features of the infection. First, the rapid development, with age, of resistance to the oncogenic effects of infection (37) means that the statistical probability of a mouse acquiring infection during the critical age period is quite low. Second, the transfer of maternal antibody is extremely effective. Antibody is transferred at high level both to the unborn fetus and to the nursing young (22). Thus, in a heavily infected population, maternal protection will be a major factor in preventing infection during the critical first days of life. Third, the dose acquired spontaneously is probably much smaller than that usually employed in tumorgenic experiments; and fourth, the intranasal route of inoculation is very ineffective for induction of tumors (Law, L. W., and Rowe, W. P., unpublished data).

However, this is not to say that the virus cannot induce tumors under spontaneous conditions. The characteristic parotid tumors have been observed in uninoculated animals in several laboratories (4, 14, 21; Ward, T. G., personal communication; Rowe, W. P., unpublished data). It is interesting to consider the conditions under which these tumors arose. Considering the efficacy of maternal antibody transmission to the young, and the effects that a high prevalence of antibody would have on the number of susceptible young, one can anticipate that the maximal opportunity for infection of infant mice would be a colony in which the incidence of infection is sharply rising; that is, shortly after the virus is introduced and is becoming well disseminated. At this time there would be maximal environmental contamination and minimal frequency of immune mothers. Apparently it is exactly under these conditions that the spontaneous parotid tumors have been observed. In each of the laboratories in which these tumors have been described, they occurred shortly after the laboratory began working with the virus. After work for more than
a year with the virus, the spontaneous tumors were no longer encountered.

Thus we have the picture of polyoma virus as an agent which is highly prevalent in certain focal areas, but with an ecologic pattern that strongly works against expression of the infection as overt disease. Actually, the focal infections of polyoma virus in mouse colonies may be more of a danger to other rodent species in contact with these animals than to the mice themselves. For example, hamsters readily develop tumor after intranasal instillation of virus (27), and there is no sharp development of resistance with age (37). Spontaneous polyoma-induced tumors have been observed several times in hamsters housed in our experimental mouse rooms.

The pattern of infection in the hamster demonstrates a very interesting comparison with that in the homologous species, as shown in figure 7. Again the curve of virus growth in newborn mice is shown for comparison. This chart is a composite of several experiments (29), all of which were done with very large inocula of virus, which resulted in rapid tumor response. In contrast to the mouse pattern, the virus titer declined sharply after the 4th or 6th day of infection, and by the 20th day the tissues had lost their infectivity. Also, the peak titers obtained were several powers of 10 lower than that in the mouse, even though the tumor response was much quicker and more uniform.

Table 10 shows the results of testing tumors from individual hamsters. These tumors were tested by the mouse antibody production test, mouse embryo tissue culture, and some by inoculation into suckling hamsters with observation for tumors and development of antibody. In agreement with the growth curve just shown, the tumors removed 16 and 17 days after infection were generally infectious, whereas those removed at 6 or 11 weeks were rarely positive. Only one of the 10 late tumors was positive in any test. Thus, both in newborn mice and the suckling hamster an unusual pattern of relation of virus growth to appearance of tumor is observed, i.e., a peak of virus titer preceding appearance of tumor. To my knowledge this pattern has not been described for other tumor viruses. To compare this pattern with that of some other tumor viruses, similar experiments were carried out with the Friend mouse leukemia virus and with the Shope rabbit papilloma virus.

Figure 8 shows results of two experiments with the Friend mouse leukemia virus. The development of disease, as indicated by weight of the spleen, is shown in relation to the concentration of virus in the spleen (26). There is a rather remarkable parallel of increase in log spleen weight and increase in virus titer during the first 10 to 14 days after inoculation. After this time the virus titer declines and is maintained at a plateau. This then is in marked contrast to the polyoma pattern, as is the pattern of the Shope papilloma virus as shown in figure 9. In this experiment, which was done by Dr. David White in our laboratory, a cottontail rabbit was massively sacrificed and infected. At intervals, skin
biopsies were taken and tested for content of virus. This figure represents only the result of the preliminary test of the samples, showing the number of tumors developing per site inoculated with the undiluted suspension of biopsy tissue. Again the appearance of tumor and increase of virus coincided very closely, with no significant evidence of a preliminary peak before the appearance of disease. The small amount of virus recovered on the 2nd day after inoculation probably represents residual virus from the inoculum.

I would like now to turn to the question of what significance these studies may have for the over-all problem of tumor viruses. First, I should point out that the results with polyoma virus, taken by themselves, have little "importance" except in a negative sense of dispelling over-optimistic hypotheses. Polyoma virus is not the answer to the problem of spontaneous tumors in mice, and so far as known, in any other species. The greatest importance appears to lie in the influence that these findings may have on our thinking about studying the relation of viruses to neoplasms.

It seems fair to say that most searches for tumor-inducing viruses have been based on the tacit assumption that the place to look for tumor viruses is in the tumor. This, of course, is merely a corollary of the theories that in virus-induced tumors, neoplastic growth is dependent on persistence of virus, and that neoplastic transformation is a necessary accompaniment of tumor virus growth. Although this pathogenetic mechanism is perhaps operative in most of the known tumor virus systems, we must remember that these "known tumor virus systems" are

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**Table 10**

*Attempts to recover polyoma virus from tumors of inoculated hamsters*

<table>
<thead>
<tr>
<th>Day after inoculation when removed (days)</th>
<th>80 - MOP (wk)</th>
<th>60 - Titer (Log MOP)</th>
<th>40 - Titer (Log MOP)</th>
<th>30 - Titer (Log MOP)</th>
<th>16 - Titer (Log MOP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>2/2</td>
<td>+ (17-20 Days)</td>
<td>+ (17-21 Days)</td>
<td>+ (Blind Passage)</td>
<td>+ (Blind Passage)</td>
</tr>
<tr>
<td>16</td>
<td>1/2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0/2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Reprinted, with permission from the National Cancer Institute Monograph No. 4, Symposium on Phenomena of the Tumor Viruses (29).

**Figure 8.** Growth curve of Friend leukemia virus in mouse spleen.
known precisely because they have this pattern. Thus, we are viewing a highly selected group of models, and it does not follow that the pattern most frequently seen in our models is necessarily the most frequent pattern occurring in nature.

At this point it is worth injecting another caution against the injudicious extrapolation of our model systems. From experience with mouse tumor virus systems, we come to think of tumor virus infections as latent or chronic infections, and conversely, to exclude acute infectious processes from consideration as possible tumorigenic systems. But again, there is a strong selective force in the availability of our experimental models. For why should not the mouse tumor viruses be producers of latent infections, when the great majority of "nontumorigenic" viruses of the mouse also produce latent or chronic infection? Thus, of the 10 or so "nontumorigenic" viruses indigenous to mice, six are well established as producing chronic infections: lymphocytic choriomeningitis (25, 39), mouse salivary gland virus (1), Thelier's viruses (38, 40), K virus (20; L. Kilham, personal communication), the mouse adenovirus (16; J. W. Hartley and W. P. Rowe, unpublished data), and the recently isolated mouse thymic agent (W. P. Rowe, unpublished data). For three others there is epidemiologic or other evidence of persistent infection: ectromelia (11), mouse hepatitis (12), and the pneumonia virus of mice (18).

This relative rarity of mouse viruses which produce infections of short duration is to be expected from the ecology of wild mice, which have relatively restricted movement and generally form new colonies from a small nucleus of immigrants; under these conditions acute viruses could not be maintained. The ecologic conditions of laboratory mice impose yet greater restrictions on maintenance of "acute viruses," with isolation and quarantine practices in frequent use, sterilization of containers, maintenance of colonies in semi-isolated groups of relatively small number, origination of colonies from a small nucleus of breeding pairs, and relative freedom from certain arthropods which may be potential reservoirs. Thus there is strong selection against mouse viruses producing infection of short duration, and as a consequence, the over-all virus experience of mice is of a very different nature from that of man. Another factor which is highly special about the viral experiences of laboratory mice is their relative lack of exposure to viruses of other species, because of their sheltered environments. One wonders whether we might not obtain a quite different picture of spontaneous tumor occurrence if we raised our mice in a veterinarian's office or in the middle of the Chicago stock yards! Thus because of the rarity of "acute viruses" indigenous to mice and their lack of exposure to viruses of other species, there is essentially no opportunity for the problem of spontaneous tumors in laboratory mice to include a pathogenesis pattern such as that seen in the hamster infected with polyoma virus.

The main point of the foregoing discussion has been to emphasize that the apparent pattern of polyoma virus in hamster tumors, although rarely found in tumor virus systems, has good reason for being rare in our model systems, but not necessarily in nature. The concept suggested by the hamster polyoma pattern, i.e., that an acute virus infection may trigger a cellular alteration which is later expressed as neoplasia, without necessity for continued presence of virus, is to me one of the most important outgrowths of
the polyoma studies. Although this concept is in no way new, is commonly observed with chemical and physical carcinogens, and has some experimental support from the VX2 carcinoma (24) and noninfectious tumors induced with low doses of the Rous virus (3), it has had very little influence on searches for tumor viruses. Probably this is so because this hypothesis demands searches for the tumor virus before, and possibly long before, the appearance of tumor. How then can this somewhat paradoxical problem be approached, particularly in man? I think that the answer to this application of the approach used in the Bar Harbor study mentioned above—to compare the previous viral experience of tumorous and nontumorous cohorts, either by retrospective serologic surveys or, less feasible, by long term observation. The disarraying normal behavior of polyoma virus in systems in vitro indicates that we cannot, a priori, exclude the well known, easily studied prevalent viruses of man or his associated domestic animals as potential candidates for such studies.

In conclusion, I should like to emphasize that these studies represent a team and collaborative effort; in particular, Drs. Huebner, Hartley, Brodsky, Law, White, and Murphy have contributed greatly to this work.

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34. STEWART, S. E. 1953 Leukemia in mice produced by a filterable agent present in AKR leukemic tissues with notes on a sarcoma produced by the same agent. Anat. Record, 117, 532.


