Isolation of Pleomorphic, Acid-fast Organisms from Several Strains of Mice

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SUMMARY

A pleomorphic, intermittently acid-fast organism has been isolated in this laboratory and elsewhere with great regularity from mammalian tumors and from blood of tumor-bearing hosts. In the present study involving 1500 randombred and inbred mice, the percentage of animals harboring the organisms has been found to parallel rather closely the tumor incidence. In some groups bleedings were repeated at intervals throughout the life span and compared with tumor production as demonstrated microscopically in the same individuals. For example, 56/100 ICR/albino female mice produced tumors and 49 of the 56 yielded the organism from blood prior to appearance of tumors; whereas in males, where only 19/100 became tumor-bearing, there was a corresponding drop in number of positive bloods to 16.

When the isolate from mouse Sarcoma 180 was injected intraperitoneally into newborn ICR/albino males, the tumor incidence was increased—up to 83% of individuals surviving beyond 18 months, as compared to 20% in controls.

The possible significance in the neoplastic process of filter-passing forms of these bacteria, and of phages which they have been shown to harbor, is under investigation.

INTRODUCTION

In recent years several investigators have reported the isolation of pleomorphic, acid-fast organisms from mammalian tumors and blood of tumor-bearing hosts (2, 9, 14, 19, 22, 23). Mazet (17) observed acid-fast organisms in stained preparations of leukemic tissues and Hodgkin's nodes, but did not culture them, and in 1955 Inoue et al. (12) reported the isolation of acid fast organisms from tumors of the newt. Other investigators isolated pleomorphic organisms from malignant tissues without noting or testing for acid-fastness, and still others, e.g. Carpenter et al. (6), identified the pleomorphic organisms which they isolated from cancerous patients as belonging to the corynebacteria. All but the most recent of these findings have been reviewed in an earlier paper by Diller (8).

Although such observations have been reported repeatedly, animal experimentation has for the most part been meager. It seemed important, therefore, to attempt to study these organisms in a species such as the mouse, where the average tumor incidence has long been established in many strains, and where possible variables due to age, sex, and strain differences could be explored in adequate numbers of animals. Over 1500 mice were involved in the present experiment, which required almost twelve years to complete. Repeated sampling of bloods from randombred mice necessitated the handling of more than 3000 cultures, and approximately 800 cultures from inbred mice were also studied.

MATERIAL AND METHODS

Methods of culturing the organisms, morphologic, physical, and chemical characteristics, staining capacity, growth patterns, and procedures used in reinfecting mice, were described in detail in earlier papers (8, 9).

In the first experiment 100 female mice of the ICR/Ha strain were earmarked, and were bled at one month of age from the jugular vein after the manner described by Kassel and Levitan (13) with a sterile syringe introduced into tissue cauterized immediately before puncture in an area previously depilated. The blood was inoculated in self-sealing screw-capped Kimble tubes containing three different media: Alexander-Jackson's "sensitive peptone broth," von Brehmer's medium, and a beef-heart-peptone broth enriched with 25% filtered ascitic fluid plus crystal violet and potassium tellurite (8). The tubes were immersed immediately in Dry Ice and acetone, and the contents quickly frozen. They were then brought slowly to room temperature, thus rupturing the blood cells. These preparations were incubated for ten days at 37°C and a sample of the inoculum was then withdrawn through the self-sealing cap with a sterile syringe and inoculated on solid medium, usually Trypticase-soy-blood agar, and again incubated until the organisms appeared, approximately ten days later. If growth did not appear, a second subculture of the original inoculum was attempted. Blood sampling was repeated when the mice were three, six, nine, and twelve months of age. Thereafter, blood was cultured only once, at death or sacrifice of each individual mouse. Tissues of the principal organs of each mouse were preserved, and paraffin sections were prepared for pathologic diagnosis. Quadruplicate slides were stained with hematoxylin and eosin, Alexander-Jackson's triple stain, Jayaraj's modification of Ziehl-Neelsen, and Robinow's bacterial stain, as previously described (8).

The second experiment involved 100 male ICR/Ha mice, and was conducted in the same manner, except that the mice were bled only once, at 12 months of age, since our experience with female mice indicated that the maximum percentage of positive bloods found prior to the appearance of tumors occurred when the mice were 12 months old. This experiment was twice repeated,
once with ICR/Ha males from laboratory of Dr. T. S. Hauschka at Roswell Park Memorial Institute in Buffalo, and again with descendants of this substrain (ICR/albino) that had been housed for two years in our Institute’s animal colony. Tests were also made of several inbred mouse strains:

1. Fifty old C3H/HeN1cr females (mammary tumor incidence in excess of 95%) and fifty old males (tumor incidence approximately 34%). Blood was cultured from the heart.

2. C58 males and females bred in our own laboratory from stock obtained from the Cold Spring Harbor Laboratories (MacDowell stock). The incidence of spontaneous lymphatic leukemia in these mice is 95% in both sexes. Again, heart blood was cultured from sacrificed animals. Fifty males and fifty females were tested.

3. Fifty males and fifty females of the “low-tumor” C57BL/6JN1cr strain.

4. In addition, two hundred males and females of C3H/HeN1cr and two hundred C57BL/6JN1cr males and females were treated with cortisone (Hydrocortone Merck, 1:1 dilution in sterile saline, subcutaneously, 0.40 ml/mouse). Fifty mice in each group were treated at two months of age and fifty in each group at nine to twelve months of age, and they were sacrificed ten days post-treatment. A corresponding number of untreated mice was tested in each age group. The heart blood of these mice was cultured as before.

Unless one has unlimited space, storage of mice until maturity is out of the question, and dealers cannot furnish older mice for the same reason. We were therefore compelled to compile our data a little at a time from retired breeders furnished by our Institute colony.

Slides were prepared from every one of the inocula and stained by means of one of the acid-fast techniques, regardless of whether growth was macroscopically detectable. Many of the cultures were subcultured for a number of generations, usually on glycerol agar. In the latter part of the study it was found that semi-fluid Trypticase agar base was a useful addendum to our list of media for primary isolation, as well as for subculture.

To minimize possible contamination, all operations were carried out in a hood that had been previously sterilized by ultraviolet irradiation. The mice were immersed in Wescodyne disinfectant and their skins were laid back with sterile instruments. A fresh set of sterile instruments was employed to make a small opening above the heart, from which blood was withdrawn by means of a sterile hypodermic needle.

RESULTS

Correlation of Incidence of Organisms in Blood and Tumor Incidence in the Same Individuals. The result of culturing blood of 100 ICR/Ha female mice appears in Table 1. Only one of the mice yielded the organism at one month of age; this was the first to develop a tumor, at eight months. However, on the basis of our overall results, no correlation could be established between early appearance of organisms in the blood and early onset of neoplasia. One mouse, whose blood yielded the organism from six months onward, survived to the advanced age of 34 months, when postmortem examination revealed the presence of a lung tumor. At six months the organism was isolated from 12 of the mice and at nine months from 34 of them. By 12 months some of the mice were dead, but the number of recoverable positives had risen to 41. Thereafter blood was cultured only when the mice appeared moribund or were sacrificed because a tumor had been noted. Once the organism had been isolated from an individual, all successive bleedings were positive. The total number of individuals that developed tumors was 56. Organisms were isolated from 52 of the 56 tumor tissues. Forty-nine of the mice that became tumor-bearing yielded organisms from blood at the time of death, and, except that the number of positives was greater, these included the same individuals that had been positive at earlier bleedings. In addition, three mice had positive bloods prior to death from other causes. Failure to isolate acid-fast organisms from either tumor or blood occurred in only two tumor-bearing individuals. It is possible that the greater yield of organisms from blood at time of sacrifice may have been the result of experimental conditions, i.e. at exitus blood was withdrawn from the heart rather than from the jugular vein, thus providing a considerably larger sample.

Incidence of Organisms in Blood of Randombred and Inbred Strains. The incidence of organisms in blood of male mice of the ICR/Ha strain appears in the first part of Table 2. In the first instance, 19 of these individuals developed neoplasms: 12 primary lung tumors, 6 lymphosarcomas, and 1 leukemia. Blood cultures yielded organisms from only 16 of them. When tests of male mice were repeated at two-year intervals, only 12 of 100 bloods were positive in one series, 14 out of 100 in the other. In the later tests, blood was withdrawn only once, at 12 months of age, from sacrificed animals which were not examined for the presence of tumors.

It would appear from the experiments on this particular strain of randombred mice that the percentage of bloods positive for acid-fast organisms approximates the incidence of tumor, since...
in females, 49/56 were positive after presence of tumors was demonstrable; whereas, in the males, whose tumor incidence is only 18–20%, study of 300 bloods revealed the presence of the acid-fast organism in an average of only 14% of the individuals. In all cases in which neoplasms developed, acid-fast organisms could be isolated from a greater percentage of actual tumor tissues than from the bloods of the corresponding hosts.

Since the greatest percentage of positive bloods appeared to be demonstrable in the 9- to 12-month period, and since the task of repeated sampling is an enormous one, we cultured mice of the C3H/HeNicer, C58, and C57BL/6JNicer strains only once, from heart blood, at approximately one year of age. In some groups two-month-old mice were sampled for comparison.

The percentage of bloods positive from the different samples was remarkably constant, and the figures given in Table 2 represent averages of the samples at different intervals, as compared to the expected tumor incidence established either in our laboratory or in the Roscoe B. Jackson Memorial Laboratory (See footnote to Table 2). As before, the organism could be isolated from a high percentage of mice of high tumor incidence, and from corresponding hosts. As before, the organism could be isolated from the bloods of the corresponding hosts.

Similarly in C58 mice with an incidence of lymphatic leukemia in excess of 95%, both males and females showed a high percentage of positive bloods prior to the onset of leukemia in the 9- to 12-month period (76% in males and 78% in females). In the case of C57BL/6JNicer strain females the percentage of positive bloods was almost identical (30%) with the expected tumor incidence (31.6%) reported by the Roscoe B. Jackson Memorial Institute. The males of this strain showed the only exception to our general finding, i.e., 21% of the bloods of mature males were positive, but the tumor incidence reported by the Jackson Laboratory is only 16%. This strain of mice appears to be resistant to experimental infections with Corynebacterium kutscheri, judging by the work of Pierce-Chase et al. (18) to be discussed later in this paper, though activation of a pre-existing latent infection could be evoked by administration of cortisone.

**Effect of Cortisone on Experimental and Latent Infection.** In our own laboratory (7) similar lethal infections were induced when one of the organisms isolated from a mouse tumor was injected into ICR/albino mice five weeks of age. Prior to infection, each mouse received intramuscularly Hydrocortone acetate Merck (0.2 ml/dose of a 1:1 saline dilution) daily for five days preceding infection (total dosage 12.5 mg). Eight of ten mice were dead on the sixth day following infection and the remaining mice died on the seventh day.

In order to test whether administration of cortisone would reveal an increased percentage of latent organisms in our strains, we repeated our blood tests with two of the inbred strains as shown in Table 3. Cortisone was administered subcutaneously (0.4 ml/mouse), and the mice were sacrificed ten days later for heart blood culture. Liver and kidney were also tested in these same mice. The difference in percentage of specimens positive for the

### TABLE 2

**Incidence of Pleomorphic Acid-fast Organisms in Bloods of Several Mouse Strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of mice</th>
<th>Sex</th>
<th>Percentage of bloods positive at death or sacrifice</th>
<th>Tumor incidence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICR/Ha</td>
<td>100</td>
<td>F</td>
<td>49</td>
<td>56%: 34 mammary tumors; 12 primary lung tumors; 8 lymphosarcomas; 1 leukemia</td>
</tr>
<tr>
<td>ICR/Ha</td>
<td>100</td>
<td>M</td>
<td>10</td>
<td>19%: 12 primary lung tumors; 6 lymphosarcomas 1 leukemia</td>
</tr>
<tr>
<td>ICR/Ha</td>
<td>100</td>
<td>M</td>
<td>12 av. 14%</td>
<td>95%: lymphatic leukemia</td>
</tr>
<tr>
<td>C58</td>
<td>50</td>
<td>F</td>
<td>78</td>
<td>95%: lymphatic leukemia</td>
</tr>
<tr>
<td>C58</td>
<td>50</td>
<td>M</td>
<td>76</td>
<td>95%: lymphatic leukemia</td>
</tr>
<tr>
<td>C3H/HeNicer</td>
<td>50</td>
<td>M</td>
<td>30</td>
<td>34%: 8 primary lung tumors; 3% skin carcinomas; 21% hepatomas; 2% lymphatic leukemias</td>
</tr>
<tr>
<td>C3H/HeNicer</td>
<td>50</td>
<td>F</td>
<td>81</td>
<td>95%: mammary carcinomas, plus primary and metastatic tumors of the lung</td>
</tr>
<tr>
<td>C57BL/6JNicer</td>
<td>50</td>
<td>M</td>
<td>21</td>
<td>16%: 1.5 primary lung tumors; 3% skin tumors; 3.5% hepatomas; 3% leukemias; 5% reticulum cell sarcomas</td>
</tr>
<tr>
<td>C57BL/6JNicer</td>
<td>50</td>
<td>F</td>
<td>30</td>
<td>31.6%: 11% skin tumors; 6% lymphatic leukemias; 9% reticulum cell sarcomas; 9% hepatomas; 1.6% mammary and lung tumors and an occasional ovarian tumor</td>
</tr>
</tbody>
</table>

*Unless marked with an asterisk (*), the tumor incidence was established in our own laboratories; the other figures are taken from a mimeographed report of the Roscoe B. Jackson Memorial Laboratory on mice of which our current counterparts are presumably substrains. Their percentages were based on the study of 350 C3H females and 184 C3H males, 379 C57BL/6J females and 251 C57BL/6J males.
organism in cortisone-treated and untreated mice is insignificant, with the possible exception of the blood of C57BL/6J icr mice, both young and old, where the values are increased 6% and 9%, respectively, by cortisone administration.

The effect of cortisone in smaller doses upon course of infection with some of the organisms isolated from tumors was tested on ICR/Ha males at two different ages, 2 months and 12 months, respectively. Strains S180 from a mouse sarcoma and T6, a similar organism isolated from a sarcoma of the rat, were injected subcutaneously and intraperitoneally into mice that had received three doses each of 0.2 ml of Hydrocortone acetate (total dose, 7.5 mg). Two groups of controls were maintained: (a) mice treated with Hydrocortone acetate in the same amount, but without infection and (b) untreated controls of the same age. Sixteen mice were included in each group. The results appear in Table 4. When mice were infected at one year of age without cortisone there was a mean survival time of 6—8 months; those infected at two months of age survived 4.5—7.0 months. The shortest survival (two months post-treatment) was noted in cortisone-treated mice infected subcutaneously with the S180 organism. Mice that were injected with cortisone but not infected lived 8.5 months, and uninfected control mice, 14 months. No changes in tumor incidence were noted in the treated groups (possibly because, in general, death occurred in less than one year), but abscesses were found in liver, lung, and kidney when both organisms and cortisone had been administered. These findings are similar to those reported by Fauve et al. (13) when mice were treated with cortisone and infected with C. butchers. Failure to elicit the extreme disease changes noted by these authors may have been due to the difference in dosage.

When this entire series was repeated with female mice of the same strain, no decrease in survival time or increase in pathogenic change was noted, with the exception of a group treated with

### TABLE 3

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Treatment</th>
<th>Age of mice</th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 months</td>
<td>9-12 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. of mice</td>
<td>Blood (%)</td>
<td>Liver (%)</td>
<td>Kidney (%)</td>
<td>No. of mice</td>
</tr>
<tr>
<td>C3H/HeN1cr</td>
<td>M</td>
<td>Cortisone</td>
<td>50</td>
<td>26</td>
<td>33</td>
<td>44</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>Cortisone</td>
<td>50</td>
<td>26</td>
<td>45</td>
<td>46</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>Cortisone</td>
<td>50</td>
<td>44</td>
<td>35</td>
<td>43</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>Cortisone</td>
<td>50</td>
<td>45</td>
<td>33</td>
<td>45</td>
<td>50</td>
</tr>
<tr>
<td>C57BL/6JN1cr</td>
<td>M</td>
<td>Cortisone</td>
<td>50</td>
<td>20</td>
<td>33</td>
<td>37</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>Cortisone</td>
<td>50</td>
<td>26</td>
<td>30</td>
<td>38</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>Cortisone</td>
<td>50</td>
<td>30</td>
<td>35</td>
<td>39</td>
<td>50</td>
</tr>
</tbody>
</table>

### TABLE 4

<table>
<thead>
<tr>
<th>No. of mice</th>
<th>Organism</th>
<th>Source</th>
<th>Route of injection</th>
<th>Cortisone</th>
<th>Age at infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 months</td>
</tr>
<tr>
<td></td>
<td>Strain S180</td>
<td>Mouse Sarcoma 180</td>
<td>i.p.</td>
<td></td>
<td>7.0</td>
</tr>
<tr>
<td>16</td>
<td>Strain S180</td>
<td>Mouse Sarcoma 180</td>
<td>s.c.</td>
<td></td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>Strain T6</td>
<td>Rat Sarcoma 6</td>
<td>i.p.</td>
<td></td>
<td>6.7</td>
</tr>
<tr>
<td>16</td>
<td>Strain T6</td>
<td>Rat Sarcoma 6</td>
<td>s.c.</td>
<td></td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td></td>
<td>s.c.</td>
<td>0.2 ml, 3 doses (7.5 mg)</td>
<td>8.5</td>
</tr>
<tr>
<td>16</td>
<td>Strain S180</td>
<td>Mouse Sarcoma 180</td>
<td>i.p.</td>
<td>0.2 ml, 3 doses (7.5 mg)</td>
<td>8.0</td>
</tr>
<tr>
<td>16</td>
<td>Strain S180</td>
<td>Mouse Sarcoma 180</td>
<td>s.c.</td>
<td>0.2 ml, 3 doses (7.5 mg)</td>
<td>2.0</td>
</tr>
<tr>
<td>16</td>
<td>Strain T6</td>
<td>Rat Sarcoma 6</td>
<td>i.p.</td>
<td>0.2 ml, 3 doses (7.5 mg)</td>
<td>4.0</td>
</tr>
<tr>
<td>16</td>
<td>Strain T6</td>
<td>Rat Sarcoma 6</td>
<td>s.c.</td>
<td>0.2 ml, 3 doses (7.5 mg)</td>
<td>4.5</td>
</tr>
<tr>
<td>16</td>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
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</table>
Sex Infected with

Development of Survival and tumor production at different ages

Types No. % Prior to 18 mo. No. dead No. tumors % Surviving No. tumors %

M 50 Sarcoma 180 <72 hr
Adenocarcinomas, lung 13 32 7/32 20 18 15/18 83
Osteosarcoma of jaw 1 1
Spindle cell sarcoma 1
Reticuloendothelial sarcomas 5 1
Lymphatic leukemia 1
Papillary squamous cell carcinoma 1 22 44
Total 23

M 50 (Placebo) Controls <72 hr
Adenocarcinomas, lung 7 3/15 20 35 7/35 20
Reticuloendothelial sarcomas Total 3 10 20

F 30 Sarcoma 180 <72 hr
Mammary adenocarcinomas (metastases to lung, 6) 19 17 13/17 77 13 10/13 77
(metastases to lung, 6)
Anaplastic carcinoma, breast 3
Reticuloendothelial sarcomas Total 23 76

F 30 (Placebo) Controls <72 hr
Mammary adenocarcinomas (metastases to lung, 2) 7 5 2/5 40 25 11/25 44
(metastases to lung, 2)
Anaplastic carcinoma, breast 1
Primary lung carcinoma 1
Uterine sarcoma 1
Uterine carcinoma Total 13 46
infection. Loss of hair, abscess formation, disorientation symptoms (such as twirling when held by the tail or circling movements), and blindness were observed. According to Pierce-Chase et al. (18) C57BL/6J mice of the strain studied at Rockefeller Institute are much more resistant to corynebacterial infections than are Swiss mice, and though renal abscesses occurred during early stages of infection of C57BL/6J mice with C. kutscheri there was no residual pathology. If organisms of this nature are in any way related to neoplastic change, these observations may have some bearing on the low tumor incidence of C57BL mouse strains.

Current studies, not yet complete, indicate that pathogenic changes may be induced by injecting the organism into the footpad of mice, as was done by Shepard (20) with nasal washings from leprosy patients. Following such injections there appears to be an unusual number of mice with lung changes (abscesses, lymphocytic infiltration, and tumors) as well as bone marrow changes resembling myelogenous leukemia. Unfortunately, this was not suspected at the time of sacrifice and blood studies were not made. Such leukemias have not heretofore been observed in this strain and the experiment should be repeated and supplemented by a study of peripheral blood.

The greatest difference in survival time of experimental mice and of pathologic changes induced in them as compared to untreated mice has occurred when the organisms were administered either intraperitoneally or via footpad, which suggests that dissemination of the organism occurs by way of the lymphatic system.

DISCUSSION

The experiments reported here indicate that several strains of mice harbor pleomorphic, intermittently acid-fast organisms and that the incidence of such infections parallels rather closely the incidence of tumors in these same strains; furthermore, when ICR/albino mice are infected with these organisms under experimental conditions, tumor production is enhanced, particularly in those mice that survive more than eighteen months postinfection.

The exact taxonomic position of the organisms has not been determined. Wuerthele-Caspe (22) gave the designation Mycobacterium tumefaciens to similar organisms isolated by her from animal and human tumors, and Seibert (9) established serologically that some of our mouse and rat isolates, as well as an organism from human leukemia, were antigenically related to certain mycobacteria, notably the atypical "Battey" strain. However, though the organisms are more closely related to one another antigenically than to any of the other strains tested, they are not identical. Because of their fleeting acid-fastness and the tendency to revert quickly to the cocobacillary form, the possibility that they may represent an unclassified organism intermediate between Mycobacterium and Corynebacterium was previously discussed (8).

A latent infection of mice by Corynebacterium kutscheri, which likewise shows partial acid fastness, was reported by Pierce-Chase, et al. (18). The presence of the organism was more readily revealed, either in microscopic preparations of various organs, or through isolation technics, when cortisone (10 mg/mouse) was administered subeutaneously. Death occurred in all strains at this dose level from corynebacterial pseudotuberculosis as the result of activation of latent organisms. As was the case with our tumor isolates (10) intracellular organisms could be more readily stained in tissues that had been incubated than in freshly excised specimens. In view of the findings of these authors, further serologic tests are being undertaken by Dr. Seibert to determine whether or not any or all of the tumor isolates are related to C. kutscheri.

Whatever the significance of these organisms with respect to the neoplastic process may be, it would seem that their presence in various strains of mice and the production of neoplasia go hand in hand. It could of course be argued that the same factors that render the host differentially susceptible to infection with these organisms also predispose to the development of neoplasia, or that the presence of the organisms as latent infections may lower the immunity, thus rendering the host more susceptible to some carcinogenic agent such as an oncogenic virus. In this connection it is of interest that Mankiewicz (14) has already demonstrated that the T6 and S180 strains described in some of these experiments, as well as partially acid-fast bacteria isolated from human lung cancer, from rabbit neoplasms, and from Rous sarcoma of the chick are carrying phages which lyse Mycobacterium smegmatis. On the basis of phage typing and other considerations, Mankiewicz is inclined to believe that these particular isolates are more closely related to Corynebacterium than to Mycobacterium.

The experiments reported in this paper show that the percentage of tumor production can be increased when mice are infected under experimental conditions with some of these phage-carrying bacteria. Many questions arise: Is it possible that the phages are oncogenic agents, for which the bacterium acts only as a vector or as a sensitizing agent? Are the phages merely fellow travelers, without significance in the neoplastic process? Or, are both bacterium and bacteriophage concerned in pathogenic change as in the case of Corynebacterium diptheriae which becomes toxigenic only in the presence of a bacteriophage (4). On the other hand, Brieger and Glauert (5) found that filtrates of Mycobacterium avium that contained small bodies barely visible in the light microscope (L forms of the bacterium?) caused pathogenic changes in guinea pigs, whereas particles in ultracentrifuge, demonstrable only by electron microscopy, caused no such changes. They postulate that either the minute forms are passengers having nothing to do with infection, or that they are ultrafine stages in the life cycle of the bacterium which must form larger aggregates in order to initiate pathogenicity.

In 1945 Alexander-Jackson (1) described minute and aberrant symplastic "zooglenal" forms of Mycobacterium tuberculosis which she assumed to be filterable or "L" forms and definite corroboration of this assumption was provided by Mattman et al. (16) for M. tuberculosis and Mycobacterium phlei. One of our bacterial isolates and others cultured by Seibert (19) from human cancerous lesions, when passed through 0.1-µ filters, regrew on artificial medium. The two strains, S180 and T6, were also filtered through 0.1-µ Millipore filters in our laboratory and the filtrates were inoculated into Waymouth's synthetic medium (21) devoid of or ration of this assumption was provided by Mattrnan et al. (16) for M. tuberculosis and Mycobacterium phlei. One of our bacterial isolates and others cultured by Seibert (19) from human cancerous lesions, when passed through 0.1-µ filters, regrew on artificial medium. The two strains, S180 and T6, were also filtered through 0.1-µ Millipore filters in our laboratory and the filtrates were inoculated into Waymouth's synthetic medium (21) devoid of or...
nogeneates were cultured from mice with latent corynebacterial infections.

Recently, Alexander-Jackson (3) has isolated consistently on cell-free solid medium a pleomorphic intermittently acid-fast bacillus from Rous sarcoma tissue and from partially purified Rous virus. Chickens immunized by her with this killed isolate were protected against challenge with live Rous virus, whereas more than half of the controls succumbed to Rous disease. She concluded from this and other experiments that Rous virus may represent virus-sized units which appear to be the transitional L-forms of this mycobacterium-like organism.

In view of the constant association with neoplastic tissues of pleomorphic, intermittently acid-fast bacteria which share antigens in common with Mycobacterium and Corynebacterium (15), and the fact that the incidence of neoplasia in animals can be experimentally altered by infecting with them, it is clear that a study of their role in initiation of disease should be more intensively studied. The further fact that some of the strains are both filterable and phage carrying could afford an explanation of the apparent lack of consistency between the points of view of those who suspect a bacterial and those who suspect a viral induction of neoplasia.

REFERENCES