Acquisition of Heightened Resistance and Susceptibility to Spontaneous Mouse Mammary Carcinomas in the Original Host*

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SUMMARY

Experiments were designed to answer the following question: Can mice acquire heightened reactivity of an immunological nature to their own spontaneous mammary neoplasms?

Twenty-five spontaneous mammary carcinomas, arising from transplanted C3H/1/CrGl nodule outgrowth, were removed and subsequently retransplanted into their original C3H/CrGl/2 hosts. At the same time, the tumors were transplanted into series of isologous, previously tumor-free control animals. Some of the original tumor hosts and some of the isologous controls were treated with agents capable of increasing immunological responsiveness and with killed tumor cell preparations. The growth of the transplanted tumors in the original hosts and in their respective isologous controls was compared. The tumors grew in all the isologous control animals. Tumor growth was either appreciably retarded or appreciably facilitated in a majority of the original tumor-bearing animals, relative to their isologous controls. Several of the original hosts entirely failed to support growth of the neoplastic transplants, and in more than half of the remaining experiments the original animal ranked either first or last in order of resistance to the transplants. The lymph nodes draining the sites of tumor implantation showed exaggerated lymphoid hyperplasia in original animals exhibiting either resistance or enhancement.

Similar experiments with spontaneous mammary carcinomas appearing in old multiparous female mice also indicated the development of heightened tumor resistance in the autochthonous host.

The results of the present study strengthen the biological generality of the tumor resistance phenomena described by others working with experimentally induced neoplasms and with certain virus-initiated tumors.

The natural history of some tumors in man and in animals suggests that the host can play an active role in the course of at least some neoplastic processes and that this role may be of an immunological nature (15).

Experimental support for the operation of immunological defenses against neoplastic cells comes largely from the findings that tumors induced in the laboratory with chemical carcinogens (7, 18, 22, 30, 32, 34), arising at the site of implantation of cellophane films (17) or initiated by the polyoma or Gross viruses (10, 11, 16, 35), can evoke a state of resistance in their hosts against retransplantation following surgical removal or regression of the first tumor. Many of these experiments have been performed in systems in which heterozygosity of isoantigenic characteristics between tumor-donor and host had been eliminated or so minimized as to rule out this artifact as an explanation of the results.

Proof that natural, spontaneously arising tumors possess new antigens or can elicit acquired resistance is still sparse. The formation of new antigens in spontaneous mouse leukemias has been shown by Gorer and his co-workers (9). Hirsch et al. have reported a "small but significant" increase in the survival time of mice following removal of a first implant of a newly arisen mammary adenocarcinoma and the immediate introduction of a second, challenge implant (13). Koldovsky has claimed considerable success in eliciting immunity to spontaneous mammary carcinomas in mice of "controlled antigenic homogeneity" (19–21). Acquired resistance against several new spontaneous mammary tumors in isologous mice following a previous experience with that tumor has recently been reported by Morton (24). Evidence for the existence of tumor antigens also comes from the re-
cent demonstration by Rubin of an immunological basis for noninfective Rous sarcomas, but it is pointed out by
that author that "there is nothing in the biology of RSV infection which precludes the possibility that . . . the new
transplantation antigen in the tumor cells is viral antigen" (33). Immunity to Rous sarcomas may therefore be more a phenomenon of anti-viral resistance than of resistance to host cell components specific to the neoplastic
condition. The results of other studies on immunity to spontaneous tumors, many of which were based on sero-
logical and immunochemical approaches (38), and the difficulties of their interpretation, have been discussed critically in a number of recent reviews (8, 12, 31).

The purpose of the present study was a systematic investigation of the occurrence and nature of the resistance
which may be acquired by animals against their own spontaneous tumors, and against tumors newly arisen in
other animals of proved isogenicity. The experimental model of spontaneous mammary carcinomas in the mouse
was chosen because of the considerable information already available on the biology of these tumors and because of
their common occurrence in a number of highly inbred strains. The present communication reports the results of experiments which indicate the acquisition of heightened resistance or susceptibility to spontaneous mammary
carcinomas by hosts in which they developed from implanted hyperplastic alveolar nodules or in which they arose from the autochthonous mammary tissue.

MATERIALS AND METHODS

**Experimental design.**—The design of the study was to excise spontaneously arising mammary carcinomas from female mice of inbred strains, to preserve the tumors by storage at −79° C. or by single passage in several isologous animals (designated "tumor banks"), and to return them after intervals ranging from 30 minutes to 2 months into their original hosts, (hereafter also referred to as the "original" animal) and, simultaneously, to transplant them into groups of isologous controls of the same age and sex. The number of control animals in each experiment varied, depending on the availability from our breeding colony of isologous females of appropriate age.

In the interval between initial tumor removal and transplantation back to the original and into the isologous animals, some of the original tumor hosts, and, simultaneously, some of their isologous controls, were treated with one of several "immunological activators" and/or with preparations of cells of that tumor killed by repeated freezing and thawing ("tumor vaccines"). Other isologous control animals received simultaneous treatment with "normal-tissue vaccines" with or without an immunological activator, or with placebo.

The immunological activators were administered to both the original and to the isologous animals at the time of tumor removal from the original hosts and, again, at the time of each treatment of the animals with the tumor or normal-tissue vaccines, when these were employed. The tissue vaccines were given in one or two injections administered between the 2d and 6th weeks following removal of the tumors from the original animals; when two vaccinations were given, one or two weeks elapsed between treatments.

This experimental design permits a comparison of the behavior of transplants of a given tumor in the single
original host and in the group of isologous animals receiving that tumor at the same time. Each original host and its group of isologous control animals constitute a distinct experiment. Although this design poses considerable difficulties to the analysis of the results, it appears to be the most rigorous approach to the question of resistance to spontaneous tumors. Only by testing for such resistance in the original tumor-bearer can the possible artifacts of a residual heterozygosity of isoantigenic characteristics between tumor donors and hosts, or of physiological idiosyncrasies peculiar to the original tumor hosts, be ruled out with certainty.

**Measurement of response to tumor transplants.**—The rate of growth of the transplanted tumors was followed
by thrice-weekly measurements of the two diameters evenly bisecting the palpable growths at right angles to each other. The approximate tumor volumes were calculated from these measurements according to a method developed by Mr. M. Attia of this laboratory (1). In many of the experiments the original animals and their isologous controls were sacrificed at varying times after tumor implantation, and the tumors and draining lymph nodes (axillary and brachial) were removed, weighed, and examined histologically. In other experiments, in which all animals showed progressive tumor development, the animals were permitted to live to term, and their survival times after tumor implantation were compared.

**Variation in experimental design.**—The initial plan of the study was to deal with mammary carcinomas as these were detected upon routine inspection of multiparous breeding females. This experimental design had to be abandoned when it was found that well over half of the first 30 animals subjected to tumor removal rapidly developed one or more new mammary carcinomas in the remaining mammary glands, making a later retransplantation of the original tumor impossible. Moreover, the advanced age of the animals at the time of excision of the first tumor (9-15 months) made them poor surgical risks, and a number of the mice that did not develop new tumors before retransplantation of the first tumor succumbed during the retransplantation operation.

The design of the experiment was then changed to obtain a different source of primary hosts. Transplants of an outgrowth of a precancerous hyperplastic alveolar nodule (HAN) derived from a C3H/f/Crgl female and possessing a high tumor-producing capability, were placed into two glandfree inguinal mammary fat pads of 3-week-old C3H/2/Crgl females (3, 5). Tumors arose spontaneously in one or both of the implants of nodule outgrowth 12-50 weeks later. They were removed after reaching a diameter of 5-12 mm. When more than one tumor developed simultaneously in an animal only one was preserved for further study. When the tumor(s)

1 These tumors appeared to be bona fide new cancers, rather than metastatic foci of the first growth. These observations, and their possible immunological implications, are currently under study.
arose in only one of the nodule implants, the contralateral inguinal fat pad containing the outgrowth was also removed, to prevent later development of another tumor at that site. This was found to be a common occurrence in early phases of this study, when the contralateral fat pad was not removed at the time of primary tumor excision. The subsequent steps were the same as in the case of tumors arising in multiparous breeding females. In none of 75 animals so far subjected to surgical removal of tumors developing from the HAN transplants did new tumors arise subsequently in other mammary glands. In a few instances secondary tumors did develop in the inguinal region, apparently the result of failure to remove completely the implants of nodule outgrowth or the already developed tumor. These secondary tumors were removed and discarded, and the animal was retained in the experiment.

The young age of the C3H/2 tumor hosts at the time of tumor removal and retransplantation made them safer surgical subjects. Less than 10 per cent were lost to the operative manipulations. A further advantage of the revised experimental design lies in the fact that neither the original HAN tissue donor (C3H/f) nor the recipients (C3H/2) carry the biologically active mouse mammary tumor virus (MTV) (25, 26). This reduces the possibility that any immunity detected would be directed at a viral component in or on the tumor cells. (This possibility is remote in any case, in the light of several aspects of mouse mammary tumor carcinogenesis, and especially in view of the fact that infection with the mammary tumor virus does not cause mice to become refractory to later isografts of virus-induced mammary tumors.)

Because of these advantages to the use of spontaneous mammary tumors arising in young, MTV-free animals from HAN implants, most of the experiments in this study were carried out with such cancers.

Experimental animals.—Mice of the C3H/Crl, C3H/Crl/2, C3H/f/Crl, A/Crl, RIII/Crl, and DBA/Crl strains were employed. The isogenicity of the animals within each strain was proved by the permanent survival of first and second reciprocal skin, mammary, pituitary, and other organ isografts performed periodically on randomly selected pairs. No histoincompatibility barriers detectable by this method were found between the C3H, C3H/f, and C3H/2 sublines, even in animals treated with tubercle bacillus fractions (36).

Tumors.—The carcinomatous nature of all tumors studied was confirmed by histological examination. Transplantation of tumors was always by means of approximately equal-sized fragments of tumor tissue from which the surrounding normal tissue and excess connective tissue had been removed. The size of the fragments aimed for was 0.05 cu. mm. Although the fragments were cut under a dissecting microscope and carefully selected for appropriate size, it was not possible to assure uniformity of dimensions in every instance, and the actual size of the tumor implants may have varied within, maximally, a threefold range in both directions. As determined by Mr. Attia of this laboratory, a mass of 0.05 cu. mm. of spontaneous mammary carcinoma in C3H and A mice contains approximately 6.5 x 10^4 tumor cells. It must be noted that only a fraction of the total number of cells in a fragment of tumor tissue is likely to survive the transplantation procedure, however.

The tumor pieces used for simultaneous transplantation to an original tumor-bearing animal and to its accompanying group of isologous controls were picked at random. The order in which the animals received the implants was also random. Any effect which the unavoidable variations in the size of the tumor implants might exert on the time of appearance and subsequent development of tumors would thus also be distributed randomly among original and isologous animals throughout the study. It has already been shown, moreover, that variation in tumor size of the order here encountered does not influence subsequent tumor growth patterns (1). It seemed safe to assume, therefore, that marked differences which might be observed in the behavior of transplants of the same tumor in animals of different tumor prehistories could not be ascribed to the artifact of variation in implant size.

Transplantation was always into both thoracic (No. 3) mammary glands of the experimental animals, and into the inguinal (No. 4) glands of the tumor banks. A skin incision was made under nembutal anesthesia (28), and the tumor implants were placed into the mammary fat by means of a fine forceps. The tumor fragments were cut immediately before use and were kept moist by flooding with Morgan #199 tissue culture fluid.

Tumors preserved by freezing were sealed in glass ampoules and stored on dry ice. For retransplantation, they were thawed rapidly and implanted immediately into the host animals.

Tumor and normal tissue "vaccines".—Killing of tumors for the purpose of preparing tumor cell vaccines was by alternate freezing and thawing (2 or 3 times) of suspensions of tumor cells obtained by forcing tumor fragments through a cytosieve. In no instance did such preparations initiate new growth when placed into isologous animals. The cell suspensions were prepared in Morgan #199 solution and were used as vaccines directly or as emulsions in complete Freund's adjuvant.

Preparations of normal tissue, consisting of normal mammary gland, liver, spleen, and striated muscle, were similarly made and killed by freezing and thawing. The animals from which the normal tissues were derived were always the same ones which served as sources of the neoplastic tissues for the tumor cell vaccines. The quantities, excipients, and scheduling were the same in every experiment for the tumor and the normal-tissue vaccines. The initial vaccination was of 0.5 cc., given intraperitoneally; when a second vaccination was given, it was administered subcutaneously in each axillary area (the site of eventual tumor retransplantation) in 0.25 cc. When Freund's adjuvant was used, it was usually employed only for the first intraperitoneal (I.P.) injection; the second, subcutaneous administration was with aqueous cell suspensions in most instances. The quantity of killed tumor cells given at each vaccination varied from

1 That the normal tissues were free of metastatic foci of the tumor was confirmed by macroscopic and histological examination.
proved to be the most resistant animals among their total to their degree of resistance to the tumor transplants. The within each experiment were ranked successively according This difference became evident when all the animals the other 22 experiments, the original hosts did support in every isologous control animal. There was a marked difference in the growth of the tumor transplants in the hosts and in their respective isologous controls, as meas

Injection of the activators was always intraperitoneally, in 0.25-0.5 cc. The following quantities of the several substances were given at each time of administration: Tubercle bacillus fraction, 0.25-0.50 mg.; DNA Digest, 1.25-2.5 mg.; Nowotny endotoxin preparations, 0.5-1.0 mg.

RESULTS

Differences in tumor growth in original and isologous animals, with tumors arising in young C3H/2 females from HAN implants.—The results of the first 25 experiments of tumor removal and retransplantation are here reported. The experimental conditions in each case and the relative capacity of the tumor implants to grow in the original hosts and in their respective isologous controls, as measured at the termination of the experiments, are shown in Table 1.

At least one, and usually both, of the tumor grafts grew in every isologous control animal. There was a marked difference in the growth of the tumor transplants in the isologous and in the original animals in a large number of the experiments. In three instances (Exps. 1, 2, and 4), neither of the tumor grafts grew in the original animals, whereas both grafts grew progressively in all their controls (twelve, nine, and six animals, respectively). In the other 22 experiments, the original hosts did support tumor growth, but in many instances they did so to a different extent than did their corresponding controls. This difference became evident when all the animals within each experiment were ranked successively according to their degree of resistance to the tumor transplants. The original hosts in four experiments (Exps. 3, 5, 6, and 8) proved to be the most resistant animals among their total groups of five to thirteen mice; the original host in Exp. 7 ranked first among a group of four animals. Another original host (No. 9) placed second in order of resistance among a group of eleven animals, and the original tumor-bearing animal in Exp. 10 placed third among thirteen. In contrast, the original hosts in seven experiments (11–17) proved to be the least resistant, or fell among the two or three least refractory animals in their experimental groups—i.e., appeared to exhibit an enhanced tolerance of their tumor grafts. In a third group of experiments (18–25), the behavior of the original animals was either indifferent—i.e., their position fell toward the median of their groups (viz., 21), or remained obscure because the number of control animals and the differences between the behavior of the tumors in the original and in the isologous hosts seemed too small to warrant even a tentative evaluation. It is possible that in some of these experiments the behavior of the original host would have indicated resistance or enhanced tumor tolerance had a larger number of control animals been available or had a smaller “challenge” inoculum of tumor cells been employed (27).

In a majority of the 25 experiments the original animals thus ranked at either extreme of the spectrum of resistance. This distribution of the original animals appears skewed when compared with the even distribution expected had their previous tumor experience, together with the subsequent treatment(s), been without effect. The distribution of the original animals among their respective isologous controls as a function of their relative tumor resistance is summarized in the histogram shown in Chart 1.

The relative behavior of the original tumor hosts toward their tumor retransplants was also ascertained by a comparison of the growth rates of the transplanted tumors in these animals and in their isologous controls. In every instance where the original animal appeared to show heightened tumor resistance or susceptibility on the basis of the termination data, the same evaluation was suggested by the relative growth patterns. This is exemplified in Chart 2, which shows the increases with time in the calculated volumes of the tumor implants in an original animal (14) and in its isologous controls.

In a number of cases where the behavior of the original host via-à-vis the tumor transplants was not clearly different from that of its controls on the basis of the termination data, an analysis of the growth of the tumors did suggest a definite difference. This is illustrated by Exp. 23. The original animal in this experiment ranked second of the total group of five in survival time (Table 1), and its longevity did not greatly exceed that of a second animal of the control group. However, the size of the tumors growing in the original host was consistently less throughout the experiment than those of the control animals (Chart 3).

Differences in tumor growth among the differently treated isologous control animals.—Because of limitations in their availability, the number of control animals assigned to each original host never exceeded 12, and hence the differently treated sub-groups were limited to two to four animals. These numbers are much too small to permit a significant comparison, especially in view of the frequently large degree of variation in the onset and subsequent rapidity of growth of first-generation transplants derived...
TABLE 1

RELATIVE CAPACITY OF TUMOR IMPLANTS TO GROW IN THE ORIGINAL HOSTS AND IN THEIR RESPECTIVE ISLOGOUS CONTROL ANIMALS

<table>
<thead>
<tr>
<th>No. of experiment</th>
<th>Removal-retransplantation interval (days)</th>
<th>No. isologous controls</th>
<th>Treatment of original host</th>
<th>Retransplantaion termination interval (days)</th>
<th>Criterion of growth of tumor implants</th>
<th>Result in original host</th>
<th>Range of results among controls</th>
<th>Ranking of original animal among its controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67</td>
<td>12</td>
<td>R; 2 × TV-A</td>
<td>34</td>
<td>Weight</td>
<td>0 mg.</td>
<td>10-740 mg.</td>
<td>1st of 13</td>
</tr>
<tr>
<td>2</td>
<td>63</td>
<td>9</td>
<td>DNA; TV-A</td>
<td>21</td>
<td>&quot;</td>
<td>0 &quot;</td>
<td>0-5 mg.</td>
<td>1st of 10</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>6</td>
<td>E-5; 2 × TV-A</td>
<td>29</td>
<td>&quot;</td>
<td>5 &quot;</td>
<td>20-200 &quot;</td>
<td>1st of 7</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>6</td>
<td>R; 2 × TV-A</td>
<td>13</td>
<td>&quot;</td>
<td>0 &quot;</td>
<td>5-20 &quot;</td>
<td>1st of 7</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>4</td>
<td>None</td>
<td>—#</td>
<td>Volume</td>
<td>2200 μm.</td>
<td>2500-4300 μm.</td>
<td>1st of 5</td>
</tr>
<tr>
<td>6</td>
<td>51</td>
<td>4</td>
<td>E-2</td>
<td>—#</td>
<td>Survival</td>
<td>83 days</td>
<td>59-72 days</td>
<td>1st of 5</td>
</tr>
<tr>
<td>7</td>
<td>37</td>
<td>3</td>
<td>None</td>
<td>—#</td>
<td>&quot;</td>
<td>148 &quot;</td>
<td>80-133 &quot;</td>
<td>1st of 4</td>
</tr>
<tr>
<td>8</td>
<td>68</td>
<td>12</td>
<td>DNA; 2 × TV-A</td>
<td>56</td>
<td>Weight</td>
<td>5 mg.</td>
<td>10-1560 mg.</td>
<td>1st of 13</td>
</tr>
<tr>
<td>9</td>
<td>28</td>
<td>10</td>
<td>E-2; TV-A</td>
<td>42</td>
<td>&quot;</td>
<td>240 mg.</td>
<td>140-600 mg.</td>
<td>2nd of 11</td>
</tr>
<tr>
<td>10</td>
<td>56</td>
<td>12</td>
<td>E-2; 2 × TV-A</td>
<td>40</td>
<td>&quot;</td>
<td>230 &quot;</td>
<td>130-660 &quot;</td>
<td>3rd of 13</td>
</tr>
<tr>
<td>11</td>
<td>0†</td>
<td>4</td>
<td>None</td>
<td>—#</td>
<td>Survival</td>
<td>93 days</td>
<td>&gt;100**</td>
<td>5th of 5</td>
</tr>
<tr>
<td>12</td>
<td>0†</td>
<td>4</td>
<td>DNA</td>
<td>25</td>
<td>Weight</td>
<td>620 mg.</td>
<td>100-570 mg.</td>
<td>6th of 5</td>
</tr>
<tr>
<td>13</td>
<td>28</td>
<td>3</td>
<td>E-2</td>
<td>54</td>
<td>&quot;</td>
<td>850 &quot;</td>
<td>60-750 &quot;</td>
<td>4th of 4</td>
</tr>
<tr>
<td>14</td>
<td>37</td>
<td>3</td>
<td>R</td>
<td>85</td>
<td>&quot;</td>
<td>4800 &quot;</td>
<td>600-1570 &quot;</td>
<td>4th of 4</td>
</tr>
<tr>
<td>15</td>
<td>31</td>
<td>9</td>
<td>E-2; TV-A</td>
<td>25</td>
<td>&quot;</td>
<td>3600 &quot;</td>
<td>10-6380 &quot;</td>
<td>9th of 10</td>
</tr>
<tr>
<td>16</td>
<td>64</td>
<td>12</td>
<td>E-5; 2 × TV-A</td>
<td>28</td>
<td>&quot;</td>
<td>480 &quot;</td>
<td>70-1110 &quot;</td>
<td>11th of 13</td>
</tr>
<tr>
<td>17</td>
<td>30</td>
<td>11</td>
<td>E-3; TV</td>
<td>29</td>
<td>&quot;</td>
<td>1000 &quot;</td>
<td>50-1500 &quot;</td>
<td>10th of 12</td>
</tr>
<tr>
<td>18</td>
<td>26</td>
<td>4</td>
<td>None</td>
<td>—#</td>
<td>Volume</td>
<td>3500 μm.</td>
<td>2700-4000 μm.</td>
<td>2nd of 5</td>
</tr>
<tr>
<td>19</td>
<td>0†</td>
<td>4</td>
<td>None</td>
<td>26</td>
<td>Weight</td>
<td>130 mg.</td>
<td>60-210 mg.</td>
<td>4th of 5</td>
</tr>
<tr>
<td>20</td>
<td>0†</td>
<td>5</td>
<td>None</td>
<td>—#</td>
<td>Survival</td>
<td>128 days</td>
<td>94-148 days</td>
<td>2nd of 6</td>
</tr>
<tr>
<td>21</td>
<td>58</td>
<td>10</td>
<td>E-2; TV†</td>
<td>42</td>
<td>Weight</td>
<td>180 mg.</td>
<td>5-430 mg.</td>
<td>7th of 11</td>
</tr>
<tr>
<td>22</td>
<td>26</td>
<td>4</td>
<td>None</td>
<td>—#</td>
<td>Survival</td>
<td>106 days</td>
<td>92-123 days</td>
<td>2nd of 5</td>
</tr>
<tr>
<td>23</td>
<td>0†</td>
<td>4</td>
<td>None</td>
<td>—#</td>
<td>&quot;</td>
<td>78 &quot;</td>
<td>48-113 &quot;</td>
<td>2nd of 5</td>
</tr>
<tr>
<td>24</td>
<td>47</td>
<td>8</td>
<td>E-5; TV-A</td>
<td>28</td>
<td>Weight</td>
<td>160 mg.</td>
<td>20-1130 mg.</td>
<td>3rd of 9</td>
</tr>
<tr>
<td>25</td>
<td>37</td>
<td>2</td>
<td>R</td>
<td>—#</td>
<td>Survival</td>
<td>80 days</td>
<td>61-95 days</td>
<td>2nd of 3</td>
</tr>
</tbody>
</table>

* Treatment of original tumor-bearing animal following tumor removal. Some of the isologous controls received similar treatment simultaneously, whereas others received only partial treatment or placebo.
† Tumor weights are given in mg., and are based on the combined moist weights of both tumor implants in each animal upon termination. Survival time measurements begin with the day of tumor transplantation. Tumor volumes are the combined calculated volumes of the two tumor implants in each animal. (Tumor volumes are given only for the animals in Experiments 5 and 18. These animals were accidentally discarded on the 43rd day after transplantation. An indication of their relative resistance to the tumor grafts was obtained by a comparison of the calculated tumor values, based on measurements of their tumors on the day prior to their loss.)
† The animal in each total experimental group (original host + isologous controls) having the smallest tumor mass at termination, or the animal surviving longest after tumor transplantation in experiments in which the animals were permitted to live to term, is ranked first (= most resistant) in its group.
‡ The following abbreviations are used to designate treatment: R = methanol-insoluble fraction of killed tubercle bacilli (BCG); TV = Killed tumor cell vaccine; A = Freund's adjuvant (without tubercle bacilli); DNA = DNA digest; E-2, E-3, E-5 = detoxified endotoxin preparations.
# Animals permitted to live to term after tumor transplantation.
†† Tumor retransplantation within 30 minutes after tumor removal.
** The four isologous controls in this experiment were accidentally discarded on the 100th post-transplantation day.
†† In this experiment, the tumor cell vaccine was killed by heating (70°C. for 10 minutes) instead of by freezing and thawing.

from a single spontaneous mammary carcinoma (a variability which is also observed when such transplants are placed into different sites of the same isologous mouse, even when these sites are in similar tissue). Nonetheless, some indication of a possible effect of pretreatment of the isologous animals was suggested in those experiments in which the greatest degree of resistance was displayed by the original hosts. Thus, in Exp. 1, the combined weights at termination of the four tumor transplants in the two isologous animals which received tubercle bacillus residue and immunization with a normal-tissue preparation was 1300 mg., as compared with 120 mg. for the tumors in the
two mice receiving the residue and tumor cell vaccine; the eight tumor grafts of the four animals given only placebo weighed 1970 mg., as against 800 mg. for the eight tumors in four animals injected with tumor cell vaccine alone. As another example, the combined tumor grafts of two animals treated with DNA-Digest and normal tissue in Exp. 2 weighed 60 mg.; of the two animals given the DNA-Digest plus tumor vaccine, 10 mg.; of two normal tissue and two tumor tissue animals not given the DNA material, 1460 and 410 mg., respectively; and of a single animal given placebo only, 80 mg.

That pretreatment with killed tumor cells and/or with immunological activators can affect the tumor resistance of isologous mice was further indicated in this study by the following observation: In eleven experiments (1-4, 8-10, 15-17, and 24) the isologous controls received pretreatment with either placebo and/or normal tissue preparations, or with immunological "activators" and/or tumor cell preparations. In five of these experiments (1-4 and 8) the original host ranked most resistant, and the isologous animal placing next in resistance had been pretreated either with immunological activator or with tumor cells and activator. In the other six experiments, the original host was exceeded in tumor resistance or susceptibility by one or two of its isologous controls. In every instance, the isologous animal which showed the greatest tumor resistance or the greatest tumor support had also received previous injections of activator or tumor vaccine, or both.

A detailed report of the effects of immunological activators and of pretreatment with living and killed tumor cells on the growth of spontaneous tumor implants in isologous mice will be presented in succeeding publications.

**Appearance of draining lymph nodes in original and isologous animals at the time of termination.**—In the course of removal of the tumor transplants from groups of original and isologous hosts at the time of termination of the experiments, it was noted that the local lymph nodes (axillary and brachial) frequently appeared to be larger in the original animals. To quantitate this observation the local nodes were removed at termination from all animals in the later phases of this study. They were pooled and weighed, and then prepared for histological examination. However, of the 25 experiments here reported, lymph node studies were carried out only on the local nodes of four (Exps. 1, 8, 10, and 16). The weights of the draining lymph nodes of all animals in these four experiments are shown in Table 2.

It is seen from Table 2 that the weights of the local lymph nodes of the original animals in Exps. 8, 10, and 16, but not of the original animal in Exp. 1, was greater than that of any of the twelve isologous animals in each experiment. As seen from Table 1, the original hosts in Exps. 8 and 10 appeared to exhibit resistance to the tumor retransplantation, whereas the original animal in Exp. 16 supported the tumor better than did most of its controls.

A similar enlargement of the size and weight of the draining lymph nodes of those original animals which displayed either heightened resistance or susceptibility toward their tumor retransplants has been observed consistently in further tumor removal-retransplantation experiments which will be reported in a later communication.

The enlarged nodes of the original animals exhibited a marked hyperplasia of the lymph nodules, involving both germinal centers and reticulum cells. Numerous plasma cells were seen. These changes were also found in some of the isologous animals which had been pretreated with tumor tissue vaccine and/or immunological activators, but they were less marked than those occurring in the original hosts. A more detailed report of the lymphoid reactions of the original animals will be presented at a later date.

**Differences in tumor growth in autochthonous and isologous animals, employing tumors arising spontaneously in multiparous breeding females.**—As has been mentioned above, the original design of this study was to employ tumors arising spontaneously in aging multiparous breeding females. This plan had to be abandoned because of the development of new mammary tumors in many of the animals following excision of the primary cancer. That the original tumor host can develop heightened resistance to tumors arising spontaneously from *the own* mammary tissue (i.e., autochthonous tumors), as well as to tumors which originate from HAN implants, was evident from the fact that six of fifteen multiparous breeders in which
Chart 2.—The curve plotted for the original host shows the combined calculated volumes of its two tumor implants with time. The curve for the isologous controls shows the average combined volumes of the two tumor implants growing in each of the three control animals and the maximum and minimum deviations from the mean values on the 41st day and thereafter.

Chart 3.—The curve plotted for the original host shows the combined calculated volumes of its two tumor implants with time. The curve for the isologous controls shows the average combined volumes of the two tumor implants growing in each of the four control animals and the maximum and minimum deviations from the mean values on the 35th day and thereafter.
new tumors did not appear after removal of the autochthonous neoplasm, and in which the cycle of tumor removal and retransplantation could be accomplished accordingly, exhibited greater refractoriness to the tumors than did any of their isologous controls (three-five in each experiment). This is illustrated by the following experiment:

A spontaneous mammary carcinoma was removed from a mammary gland of a 16-month-old multiparous R11 female; the tumor measured 10 X 11 mm. and was preserved by freezing. The animal was not subjected to any further treatment. One month later pieces of tumor tissue measuring approximately 0.05 cu. mm. were transplanted into the two No. 3 mammary glands of the original animal and into both the No. 3 and the No. 4 mammary glands of each of three normal isologous females of the same age. When the experiment was terminated 8 weeks later, all twelve tumor implants in the isologous control animals had grown to considerable size (ranging from 50 to 1800 cu. mm., with an average size of 400 cu. mm.). Neither of the two implants in the autochthonous animal had initiated growth.

The finding of resistance of autochthonous animals to spontaneous cancers developing from their own mammary tissues indicates that the similar results obtained with tumors arising from nodule implants, on which most of the present study was based, are not likely to be due to special circumstances pertaining to such tumor-host relationships.

Statistical analysis.1—Individual tumors, though they may be morphologically similar, native to an isologous strain, and induced by the same stimulus, frequently differ geno- and phenotypically from one another. Each of the mammary carcinomas here studied was therefore considered a separate entity. The differences in experimental conditions from experiment to experiment also prevented comparisons of the reactions of the original and isologous animals to the transplants of the 25 different tumors. The evaluation of the capacity of the mice to react to their original neoplasms had to be based, therefore, on separate comparisons in each experiment of the behavior of two groups of animals: the single original host and its group of isologous controls.

The number of isologous control animals available for each original host was small (never more than twelve), and the growth patterns of first-transplant generation mammary carcinomas in isologous mice vary over a large range (e.g., see Table 1). Statistical analyses of the differences in terminal tumor weights or in survival times between the individual original hosts and their respective isologous controls did not, therefore, show significance at the 0.05 level in the experiments in which the tumor retransplants grew in the former, even in those instances in which the original host ranked first or last in order of tumor resistance.

In contrast, the differences between original host and isologous controls were highly significant in the three experiments (1, 2, and 4) in which both tumor implants developed in every one of the control animals (12, 9, and 6 animals, respectively) but failed to initiate growth in the original hosts: If it is supposed that there was nothing special about the original host in Exp. 1, then the failure of two implants to grow in this experiment could have occurred in any two of the 26 implant positions in the thirteen animals. The probability that these failures occurred in the two particular sites of the original host

### Table 2

<table>
<thead>
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<th>No. of experiment</th>
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<th>Isologous controls</th>
<th>Treatment and results</th>
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Combined axillary and brachial node weights of ten normal animals

<table>
<thead>
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<th>No. of experiment</th>
<th>Original host</th>
<th>Isologous controls</th>
<th>Treatment and results</th>
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*The animal in each experiment having the largest lymph node mass is ranked first.
†See Footnote § to Table 1 for abbreviations used to designate treatment. Additional abbreviations are: NV = killed normal tissue vaccine; F = tissue culture fluid
§Statistical analysis.*
animal is the probability of drawing two defective items from a set of 26 items, only two of which are defective. This equals 1/325, or 0.0031 (6). Data from Exps. 2 and 4 result in analogous probabilities of 1/190, or 0.0053, and 1/91, or 0.011, respectively.

An analysis of the distribution of the relative ranks of tumor resistance of the original animals among their respective control groups (Table 1 and Chart 1) was also undertaken by comparing the actual distribution with the uniform distribution which would be expected if the previous tumor experience had had no effect on the animals. A $X^2$ test of goodness of fit was performed. The value of the $X^2$ statistic was 8.29 and the P value approximately 0.06. The distribution of the relative ranks of the original animals thus suggests that they behave differently as a group than do the controls.

**DISCUSSION**

The experiments here reported represent only the first phase of a study on the occurrence and nature of the resistance to newly arisen spontaneous tumors in the original and in isologous hosts, and a full discussion of the present data will be postponed until the results of further phases of the program have been communicated. It appears necessary at this time to point only briefly to several aspects of the present findings.

The outcome of the first 25 attempts to retransplant mammary carcinomas into the virgin female mice in which the tumors had developed spontaneously from implants of isologous nodular outgrowths, and the similar experience with retransplantation of autochthonous mammary carcinomas to multiparous females, indicate that animals of this species can acquire a heightened capacity to react against such tumors. This heightened reactivity was manifested either by increased resistance or by increased tolerance to the tumor retransplants.

Both the increased tolerance and the increased resistance displayed by many of the original hosts can be explained in immunological terms. Certain immunological responses bring about rejection of antigenically foreign tissue, whereas others fail to do so and can act to protect a foreign graft from the destructive reactions. It is suggested that the heightened tumor susceptibility of some of the original hosts may represent a phenomenon identical with immunological enhancement (14).

An immunological interpretation of the findings is supported by the appearance of the lymph nodes draining the sites of the tumor implants. These nodes were enlarged in original animals showing either type of response to the tumor retransplants, and in both cases their histological appearance suggested that an active immunological response was in progress. It has already been reported by other workers that lymph node cells derived from mice displaying resistance to carcinogen-induced cancers can specifically neutralize the tumor cells in vitro and can transfer a state of heightened tumor resistance to normal animals (18, 20, 27). It has also been reported by Koldov-

4 Working with isografts of long-transplanted mammary carcinomas, Martinez et al. have also shown that mice may react either with exaggerated susceptibility or with heightened resistance to reinoculation of the same tumor (23).

Sky that the lymph nodes of primary hosts of benzpyrene-induced, immunogenically active tumors show considerable enlargement (22).

It must be considered, however, that absence of lymphatic hyperplasia does not necessarily indicate that an active immunological response had failed to take place. The original host may have killed early the (more) antigenic clones within the tumor transplants by means of immunological reactions. Tumors seen upon termination some weeks after retransplantation would then represent a less or nonantigenic cell population. The fact that the original animal in Exp. 1 suppressed tumor growth totally (Table 1) but did not show a marked enlargement of the axillary and brachial nodes (Table 2) may be a case in point: This tumor may have been composed entirely of cells possessing a significant antigenicity, and the sensitized host may have been able accordingly to destroy them rapidly. At the time of examination of the local nodes some weeks later, these could have returned to normal appearance.

In experiments in which the original host showed either increased tumor resistance or susceptibility, the growth rates of the palpable tumors in original and isologous animals were frequently similar (e.g., Chart 2). The differences observed throughout the course of such experiments in the size of the tumors in the original hosts and in their controls must therefore be ascribed either to large differences in the number of cells of the tumor fragments which survived implantation and/or to differences in their ability to initiate growth in the animals.4

In comparing a single original host to a group of isologous animals, it is impossible to differentiate host from tumor variables. Thus, the differences in the reactions to their own tumor retransplants among the original animals could be explained either by differences in tumor antigenicity or by differences in the native immunological abilities of the hosts. (Marked variation in immunological responsiveness of mice of even highly inbred strains is a common occurrence.) To discriminate between host and tumor factors, it is necessary to further test the behavior of the tumors here studied in groups of isologous animals with and without previous tumor experience. Statistical interpretation also becomes more feasible in such experiments. The results of the first completed experiments in this direction have shown that several of the tumors which appeared to elicit increased resistance or increased tolerance in the original host behave in the same manner in groups of isologous animals. The role of tumor-cell vaccines and of the various immunological activators in enlarging the tumor reactivity of mice is also under investigation, by variously treating different groups of isologous animals before, and after, tumor implantation. Attempts to establish beyond doubt the immunological nature of the present observations are currently directed.

4 Working with isografts of long-transplanted mammary carcinomas, Martinez et al. have also shown that mice may react either with exaggerated susceptibility or with heightened resistance to reinoculation of the same tumor.
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Acquisition of Heightened Resistance and Susceptibility to Spontaneous Mouse Mammary Carcinomas in the Original Host

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