The Survival and Metastatic Spread of Homografts of Mouse Tumors in Mice Pretreated with Lyophilized Tissue and Cortisone

NATHAN KALISS, PAULO R. F. BORGES, AND EUGENE D. DAY

(Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)

It has been demonstrated that under certain conditions the prior injection of lyophilized (11, 14, 15) or fresh tissues, or antiserums to these tissues (10), into mice of a given inbred strain will lead to a breakdown of the normal resistance of these animals to tumor homografts.

It has been further reported by Molomut and co-workers (11, 16) that the combined injection of frozen-dried tumor tissue and cortisone into mice led to the rapid appearance of "extensive intra-abdominal sarcomatosis," which they considered to be metastases from subcutaneous tumor homografts. Occasionally, intrathoracic metastases were also present (16). The sarcomatosis was found as early as 14 days after tumor grafting (12). The authors (12) state that "... we were able to predict our results based on our tentative hypothesis of mechanism ...," the hypothesis being that: (a) the injections of frozen-dried tumor and the subsequent live tumor graft constituted a "systemic trauma," permitting the successful growth of the tumor homograft; and (b) the injection of cortisone would be an additional debilitating experience which should serve to further enhance the growth of the homograft to the point where extensive metastatic spread would occur.

It is these postulates of mechanism, particularly that of a general systemic trauma, that led us initially to conduct the experiments to be reported below. Our questions stemmed chiefly from previous observations that there is a definite specificity, both at the species level (3, 8) and within the species (4, 14), which predicates whether or not prior injections of tissue will lead to a breakdown of resistance to tumor homografts. Thus, lyophilized tissues from the rabbit, hamster, guinea pig, and rat are ineffective when tested against tumor homografts in the mouse (8), and, in general, tissues from one inbred strain of mice, when tested against a transplantable tumor indigenous to an unrelated inbred strain, are also ineffective (4, 14). Could these observed specificities be abrogated by the administration of cortisone?

We also wished to ascertain whether the reported potentiating effect of cortisone would hold true for a host-tumor graft combination different from that previously used (12, 16).

The first experiments we set up were directed solely to these questions. However, it soon became clear that not only could we not negate the specificities, but we were unable to duplicate the observations of intra-abdominal sarcomatosis (12, 16) in the mice treated with lyophilized tissue and cortisone, whether or not we used the same host-tumor graft combination. It was as a result of our failures in this respect that our attention was then shifted to the problem of determining the reason for the discrepancy.

The following report is divided into two sections. The first section presents data on the survival and metastatic spread of tumor grafts in the indigenous strains and in normally resistant strains of mice. The second section presents the finally successful results of our attempts to produce intra-abdominal tumor growths of the type previously reported (12, 16) and the demonstration that in our case these could be ascribed only to the survival of tumor cells in lyophilized tumor tissue that had been incompletely dried.

MATERIALS AND METHODS

Tissues to be lyophilized were excised under aseptic conditions. They were arranged in a layer, not exceeding approximately 1 cm. in thickness, on the inner walls of Pyrex glass vessels and were frozen at −23°C. Freeze-drying was carried...
out on a Stokes cryochem apparatus at an internal gas pressure of about 100 μ of mercury, and the vessels containing the tissues were exposed to the ambient room temperature. Drying was started in the late afternoon of the same day on which the tissues were taken, and was continued for an average time of 14-15 hours. Secondary drying was carried on in vacuo, over anhydrous calcium sulfate, for at least 8 days and in most instances for several weeks.

For injection, the dried tissues were suspended in sterile 0.85 per cent NaCl and ground in glass Potter-Elvehjem homogenizers. All injections were given intraperitoneally in a volume of 0.5 ml of suspension per injection.

The cortisone used was cortisone acetate (Cortone, Merck). For injection, it was diluted with 0.9 per cent benzy alcohol in sterile 0.85 per cent NaCl. Injections were made subcutaneously in either flank. In the first two experiments, benzy alcohol (0.9 per cent in sterile 0.85 per cent NaCl) was injected into control animals in a manner similar to that used for cortisone. Injections were given daily, except Sundays.

At a given period after the last injection of lyophilized tissue, a subcutaneous inoculation of a bit of live tumor was made by trocar, under aseptic conditions, in the interscapular region. Growth of the grafts was followed by periodic palpation until the animals with progressively growing tumors died. Animals that showed no sign of a surviving graft for a consecutive period of at least 2 months were sacrificed as negative. All animals were autopsied. All gross pathological findings were checked on histological sections.

Except for one experiment (Experiment 2), the animals used as hosts were from two sublines of the C57 black strain, namely, C57BL/6Jax, and C57BL/6Ks. (For standard strain designations see [6]). The test tumor used was Sarcoma 1, which is indigenous to the A strain of mice. It is composed of solid dense sheets of spindle-shaped and polyhydral tumor cells. Mitotic figures are very numerous, and the stroma is extremely scanty. It grows progressively in 100 per cent of strain A mice, killing most of the animals by the 8th week after inoculation. In our experience, this tumor does not survive at all in untreated C57BL/6Ks animals (about one in 300).

All animals, both donors from which tumors were taken and hosts, ranged in age from 2 to 4 months at the start of the experiments. The hosts were about equally divided by sex. They appeared to be healthy and vigorous at the start of the experiments. However, in the cortisone-treated animals we occasionally ran into the problem of infections. We tried to counteract this by administering mixed antibiotics intraperitoneally (penicillin, streptomycin, terramycin), but with questionable success.

RESULTS

SECTION I: THE METASTATIC SPREAD OF HOMOGRafts AND ISOGRafts OF TRANSPLANTABLE MOUSE TumORS

(A preliminary report of these experiments has been presented elsewhere [9].) (Homografts are defined as grafts between animals of the same species but of different genetic backgrounds; isografts are grafts between animals of the same inbred strain.)

Cortisone acetate (Cortone, Merck) was generously supplied by Merck & Co., Rahway, N.J.

We are indebted to Dr. Elisabeth Fekete of our laboratory for the descriptions of the histology of the tumors used in this report.

Terramycin hydrochloride, kindly supplied by Chas. Pfizer & Co.

Four experiments are reported in this section. The first was directed to the question of whether species specificity (8) would be circumvented by the injection of cortisone. The second experiment was designed to test the effects of cortisone in a different host-graft combination. Experiments 3 and 4 were carried out to check the first two experiments, since we had not found intra-abdominal tumor masses of the type previously reported in any of the mice (12, 16).

Experiment 1: The data are presented in Table 1. Live tumor was inoculated 1 week after the last injection of guinea pig kidney (corresponding to 11 days after the last injection of frozen-dried Sarcoma 1). Cortisone was started on the day of the first injection of frozen-dried tissue, and was terminated 3 days after the inoculation of live tumor.

It is apparent that cortisone did not abrogate the normal resistance of the mice to the tumor homografts in the animals given injections of guinea pig kidney. This confirmed our previous observations (8). In the groups receiving frozen-dried Sarcoma 1, with or without cortisone, there was a breakdown of resistance, as shown by progressive growth of the tumor grafts.

Four of the fifteen mice dying with tumors had metastases. The one in the Sarcoma 1-treated group had metastases in the lungs. Of the three in the Sarcoma 1 and cortisone-treated group, one had metastases in the lungs, and two in the lungs and kidneys.

Experiment 2: The host strain was A/Jax, and the test tumor was a mammary adenocarcinoma, E5819, indigenous to the ST/Ks strain of mice. This tumor arose spontaneously in an ST/Ks female. It is composed of very small cuboidal epithelial cells forming small alveoli. The supporting stroma is loose and very vascular. The tumor grows progressively in 100 per cent of ST/Ks mice and not at all in untreated strain A animals.

Saline homogenate of frozen-dried tumor E5819 was administered intraperitoneally in four injections in the amount of 15 mg dry weight/injection/mouse, given twice weekly. Live tumor was grafted 1 week after the last injection. Cortisone was administered subcutaneously, daily except Sunday, starting on the day of the first injection of lyophilized tumor and ending 3 days after the grafting of live tumor. A total of 26 mg was given per mouse, consisting of two injections of 0.5 mg each and sixteen injections of 0.1 mg each. Control mice received subcutaneously 0.05 ml/injection of 0.9 per cent benzyl alcohol in 0.85 per cent NaCl, in the same schedule as the cortisone.

Three of twelve mice receiving frozen-dried
tumor alone died with progressively growing tumor grafts but no metastases. Of twelve mice receiving dried tumor and cortisone, four were lost by intercurrent death due to generalized infection. Of the surviving eight, one died with a progressively growing tumor graft and metastases to the lungs. Negative results were obtained in all ten mice receiving the benzyl alcohol. Apparently, cortisone did not affect the course of growth of the homografts.

Experiment 3: Since no intra-abdominal tumor growths comparable to those previously reported (12, 16) were present in the animals in our first two experiments, it was thought that perhaps the differences in the strains of mice used and in the dosages of lyophilized tumor might account for our failure to reproduce the findings. We accordingly sought to approximate more closely the experimental conditions as given (12).

The test tumor was again Sarcoma 1, and the hosts were from the C57BL/6Jax strain (the strain in which the intra-abdominal growths had been found [12, 16]) and the C57BL/6KSs strain. The total dosage of lyophilized tumor tissue per mouse was increased from 30 mg dry weight to 45 mg. In the C57BL mice, cortisone was started the same day as the first injection of lyophilized tumor and stopped 1 day before grafting of live tumor. In the A/Jax mice, cortisone was started 11 days before tumor grafting. The data are shown in Table 1.

Again, cortisone had no effect on incidence or

### Table 1

Effect of Prior Injections of Lyophilized Tissues and Cortisone in Mice on the Survival of Homografts and Isografts of a Tumor

(Sarcoma 1, indigenous to strain A mice, was used in all experiments.)

<table>
<thead>
<tr>
<th>Substance Injected</th>
<th>Amo/Infection*</th>
<th>No. Injections†</th>
<th>C57BL/6Jax Hosts Dying with Grafts§</th>
<th>Metastases</th>
<th>C57BL/6Ks Hosts Dying with Grafts§</th>
<th>Metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea pig kidney</td>
<td>15 mg.</td>
<td>4</td>
<td>0/15</td>
<td>0</td>
<td>0/15</td>
<td>0</td>
</tr>
<tr>
<td>Guinea pig kidney and cortisone</td>
<td>As above 0.5 mg. 2</td>
<td>As above 0.1 mg. 16</td>
<td>0/15</td>
<td>0</td>
<td>0/15</td>
<td>0</td>
</tr>
<tr>
<td>Sarcoma 1 and 0.9 per cent benzyl alcohol</td>
<td>15 mg. 2</td>
<td>0.05 ml. 18</td>
<td>4/14</td>
<td>1</td>
<td>11/15§ 3</td>
<td></td>
</tr>
<tr>
<td>Sarcoma 1 and cortisone</td>
<td>As above 0.5 mg. 4</td>
<td>As above 0.1 mg. 13</td>
<td>0/15</td>
<td>0</td>
<td>0/15</td>
<td>0</td>
</tr>
<tr>
<td>Nothing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 2:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoma 1</td>
<td>10 mg.</td>
<td>3</td>
<td>2/15 1</td>
<td>11/11 2</td>
<td>0/15 0</td>
<td>0</td>
</tr>
<tr>
<td>Sarcoma 1 and cortisone</td>
<td>As above 0.5 mg. 4</td>
<td>As above 0.1 mg. 13</td>
<td>0/15† 0</td>
<td>8/19 2</td>
<td>11/15§ 3</td>
<td></td>
</tr>
<tr>
<td>Nothing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 3:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoma 1 supernate</td>
<td>0.4 ml.**</td>
<td>6</td>
<td>6/14 0</td>
<td>15/15 9</td>
<td>15/15 9</td>
<td>0</td>
</tr>
<tr>
<td>Sarcoma 1 supernate and cortisone</td>
<td>As above 0.5 mg. 3</td>
<td>As above 0.1 mg. 10</td>
<td>1/17† 0</td>
<td>15/20 6</td>
<td>15/20 6</td>
<td>0</td>
</tr>
<tr>
<td>Nothing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 4:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisone</td>
<td>See text</td>
<td>See text</td>
<td>10/10 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nothing</td>
<td></td>
<td></td>
<td>14/14 3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Tissue weights are mg. dry weight.
† Frozen-dried Sarcoma 1 in Experiment 1 injected once per week; lyophilized tissues in all other cases injected twice weekly. Cortisone and benzyl alcohol injected daily except Sunday.
‡ Numerators are the numbers of mice dying with progressively growing tumor grafts; denominators are the total mice per group.
§ Two mice lost by intercurrent death, excluded from the table.
¶ Six mice lost by intercurrent death, excluded from the table.
|| Eight mice lost by intercurrent death, excluded from the table.
** Estimated total dry weight per mouse, approximately 10.0 mg.
†† Six mice in one pen died intercurrently with generalized infectious processes, excluded from table.
distribution of metastases. In the C57BL/6 mice, the metastases were found in the lungs and kidneys. In the mice of the indigenous A/Jax strain, metastases were in the lungs, mediastinum, pancreas, and axillary and left sub-maxillary lymph nodes. No intra-abdominal sarcomatosis was found.

An additional point is the greater sensitivity of the C57BL/6Ks mice to the experimental procedure in comparison with the C57BL/6Jax mice, as shown by the larger number of the former animals dying with tumor homografts.

A complicating factor in this experiment was the appearance of generalised or focal purulent peritonitis in almost all the mice that had been injected with lyophilized tumor. This was most certainly due to infectious organisms introduced with the frozen-dried tissue. The effect of cortisone in lowering resistance to infection is clearly demonstrated here, since many of the mice in the lyophilized tissue—cortisone-treated groups succumbed to the infections before they could give us valid data. These have been excluded from Table 1.

Experiment 4: It could be argued that our failure to find intraperitoneal tumor masses in the "proper" combination used in Experiment 3 might be due to the complicating factor of purulent peritonitis.

Accordingly, in Experiment 4, the hosts were given inoculations of the supernate of a centrifuged homogenate of tumor tissue—such a supernate being presumably free of bacteria. The supernate was prepared as follows: Frozen-dried Sarcoma 1 was homogenized in sterile 0.85 per cent saline in a concentration of 40 mg dry weight/ml and centrifuged at 4,500 X g at 8° C. for 20 minutes. The supernate was filtered through sterile filter paper to remove solidified fat and then recentrifuged at 8,500 X g for 30 minutes at room temperature. This supernate was diluted with sterile 0.85 per cent NaCl in equal volumes and kept frozen at −23° C. until used for injection. Bacteriological assay on nutrient agar slants gave no bacterial growths from samples taken from the homogenate before centrifugation. No bacterial growths were obtained from the second supernate.

The data are presented in Table 1. The greater sensitivity of the C57BL/6Ks mice to the experimental procedure is again evident. Cortisone did not influence the incidence, time of appearance, or anatomic distribution of metastases. These were present in the lungs, kidneys, pancreas, mesentery, lymph nodes (axillary, abdominal, mediastinal, perirenal, suprarenal), and heart. No intra-abdominal sarcomatosis was found.

The consistent repetition of results in four experiments confronted us with the problem of determining the cause of the discrepancies between our data and those of Molomut and co-workers (12, 16). The following points might be raised as possible explanations. In the first two experiments we had used either a different subline of C57BL mice as hosts (Experiment 1), or both a different tumor and host combination (Experiment 2), from those used by these investigators (12). In Experiment 3, in which we did employ the "proper" combination, the occurrence of purulent peritonitis may have acted as a negative factor. In Experiment 4, we used tumor supernatant rather than whole tumor homogenate for preparing the hosts. Nevertheless, in all these experiments we did find metastases in a variety of sites other than the abdominal cavity.

A comparison of the metastases as we found them with those previously reported (12) is pertinent at this point. First, in our case they occurred only in the C57BL mice dying at a minimum time of 6 weeks after tumor grafting and in strain A mice at a minimum of 4 weeks after grafting. Table 2 shows the relation between the length of survival of mice with progressively growing tumor grafts and the appearance of metastases for all experiments reported here. Secondly, cortisone did not influence either the incidence, time of appearance, or distribution of the metastatic foci. Thirdly, metastases were found only in animals that died with progressively growing tumor grafts, and in only about a third of these. They were present chiefly in the lungs and kidneys.

The foregoing is in contrast with the finding (12) of intra-abdominal sarcomatosis in the lyophilized tumor—cortisone-treated C57BL/6Jax mice. Such tumor growths were reported as occurring in all the mice thus treated, and they appeared as early as 2 weeks after tumor grafting. Occasionally, "intrathoracic metastases" were found in these mice (10). The subcutaneous tumor grafts "... grew slowly and in many began to become soft and necrotic" (12). The animals with the intraperitoneal tumor growths are described as having "... ascitic fluid and extensive intra-abdominal sarcomatosis involving mesentery, serosa of intestines, diaphragm, mesenteric nodes, pelvis, retroperitoneal tissues, peri-renal area, right kidney, liver." It is further stated that no abnormalities were found at autopsy in indigenous A/Jax or in noncortisone-treated C57BL/6Jax mice that had progressively growing tumors (12).

These findings differ markedly from ours. In fact, the above-quoted description of the intra-abdominal tumor masses could fit exactly the types of growths observed by us in untreated,
normally susceptible mice which had received intraperitoneal injections of a suspension of live Sarcoma 1 tumor tissue.1

Since we could not account for the differences in results on the basis of experimental design, we turned our attention to a possible difference in the manner in which the experiments had been done. The experiments reported in (12) were conducted in the summer of 1952, while three of the investigators (N. M., L. K., and S. D. G.) were working in our laboratory. We were acquainted with the freeze-drying technics these investigators had used.4 Their procedures did not permit sufficient drying of the tissues, since the containing vessels, which could be heated only by conduction, were kept in vacuo during the primary drying run. Furthermore, examination of our records of tissue preparations revealed that in at least two instances these investigators had used tumor tissues shortly after the primary drying run without subjecting them to secondary drying. It was questionable whether these lots of material had been sufficiently dried to ensure the death of all the tumor cells.

(Such a supposition has precedence in the work of Passey, Dmochowski, and co-workers [7, 13] by which they demonstrated that tumor cells survived in frozen-dried tumor tissue which superficially appeared to be “dust dry.” Billingham and Medawar [2] have reported the survival of frozen-dried rabbit skin if not more than 70 per cent of the original water content were removed.)

We therefore addressed ourselves to the question of whether tumor cells could survive these conditions of lyophilization if the tissues were incompletely dried, and whether the intraperitoneal injection of such tissues would result in the types of intra-abdominal growths previously described (12). The results of our efforts are detailed in Section II.

### TABLE 2

<table>
<thead>
<tr>
<th>SUBSTANCE INJECTED</th>
<th>TOTAL NO. DYING WITH TUMORS†</th>
<th>PROPORTION OF MICE DYING WITH TUMOR GRAFTS THAT ALSO HAD METASTASES, DISTRIBUTED BY TIME OF DEATH (WEEK) AFTER TUMOR GRaFTING†</th>
<th>TOTAL 5d 6th 7th 8th 9th 10th 11th 12th 13th 14th 15th 16th</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. C57BL/6j mice:</td>
<td>Z</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lyophilized tumor</td>
<td>48/102</td>
<td>17/40 0/1 3/5 2/5 2/5 3/5 5/5 2/5 2/5 2/5 0/1 0/1</td>
<td></td>
</tr>
<tr>
<td>Lyophilized tumor plus cortisol</td>
<td>8/27 0/3 0/1 0/1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. C57BL/6Ks mice:</td>
<td>Z</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lyophilized tumor</td>
<td>30/40</td>
<td>10/30 0/6 0/2 0/1 1/7 1/8 5/8 1/7 0/1</td>
<td></td>
</tr>
<tr>
<td>Lyophilized tumor plus cortisol</td>
<td>34/45 11/54 0/3 0/1 1/7 1/8 6/7 5/7 0/1 1/2 1/1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. A/Jax mice:</td>
<td>Z</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisone</td>
<td>10/10</td>
<td>4/10 0/1 1/3 2/3 1/1 0/1</td>
<td></td>
</tr>
<tr>
<td>Nothing</td>
<td>25/25 9/25 1/1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. A/Ks mice:</td>
<td>Z</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nothing</td>
<td>6/6</td>
<td>3/6 0/1 0/1 0/1 1/2</td>
<td>1/1</td>
</tr>
</tbody>
</table>

† Data from Experiments 1, 5, 4, 5, and 6.

The total number of mice dying with progressively growing tumor grafts (with or without metastases); denominator represents total number of mice in the group.

The total number of mice dying with tumor grafts at a given week after tumor inoculation is shown in the denominator; the proportion having metastases, as well as tumor grafts, is shown in the numerator.
i.e., tissue subjected to secondary drying in vacuo over anhydrous calcium sulfate for at least 3 weeks. For injection, both the F-L and W-D tissue were homogenized in sterile 0.85 per cent NaCl in glass homogenizers, and the homogenates were injected intraperitoneally. Subcutaneous grafts of live tumor were made by trocar in the interscapular region.

Experiment 5: The hosts were mice of the C57BL/6Jax and A/Jax strains, and the test tumor was Sarcoma 1 (the same combination employed by Molomut et al. [12]). Injections of lyophilized tumor and cortisone were started 2 weeks before inoculation of live tumor. The last injection of dried tumor was administered 2 days before tumor grafting and cortisone injections were continued for 1 week thereafter. The F-L tissue for the four injections came from vials containing 1.5–6 gm. wet weight of tumor each before freeze-drying, and the material appeared to be “bone-dry” at the end of the freeze-drying run.

The data are presented in Table 3. The effect of the larger dose of lyophilized tumor (60 mg dry weight/mouse, as compared to 45 mg. in Experiment 3, reported above) is reflected in the larger numbers of mice dying with progressively growing tumor grafts. There were large losses of animals by intercurrent deaths in the cortisone-treated groups. All these showed generalized infectious processes in the liver, kidneys, spleen, and lungs.

Metastases were found, but only in the C57BL/6Jax mice with progressively growing tumors, surviving at least 6 weeks after tumor grafting, and in the A/Jax mice at least 4 weeks after grafting. These were present chiefly in the lungs and kidneys (more frequently in the left kidney) and in various lymph nodes (axillary, mediastinal, and abdominal). No intra-abdominal sarcomatosis

### TABLE 3

**Effect of Prior Injections of Cortisone and “Well Dried” or “Freshly Lyophilized” Sarcoma 1 on the Growth of Grafts of Sarcoma 1**

<table>
<thead>
<tr>
<th>Hosts</th>
<th>W-D Sarcoma 1</th>
<th>F-L Sarcoma 1</th>
<th>Nothing</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS7BL/6Jax hosts</td>
<td>15 mg. dry wt.</td>
<td>15 mg. “dry” wt.</td>
<td>Nothing</td>
</tr>
<tr>
<td>As above</td>
<td>As above</td>
<td>No. DYING WITH SUBSTANCE INJECTED</td>
<td>No. DYING WITH SUBSTANCE INJECTED</td>
</tr>
<tr>
<td>Numb.</td>
<td>4</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>of mice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>experiment</td>
<td>No. injections*</td>
<td>No. injections*</td>
<td>No. injections*</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>CS7BL/6Jax hosts</td>
<td>16/19</td>
<td>3/7</td>
<td>0</td>
</tr>
<tr>
<td>A/Jax hosts (tumor viability controls)</td>
<td>0/12</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Frozen-dried tissues injected twice weekly; cortisone injected daily except Saturdays and Sundays.
† Numerators are the numbers of mice dying with progressively growing tumor grafts; denominators are the total mice per group.
‡ Thirteen mice died with generalized infections, excluded from the table.
§ The amounts per injection per mouse were 4.8, 16.0, 13.0, and 25.5 mg., respectively, depending upon the amount of fresh tumor in each of the four vials before freeze-drying (see text).
∥ Seven mice died intercurrently with generalized infections, excluded from the table.
★ Four mice died intercurrently with generalized infections, excluded from the table.
\* One of the three mice was moribund and was killed 19 days after tumor grafting. Its abdomen contained ascitic fluid, tumor nodules, and two sarcomatous foci in the liver. All other organs were normal.
†† The one mouse died 13 days after tumor grafting. Its abdomen contained ascitic fluid and tumor nodules. All organs were normal.
was observed. In no instance had the subcutaneous tumor graft invaded the body wall.

Experiment 6: This was a repetition of Experiment 5. To ensure a higher residual moisture content in the F-L material after freeze-drying, each vial was packed with 9–12 gm. wet weight of fresh tumor. This time, after the drying run, the cores of tissue in each of the four vials had small, obviously wet pieces in their centers and near the bottom. These wet pieces, together with what appeared to be dry tissue in the same samples, were homogenized in sterile 0.85 per cent NaCl and injected intraperitoneally into the animals.

The data are presented in Table 3. For the first time we found two animals, one each in the groups treated either with F-L tissue and cortisone or with F-L tissue alone, that had intraperitoneal tumor masses and ascitic fluid whose characteristics and distribution fitted the description given in (12). One mouse (F-L tissue-cortisone group) died 18 days after grafting of live tumor. It also had a small subcutaneous tumor nodule at the site of injection of the F-L material. The second mouse (F-L tissue group) was moribund and was killed 19 days after tumor grafting. It also had two small sarcomatous areas in the liver. Both mice had good-sized subcutaneous tumor growths at the site of grafting of live tumor. These had not invaded the body wall. In both animals, all other organs were normal.

There were also metastases of the type we have described in the experiments above. These occurred in three C57BL/6Jax hosts that died from 10 to 11 weeks and in three A/Ks control mice that died 7–11 weeks after grafting of live tumor. In the three C57BL/6Jax mice, they were present in axillary and inguinal lymph nodes. In the three A/Ks mice they were in the lungs, kidneys, and perirenal and suprarenal lymph nodes.

Just as there sometimes is initial growth for 2–3 weeks before eventual regression, in those C57BL/6Jax mice in which subcutaneous grafts of Sarcoma 180 fail to survive, so there may have been initial intraperitoneal growth and regression in some of the C57BL/6Jax hosts receiving the F-L tissue. Five of the animals in the F-L tissue cortisone-treated group exhibited definitely distended abdomens 2 weeks after the last injection (one mouse also had a palpable mass at the injection site). The swellings thereafter subsided, and the animals appeared normal at autopsy 3 months later. This was also true of three mice in the F-L tissue-treated group (two of these three also had palpable masses at the injection site).

Experiments 7 and 8: To further verify the survival of cells in the F-L tumor tissue, it was injected intraperitoneally in mice of the indigenous A/Jax strain. No fresh tumor was grafted subcutaneously. These animals were given injections simultaneously with those of Experiments 5 and 6, respectively (described above), and with the same batches of F-L and W-D material.

Experiment 7: W-D tumor was administered intraperitoneally to ten A/Jax mice (designated Group 1), and “bone-dry” F-L tumor to nine A/Jax mice (designated Group 2), the injections being given at the same time and from the same batches of frozen-dried tumor. Both groups also received cortisone injections according to the same protocol as for Experiment 5 (see Table 3).

All nineteen A/Jax mice appeared normal 1 month after the last injection of frozen-dried tissue (corresponding to 3 weeks after the last injection of cortisone). Four mice of Group 1 and all nine mice of Group 2 were at this time transferred to Experiment 8 (described below). The remaining six mice in Group 1 were normal at autopsy 4 months after the last injection of frozen-dried tumor. The negative findings in Experiment 7 are in accordance with those of Experiment 5, as regards the absence of intra-abdominal sarcomatosis in the latter experiment.

Experiment 8: The A/Jax animals in this experiment consisted of four mice from Group 1 and all nine mice of Group 2 (see Experiment 7). One month after these mice, as members of Experiment 7, had received the last injection of frozen-dried tumor (corresponding to 3 weeks after the last injection of cortisone), they were given a single injection of the obviously wet F-L tumor tissue which came from the same batch of material, and was administered at the same time as the last injection given to the C57BL/6Jax animals in Experiment 6. No cortisone was administered. The four animals of Group 1 received F-L homogenate intraperitoneally. The nine animals of Group 2 received subcutaneous injections of obviously wet F-L tissue by trocar. None of the mice received grafts of fresh tumor.

All four of the mice of Group 1 died with distended abdomens, three of the four dying in succession on the 19th, 20th, and 21st days, and the 4th, 32 days after injection. (Coincidentally, the first C57BL/6Jax mouse in Experiment 6 to die with intraperitoneal tumor masses succumbed 19 days after the last injection of F-L homogenate.)

On autopsy, they exhibited a picture identical to that previously described (12), namely, the peritoneal cavity filled with blood-stained fluid and scattered nodules of tumor. The lungs and kidneys were normal in all four animals.

Three of the nine mice (Group 2) that had re-
ceived subcutaneous injections by trocar developed tumor masses and died with progressively growing tumors. Nodules were first palpable at 6 weeks after injection, and the animals died between the 4th and 5th weeks afterwards. One mouse had metastases in the lungs and several small nodules on the mediastinum. None of the three had intraperitoneal growths, nor did the subcutaneous growths invade the body wall. The remaining six mice in Group 2 were sacrificed 3 months after injection and were normal at autopsy.

Experiment 8 demonstrates that tumor cells will survive the rigors of the lyophilization procedure if the tissue is insufficiently dried.

Experiment 9: We reproduced the conditions of Experiment 8, this time using one group each of twenty C57BL/6Ks and 40 A/Ks mice as the hosts. No cortisone was injected, and no fresh tumor was grafted. Each of the mice received a single intraperitoneal injection of 20 mg. “dry” weight of F-L tissue homogenate.

None of the 20 C57BL/6Ks mice developed intraperitoneal tumors. One did develop a very large subcutaneous tumor mass at the injection site but had no other growths when it was sacrificed 2 months after the injection. The other nineteen mice were normal at autopsy, 5 months after the injection.

Of the 40 A/Ks mice, four died with intraperitoneal tumor masses and ascitic fluid. One of the four had a subcutaneous tumor nodule at the injection site which was penetrating the peritoneal wall. Another had a few tumor nodules on the diaphragm which were penetrating into the thorax. The organs were normal in all four mice. The remaining 36 animals were sacrificed 5 months after the injection. At this time they were 9 months old. Except for three mice which had spontaneous lung tumors, all were normal at autopsy.

Experiment 9 again demonstrates the ability of tumor cells to survive freeze-drying if the tissue is insufficiently dried.

**DISCUSSION**

Whatever interpretation one may put upon the findings of Molomut and co-workers, the fact remains that tumor cells can survive the rigors of freeze-drying if the tissues are insufficiently dried and that the intra-abdominal injection of such preparations will give rise to the types of growths previously recorded (12, 16).

There remains one discrepancy between our findings and those previously reported which we cannot reconcile. These investigators stated (12) that all 70 C57BL/6Jax mice treated with cortisone and frozen-dried tumor showed intra-abdominal sarcomatosis, while none appeared in 27 mice receiving frozen-dried tumor but no cortisone. (Incidentally, in another experiment [12] in which the hosts were adrenalectomized, none of the mice treated with frozen-dried tumor, with or without cortisone, showed “... any gross evidence of metastases.” No explanation for this is given.)

In our case, cortisone did not alter the incidence or distribution of metastases nor did it influence the incidence of intra-abdominal growths in those animals receiving F-L tissue injections. Indeed, only three out of 60 C57BL/6 mice developed such growths from the F-L tissue in the experiments in which cells could be definitely shown to have survived. That the cortisone was administered at a level which debilitated the recipients is shown both by the large number of intercurrent deaths and by either weight loss, or failure to gain weight, in the survivors. (It should be emphasized that the protocols of cortisone injections we used are similar to the one employed by Molomut et al. [12].)

As for the possible potentiating effect of cortisone on the breakdown of resistance to tumor homografts in mice simultaneously injected with frozen-dried tissues, our experiments demonstrated that: (a) cortisone did not negate the phenomenon of specificity in the breakdown of resistance to a homograft, as induced by our techniques; (b) homografts did metastasize, but only in the hosts with progressively growing tumor grafts that survived for a minimum of 6 weeks after grafting; (c) cortisone did not influence either the time of appearance, incidence, or distribution of anatomical sites in which the metastases appeared.

We now wish to consider another report by a group of Chilean investigators, Agosin et al. (1), on the possible positive effect of cortisone on the appearance of extensive organ metastases from tumor grafts. These workers used a transplantable C3H tumor, a suspension of which was injected into the flank of C3H hosts. Cortisone treatment was started 8–9 days after the tumor injection. Eight of eighteen animals so treated developed metastases, which “... involved axillary, inguinal, mesenteric, and retroperitoneal nodes, the mediastinum, peritoneum, pleura, liver, spleen, kidney, lung, diaphragm, and the muscles of the thigh.” The earliest metastases were found in two of the eight mice which died 5 weeks after tumor grafting. This is comparable to our observations of the earliest time of appearance of homografts (6 weeks) and isografts (4 weeks) (see Table 2). These investigators state that the grafts in the cortisone-treated animals showed a marked inhibition of growth. (We have observed a like effect on...
Homografts in the C57BL/6 mice.) It is possible that this inhibitory effect on the graft, with a consequent increase in survival time of the host, may account for the appearance of metastases in the cortisone-treated mice (though Agosin et al. [1] believe that they have demonstrated that this is not so).

A comparison of our data for the metastatic spread of Sarcoma 1 in strain A mice, treated with cortisone or untreated (see Table 2), with those of Agosin et al. (1) is illuminating. Table 4 presents the data which Agosin et al. (1) reported in their Tables I and II and which we have rearranged to illustrate our point.

It is evident that metastases were not present in mice sacrificed before the 6th week or in mice dying before the 6th week after tumor grafting, whether or not they had received cortisone. No mice were sacrificed beyond 6 weeks after grafting, and the numbers sacrificed at this time were small (two mice each in the treated and untreated groups). No untreated mice survived beyond 6 weeks after grafting.

In our case (Table 2) where cortisone apparently does not affect the growth of Sarcoma 1 in the indigenous A strain mice, thus not affecting the length of survival of the hosts, metastases appeared equally in the treated and untreated animals, and again chiefly in animals that survived the longest.

SUMMARY

Homografts of a tumor indigenous to the inbred A strain of mice grew progressively in a large proportion of mice of two C57BL/6 sublines that had received injections of frozen-dried homologous tumor tissue prior to grafting. The homografts often metastasized, metastases appearing only in animals surviving a minimum of 6 weeks after grafting. They were found most frequently in the lungs and kidneys, and also occurred in other sites (various lymph nodes, pancreas, heart, mediastinum). The injection of cortisone along with frozen-dried tumor tissue, prior to grafting, did not affect either the incidence, time of appearance, or distribution of anatomic sites of the metastases.

Intra-abdominal tumor growths, of the type reported by Molomut and co-workers, appeared only in animals receiving intraperitoneal injections of incompletely dried lyophilized tumor tissue in which cells had survived. These growths were present in C57BL/6 mice and in mice of the indigenous A strain that had not received any grafts of fresh tumor. Cortisone injections in mice so treated did not affect the incidence, time of appearance, or nature of the intra-abdominal growths.

REFERENCES

4. ———. Failure of a Mouse Carcinoma Material To Enhance a Mouse Sarcoma. Ibid., pp. 1025–46, 1953.
The Survival and Metastatic Spread of Homografts of Mouse Tumors in Mice Pretreated with Lyophilized Tissue and Cortisone

Nathan Kaliss, Paulo R. F. Borges and Eugene D. Day


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