Studies on the Mechanism of Cortisone-induced Metastases of Transplantable Mouse Tumors*  

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During the course of investigations on the relative therapeutic effectiveness of various bacterial polysaccharide fractions on “metastatic” pulmonary tumors induced by the intravenous injections of ascites tumor cells in Swiss mice and on the protective ability of cortisone and hydrocortisone in combating the toxicity of the bacterial products (16), it was noted that widespread blood-borne metastases occurred with Krebs-II carcinoma but not with Sarcoma 87 (Table 1). These widely distributed tumors in many cases were found to be much larger than the pulmonary tumors, which fact suggested that they were not metastases embolic from the established pulmonary tumors but had been delivered to the various sites by immediate transpulmonary passage of tumor cells at the time of the original intravenous injection. Zeidman and Buss (19) have reported the immediate passage through the pulmonary circulation of the cells of two rabbit carcinomas and one rat carcinoma. The varying rates of growth of metastases in different sites are reflected by the considerable variation in size of tumors in the various sites in the mice illustrated in Figures 1 and 2. Lucké et al. (14) have demonstrated the marked variation in growth rate of induced metastases of the V-2 rabbit carcinoma in the lung and liver and have compared the average size of tumors in human autopsies in these two organs. These studies indicate that, once the tumor is established, the environment in the liver supports more rapid growth than that of the lung.

The “soil” hypothesis of tumor metastasis seems well established from the standpoint of the rate of metastatic growth (18). However, there is much controversy concerning the predominance of mechanical factors or of “soil” factors in allowing embolic tumor cells to “take” and grow in the various organs. Hackmann (9) has demonstrated the marked difference in development of resistance of the various organs to “metastases” from intravenous injections of Brown-Pearce tumor suspensions in rabbits. The numbers of tumors developing in the livers and lungs of the rabbits were reduced by immunization procedures consisting of prior injections of small amounts of tumor suspension in the orbit. This variation in “organ immunity” could be masked by more aggressive immunization procedures so that all organs became immune. Coman et al. (4) have reported on the correlation of numbers of Brown-Pearce tumor cell emboli in a given rabbit organ and the number of gross metastases developing in these same organs following left ventricular injections of tumor suspensions. Their findings seemingly give strong support to the mechanical hypothesis, which would argue for dependence of metastatic frequency on lodgment of the tumor emboli in small arterioles or in capillaries. However, no evaluation of liver or lung metastases was included, so that their results apply only to the

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**TABLE 1**

**Comparative Incidence of Metastasis of Krebs-II Carcinoma and Sarcoma 87 Following Treatment with Bacterial Polysaccharide and Cortisone**

<table>
<thead>
<tr>
<th>Tumor-free Lung Metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcoma 87: 1X10⁴ cells intravenously</td>
</tr>
<tr>
<td>Control: Cells on day 1</td>
</tr>
<tr>
<td>Bacterial polysaccharide and cortisone: day 6</td>
</tr>
<tr>
<td>Krebs-II: 2.5X10⁵ cells intravenously</td>
</tr>
<tr>
<td>Control: Cells on day 1</td>
</tr>
<tr>
<td>Bacterial polysaccharide and cortisone: day 6</td>
</tr>
</tbody>
</table>

* Corrected—complete autopsies. Thorax only examined at death. Complete autopsies performed on survivors on day 28.

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organs studied and cannot be compared to the results of Hackmann. In addition, these counts were made on cross-sectional areas and did not take into account the distortion of tumor numbers produced by even small variations in growth rate of metastases in different organs.

A number of workers have reported upon the effects of adrenal cortical steroids, notably cortisol, on the growth and transplantation behavior of tumors. There is no longer any doubt that cortisol retards the growth of some tumors, particularly the lymphomas (5, 11, 13). In other instances, cortisol promotes their transplantability to alien strains (6, 7, 8, 12) and even allows human tumors to grow in animals under continuous administration of cortisol (17). Agosin et al. (1) described the development of metastases in many organs concomitant with regression of a primary transplantable breast adenocarcinoma in C3H mice receiving cortisol. They concluded that there was a greater tendency of the tumor to shower emboli into the blood stream due to breakdown of the natural barriers around the primary tumor under the influence of cortisol on the hyaluronidase enzyme system and mucopolysaccharides in the connective tissues. This seemed contradictory to the established evidence of an inhibitory action of cortisol on the hyaluronidase system as reported by Anderson et al. (2), Benditt et al. (8), and others. This conclusion also ignores the possibility of continual tumor cell embolism from most tumors regardless of the administration of cortisol. Molomut et al. (15) have observed visceral metastases from scapular implants of Sarcoma I in mice treated with cortisol, and they relate this phenomenon to a breakdown of resistance to heterologous tumor transplantation affecting implantation of tumor emboli as well as the growth of the primary implant.

MATERIALS AND METHODS

To determine whether the metastases noted in Table 1 were the result of the administration of bacterial polysaccharides, cortisone, or both, the experiment was repeated with control groups of polysaccharides alone and cortisone alone (Table 2). Equal numbers of male and female Swiss mice were selected, and half of them were injected daily with 2.5 mg. of cortisone acetate beginning the day after and continuing for 3 days after the intravenous injection of two million Krebs-II ascites tumor cells suspended in mammalian Ringer’s solution. Over 40 per cent of the cortisone-treated mice developed widespread metastases. In addition, there was a significant reduction in the number of tumor-free mice and an increase in the number of mice with pulmonary tumors.

In order to test the “soil” hypothesis as the predominating mechanism in the production of metastasis by cortisol, a suitable method for direct introduction of the tumor cells into the systemic circulation was needed. Direct injection into the left ventricle of the heart was finally successful by utilizing an abdominal approach and introducing the needle through the diaphragm into the heart so that the tip of the needle would lie within the left ventricle during the injection. The technic is

**TABLE 2**

PRODUCTION OF METASTASES OF KREBS-II CARCINOMA BY CORTISONE

(9 x 10⁶ Krebs-II carcinoma cells injected intravenously. All animals autopsied at death or when sacrificed on day 81.)

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Long Metastases-free</th>
<th>Lung</th>
<th>Liver</th>
<th>Ovary</th>
<th>Kidneys</th>
<th>Adrenals</th>
<th>Mult. heart</th>
<th>Mesentery</th>
<th>Pancreas</th>
<th>Lungs</th>
<th>Bone, muscle, eye, subcut., etc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Cells on day 1: 9/81</td>
<td>78/81</td>
<td>9/81</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisone: Cells on day 1; 2.5 mg. cortisol subcutaneously on days 2, 3, and 4</td>
<td>9/77</td>
<td>76/77</td>
<td>92/77</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

TABLE 3

EFFECT OF CORTISONE ACETATE ON DISTRIBUTION OF TUMORS AFTER LEFT VENTRICULAR INTRACARDIAC INJECTION OF KREBS-II AND SARCOMA 87 ASCITES TUMOR CELLS

<table>
<thead>
<tr>
<th></th>
<th>KREBS-II (9 x 10⁶ cells)</th>
<th>SARCOMA 87 (1.5 x 10⁷ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Cortisone</td>
</tr>
<tr>
<td>Liver</td>
<td>0/10</td>
<td>7/7</td>
</tr>
<tr>
<td>Ovaries</td>
<td>5/6</td>
<td>2/2</td>
</tr>
<tr>
<td>Adrenals</td>
<td>0/10</td>
<td>6/7</td>
</tr>
<tr>
<td>Mult. heart</td>
<td>0/10</td>
<td>0/7</td>
</tr>
<tr>
<td>Mesentery</td>
<td>0/10</td>
<td>6/7</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0/10</td>
<td>5/7</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0/10</td>
<td>4/7</td>
</tr>
<tr>
<td>Lungs</td>
<td>1/10</td>
<td>5/7</td>
</tr>
<tr>
<td>Bone, muscle, eye, subcut., etc.</td>
<td>0/10</td>
<td>2/7</td>
</tr>
</tbody>
</table>

*Cells were injected on day 1; cortisone (2.5 mg. subcutaneously) was administered on days 1, 5, 3. All animals were autopsied at death or when sacrificed on day 81. The numerator in each instance indicates the number of animals showing gross metastasis in the organ indicated. The denominator indicates the number of animals in which a successful injection was performed. Failures were easily verified by presence of tumor in chest cavity or heart wall at autopsy. Metastatic tumors were verified histologically except where indicated.

† Gross observation. Not studied histologically.
of the cortisone acetate used for these experiments has been supplied through the courtesy of Merck & Co., Rahway, N.J.) The injection technic for the ascites cells was successful in the number of animals indicated in each column. Survivors were sacrificed 11 days after injection.

RESULTS

The most striking effect of the administration of cortisone was seen in the livers of the animals. All the animals in the cortisone-treated groups had hundreds of metastatic tumors, while no tumors were found in the control groups with either Krebs-II carcinoma or Sarcoma 37 (Figs. 3, 4, and 5). The widespread metastatic tumors after intracardiac injection of Sarcoma 37, in contrast with those after intravenous injection, provide clear evidence of the more efficient filtration of the lungs for this tumor than for the Krebs-II ascites tumor. Metastases in the ovaries and adrenals showed little difference between control and cortisone-treated groups except for frequent hemorrhage in the cortisone-treated ovaries and larger tumor masses in the adrenals in the cortisone-treated groups (Figs. 6 and 7).

Histologic study of the metastatic lesions revealed a reduction of "resistance" of the host tissues under the influence of cortisone and also indicated a marked variation in tissue reactivity in and around the various metastases at 9 days after the last injection of cortisone. Figures 8 and 9 represent different patterns of reaction in cardiac wall metastases in cortisone-treated mice, one showing tumor necrosis with marked lymphocyte and plasma cell infiltration, the other no tumor damage and no appreciable lymphocyte infiltration. Figures 10, 11, and 12 are all from the same section of one cortisone-treated mouse liver. Most of the metastases in the livers exhibited an intermediate pattern like Figure 11. Extreme patterns like Figure 10 (no reaction) and Figure 12 (almost complete destruction of the tumor with only an occasional recognizable tumor cell) were encountered more rarely.

DISCUSSION

The large discrepancy between the development of metastases beyond the lung filter in cortisone- and noncortisone-treated mice suggested the importance of the "soil" in determining the effective establishment of tumor cells or emboli in new locations. That this was not the result of breaking down of mechanical barriers around the pulmonary metastases with subsequent systemic spread was proved by direct injection into the left ventricle, which guarantees immediate delivery of the tumor cells to the host tissues, so that the effect seen must be due to changes in the resistance of these tissues. Since cortisone has been demonstrated by many workers to have a profound, though temporary, destructive effect upon the lymphoid tissues and the reticuloendothelial cells, it seems probable that this affords an explanation for the results obtained. It appears further that antigen-antibody reactions may participate in this phenomenon. This view is strengthened by the histologic patterns observed. Metastases arising from different embolic cells may be antigenically different and may engender different degrees of defense reaction in the various host tissues and indeed even in a single organ such as the liver. Hauschka and Levan (10) have shown a great variability in the chromosome numbers of ascites tumor cells tantamount to genetic multiplicity of each malignant cell population. The spread of the patterns seen in a single section of liver (Figs. 10, 11, and 12) is reminiscent of the wide spread of chromosome numbers for the same tumor. (The ascites tumors used in these experiments were obtained from Hauschka.) Microscopic study suggests that the least damaged tumors probably arose from polyploid cells and that the most damaged ones arose from cells with near-diploid numbers of around 40, while the vast majority with intermediate damage arose from cells with near-tetraploid numbers of around 80. This also coincides with Hauschka's conclusions that aneuploid, genetically imbalanced tumors have a higher transplantability percentage in alien strains of mice than do diploid tumors.

The further elucidation of tissue- and organ-specific antigen-antibody reactions and the role of cortisone and other hormones in the metastatic mechanism exerted, perhaps through their effects on antibody-producing tissues, should promote clarification of seemingly contradictory data concerning hormonal effects on tumor transplantability and on growth of established tumors. Studies are now in progress in this laboratory on the detailed histologic reactions obtained after the intracardiac inoculation technic and cortisone treatment. More precise correlation of tissue-specific antigen-antibody reactions with these patterns and their possible analysis in terms of histocompatibility genes will have to await the development of more reliable methods for demonstrating tissue antibodies and antitumor antibodies.

1 Microscopic comparison of the living cells under phase contrast gives an explanation for this, as the Krebs-II cells are smaller and have a smooth surface, whereas the S-37 cells are not only larger but have a rough surface with many filamentous pseudopodal projections (T. S. Hauschka, personal communication).
SUMMARY

1. Cortisone acetate administered to Swiss mice concomitant with intravenous injections of tumor cell suspensions produced widespread metastasis beyond the lung filter in the case of Krebs-II adenocarcinoma, but not with Sarcoma 37.

2. That this was an effect on local tissues or the "soil" was indicated by a marked discrepancy in number and distribution of both tumors after left ventricular injection of the tumor cells with and without cortisone treatment.

3. A marked variation in tissue reactivity around individual "metastases" in a single organ developed concomitant with recovery of the reticuloendothelial tissues.

4. It was concluded that the major mechanism involved may be an effect of cortisone on the reticuloendothelial tissues resulting in failure of tissue-specific antibody production. The latter may also be dependent upon the variable "antigenicity" of the tumor cells giving rise to each metastasis and thus may be an immunological selection phenomenon.

ACKNOWLEDGMENTS

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REFERENCES


FIG. 1.—Variation in growth rate of metastases in different sites. Small lung tumors as contrasted with large tumors in the mesentery. Krebs-II carcinoma.

FIG. 2.—Large metastatic tumor on chest wall contrasted with small lung and liver metastases. Krebs-II carcinoma.

FIG. 3.—Livers of untreated Swiss mice 11 days after left ventricular injection of Sarcoma 37 ascites cells. Absence of tumors confirmed histologically.

FIG. 4.—Same. Cortisone-treated. For histology, see Figures 10, 11, and 12.
FIG. 5.—Same. Cortisone-treated after partial fixation with Bouin's solution to bring out contrast between tumor and liver tissue.

FIG. 6.—Ovaries of untreated Swiss mice 11 days after intracardiac injection of Sarcoma 37 ascites cells.

FIG. 7.—Cortisone-treated ovaries. Sarcoma 37. Only gross difference was hemorrhagic tendencies in cortisone-treated animals.

FIG. 8.—Cardiac wall metastasis of Sarcoma 37 ascites. Cortisone-treated. Tumor infiltrating between muscle fibers with no reaction (H & E X100).
FIG. 9.—Same. Note the cellular reaction in and around the
tumor. (H & E×100).

FIGS. 10, 11, and 12.—Three liver metastases from the same
tissue section from one cortisone-treated mouse 11 days after
injection of Sarcoma 37 ascites cells and 9 days after the last
injection of cortisone acetate. Note the marked variation in the
reaction in and around the tumors. See text for discussion
(H & E×100).
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