Enhancing Effect of Hydrocortisone on Hematogenous Metastasis of Ehrlich Ascites Tumor in Mice

Mitsuo Kodama and Toshiko Kodama

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SUMMARY

The effect of hydrocortisone on blood-borne tumor metastasis was studied in an i.v. inoculation experiment with Ehrlich hypotetraploid clone 1, Ehrlich hypotetraploid stock, and Ehrlich hyperdiploid stock tumors. The administration of hydrocortisone before tumor inoculation resulted in increased tumor take, reduced mean survival time of mice, and concentration of tumor metastasis in a specific organ (i.e., lung metastasis for Ehrlich hypotetraploid clone 1 tumor, and liver metastasis for Ehrlich hypotetraploid stock and Ehrlich hyperdiploid stock tumors). Enhancement of tumor metastasis, as induced by hydrocortisone pretreatment, was not reproduced by the administration of 6-mercaptopurine, testosterone, or estradiol. The progress of tumor death in hydrocortisone-conditioned mice was not affected by either heparin or dextran sulfate. This indicated that the effect of hydrocortisone on tumor metastasis was independent of the effect of these agents on immune reaction or blood coagulation.

In the tracer experiment with 125I-labeled tumor cells, hydrocortisone pretreatment significantly increased over the control the intrapulmonary retention of Ehrlich hypotetraploid clone 1 tumor cells from 1 through 72 hr after tumor inoculation, the time lag required for the establishment of metastatic foci in the lung. The arrest of Ehrlich hypotetraploid stock and Ehrlich hyperdiploid stock tumors in the liver was also temporarily increased by hydrocortisone pretreatment. No correlation was found between tumor cell size and differential distribution of metastatic tumors with 3 Ehrlich tumors.

An attempt was made to use this blood-borne metastasis system for chemotherapeutic study. Administration of cyclophosphamide gave rise to a significant prolongation of survival time and often to complete prevention of tumor metastasis in hydrocortisone-conditioned mice.

INTRODUCTION

The development of tumor metastasis, as fatal as it is to the host, is a complex phenomenon that can be reproduced only under appropriate conditions. Follow-up studies of many cancer patients indicate that the presence of cancer cells in the circulating blood does not necessarily mean a poor prognosis for a patient. In spite of an abundance of cancer cells in the blood, the patient may survive for many years without any evidence of metastasis (5). Apparently, these blood-borne cancer cells are incapable of growing in the extravascular tissue and are destined to fade away in the long run. This study was initiated to investigate the mechanism of blood-borne metastasis by using 3 cell lines of Ehrlich ascites tumors. It was found that the administration of hydrocortisone before tumor inoculation significantly enhanced the tumor lodgment as well as further development of metastasis in mice and that the major loci of metastasis varied from one cell line to another in the same host environment.

MATERIALS AND METHODS

The hypotetraploid Ehrlich ascites stock tumor was supplied in 1963 by the second Department of Pathology, Nagoya University School of Medicine. Ehrlich ascites hypotetraploid clone 1 tumor, a fast-killing tumor in the i.p. experiment, was isolated in our laboratory from the above hypotetraploid stock tumor in January 1969. The history of these hypotetraploid tumors was described in a previous paper (11). The hyperdiploid Ehrlich ascites stock tumor was supplied in 1963 by Dr. K. Kajiwara of the Takeda Research Laboratories, Osaka, Japan. The tumors were passed through female Swiss/ICR mice at an interval of 3 to 10 days by i.p. transplantation. Female Swiss/ICR mice from Chubu-kagaku and Co., Nagoya, Japan, were used throughout the experiment. Metastatic tumors were produced by inoculating 5 x 10⁶ tumor cells via the tail vein of a mouse (Inoculation Day 0). All deaths before Day 60 were recorded together with macroscopic and microscopic findings. The 60-day survivors were sacrificed with ether and dissected to detect metastatic tumors.

The pharmaceutical agents used in this study were as follows: hydrocortisone acetate (Merck Sharp & Dohme, West Point, Pa.); testosterone (Sigma Chemical Co., St. Louis, Mo.); estradiol (Organon International B.V., Oss, Holland); heparin (Novo Industry, Bagsved, Denmark); dextran sulfate (MDS-A), M.W. of 3500 to 4000 (Kowashinyaku and Co., Nagoya, Japan); cyclophosphamide (Asta-Werke AG, Brackwede, Germany); and 6-mercaptopurine (Takeda Pharmaceutical Co., Osaka, Japan). [125I]IUDR* (specific activity, 19.8 Ci/mmole) was obtained from Schwarz/Mann, Orangeburg, N. Y.

*1 Supported in part by a grant for Cancer Research from the Ministry of Education of Japan.

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The radioactivity of cell suspensions and organs was measured with a well-type scintillation counter (Nihon-musen, Tokyo, Japan) or with a Biogamma T.M. (Beckman Instruments, Inc., Fullerton, Calif.). The ascitic tumor cells were labeled in vivo by i.p. injection of $[^{125}I]UdR, 4 \mu Ci/mouse, on Day 4. After 4 hr, the labeled ascites was collected from each mouse, washed 3 times with 0.9% NaCl solution, and resuspended in M-199 medium to yield an inoculum dose of $5 \times 10^6$ cells/0.2 ml. The radioactivity for the above inoculum ranged from $0.92 \times 10^6$ to $3.20 \times 10^6$ cpm, and autoradiographic tests revealed that most of the above tumor cells accumulated radioactive substance within the nuclei. In the tracer experiment, each mouse received an injection of $5 \times 10^6$ cells via the tail vein. Five mice were sacrificed at intervals to estimate the distribution of labeled tumor cells in the lung, liver, spleen, and kidney for each mouse. The excised organs were stored in individual vials containing 70% ethanol. The alcohol was replaced once a day for 3 days to remove acid-soluble $^{125}I$. The number of labeled tumor cells in each specimen was calculated by comparing the radioactivity of the specimen with that of the original inoculum.

The distribution of tumor cell size was plotted by a Coulter counter type B connected with an automatic size distribution analyzer (Model J) (Coulter Electronics, Inc., Hialeah, Fla.). Histological specimens were prepared by a routine technique and stained with hematoxylin and eosin.

RESULTS

Ability of Ehrlich Ascites Tumor to Metastasize and Effect of Hydrocortisone Administration on Metastatic Process. A striking change of metastatic process was induced by the administration of hydrocortisone with all cell lines tested. Charts 1 to 3 show the effect of hydrocortisone administration on the survival of mice that were inoculated with $5 \times 10^6$ tumor cells via the tail vein on Day 0. The mice of the hydrocortisone pretreatment group received 4 injections of hydrocortisone, 1 mg/day/mouse, before tumor inoculation (from Day $-4$ to Day $-1$). Those of the hydrocortisone posttreatment group were given the same amount of hormone between Days 1 and 4. The hormone pretreatment resulted in a remarkable acceleration of tumor death regardless of the strain of tumor inoculated, and the difference in survival rate became significant at early stages of the experiment (10 to 20 days after inoculation). The hormone posttreatment also reduced the survival rate of mice, but to a lesser extent than did the pretreatment. Another feature of hydrocortisone effect was observed in the localization of metastatic foci. Tables 1 to 3 indicate the loci of metastatic lesions together with the incidence for each category. There exists an apparent difference between the Ehrlich hypotetraploid clone 1 tumor and the other 2 tumors in the rate of tumor take of the control groups, and hydrocortisone pretreatment induced tremendous changes in the distribution of metastatic tumors as well as in the rate of tumor take for all 3 tumor lines tested. That is, over 90% of the mice succumbed to tumor metastasis within 60 days after tumor inoculation. It was found by dissection that mice with Ehrlich hypotetraploid clone 1 tumor were predominantly of lung metastasis type, and those with Ehrlich hyperdiploid stock tumor had mostly extensive liver metastasis. Deaths with Ehrlich hypotetraploid stock tumor consisted of metastases in liver (57.5%), lung (12.5%), pelvic lymph nodes (12.5%), and other tissues (10.0%). The above differences in the distribution of metastatic tumors between the pretreatment group and the

Chart 1. Influence of hydrocortisone administration on the survival of mice with i.v. inoculated Ehrlich hypotetraploid clone 1 tumor. Each group consisted of 40 mice that received $5 \times 10^6$ tumor cells via the tail vein on Day 0. Hydrocortisone acetate, 1 mg/day/mouse, was administered s.c. on Days $-4, -3, -2,$ and $-1$ in the pretreatment group ($\times$), and on Days 1, 2, 3, and 4 in the posttreatment group ($\bullet$). $\circ$, no hormone treatment. The experiment was terminated on Day 60. Details on the origin of the tumor are given in the text.

Chart 2. Influence of hydrocortisone administration on the survival of mice with i.v. inoculated Ehrlich hypotetraploid stock tumor. The experimental conditions are the same as those described in Chart 1.

Chart 3. Influence of hydrocortisone administration on the survival of mice with i.v. inoculated Ehrlich hyperdiploid stock tumor. The experimental conditions are the same as those described in Chart 1.
Table 1

<table>
<thead>
<tr>
<th>Hydrocortisone administration</th>
<th>Lung</th>
<th>Pelvic lymph nodes</th>
<th>Other tissues</th>
<th>60-day survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 None</td>
<td>17 (42.5)</td>
<td>7 (17.5)</td>
<td>4 (10.0)</td>
<td>12 (30.0)</td>
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<tr>
<td>Group 2 Pretreatment*</td>
<td>38 (95.0)</td>
<td>0</td>
<td>1 (2.5)</td>
<td>1 (2.5)</td>
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<tr>
<td>Group 3 Posttreatment*</td>
<td>22 (55.0)</td>
<td>10 (25.0)</td>
<td>2 (5.0)</td>
<td>6 (15.0)</td>
</tr>
</tbody>
</table>

*a Numbers in parentheses, percentage of mice.
*b The experimental conditions for Groups 2 and 3 are described in the legend of Chart 1.

Table 2

<table>
<thead>
<tr>
<th>Hydrocortisone administration</th>
<th>Lung</th>
<th>Liver</th>
<th>Pelvic lymph nodes</th>
<th>Other tissues</th>
<th>60-day survivors</th>
</tr>
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<tbody>
<tr>
<td>Group 1 None</td>
<td>1 (2.5)</td>
<td>0</td>
<td>6 (15.0)</td>
<td>1 (2.5)</td>
<td>32 (80.0)</td>
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<tr>
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<td>5 (12.5)</td>
<td>23 (57.5)</td>
<td>5 (12.5)</td>
<td>4 (10.0)</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>Group 3 Posttreatment*</td>
<td>17 (42.5)</td>
<td>3 (7.5)</td>
<td>4 (10.0)</td>
<td>7 (17.5)</td>
<td>9 (22.5)</td>
</tr>
</tbody>
</table>

*a Numbers in parentheses, percentage of mice.
*b The experimental conditions for Groups 2 and 3 are the same as those described in the legend of Chart 1.

Table 3

<table>
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<tr>
<th>Hydrocortisone administration</th>
<th>Lung</th>
<th>Liver</th>
<th>Pelvic lymph nodes</th>
<th>Other tissues</th>
<th>60-day survivors</th>
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</thead>
<tbody>
<tr>
<td>Group 1 None</td>
<td>7 (17.5)</td>
<td>0</td>
<td>5 (12.5)</td>
<td>4 (10.0)</td>
<td>24 (60.0)</td>
</tr>
<tr>
<td>Group 2 Pretreatment*</td>
<td>1 (2.5)</td>
<td>32 (80.0)</td>
<td>1 (2.5)</td>
<td>5 (12.5)</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>Group 3 Posttreatment*</td>
<td>6 (15.0)</td>
<td>2 (5.0)</td>
<td>9 (22.5)</td>
<td>14 (35.0)</td>
<td>9 (22.5)</td>
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</tbody>
</table>

*a Number in parentheses, percentage of mice.
*b The experimental conditions for Groups 2 and 3 are the same as those described in the legend of Chart 1.

Hydrocortisone and Tumor Metastasis

Influence of hydrocortisone administration on the hematogenous metastasis of Ehrlich hypotetraploid clone 1 tumor in mice

Influence of hydrocortisone administration on the hematogenous metastasis of Ehrlich hypotetraploid stock tumor in mice

Influence of hydrocortisone administration on the hematogenous metastasis of Ehrlich hyperdiploid stock tumor

Control group, as checked by the X² test, were statistically significant with all 3 tumor cell lines. Next, the enhancing effect on tumor metastasis was retested with reduced doses of the same hormone. Prior to tumor challenge, mice in the experimental groups had been conditioned with 1 to 4 injections of hydrocortisone, and the last hormone injection was given 24 hr before tumor inoculation. Each group consisted of 10 mice. As seen in Chart 4, 1 injection of hydrocortisone, 1 mg/mouse, 24 hr before tumor inoculation was not effective enough to induce the enhancement of tumor metastasis, but the survival for the groups with 2 to 4 injections revealed a similar drop by Day 15.

Nature of Hydrocortisone Effect on Tumor Metastasis.

The action of hydrocortisone on a living organism is essentially multidirectional, and it was hoped that a clue for that problem would be available from a comparative study of agents that may have a physiological action either similar to or antagonistic to that of hydrocortisone. The possibility that the enhancing effect of hydrocortisone on tumor metastasis could be related to the process of blood coagulation was tested with heparin, which might prevent the formation of tumor thrombus in the capillary. As before, mice had been conditioned with 4 injections of hydrocortisone, 1 mg/day/mouse, before tumor inoculation. Heparin lente, 500 units/mouse, was administered s.c. 3 hr before tumor inoculation. The metastatic process of Ehrlich hypotetraploid clone 1 in hydrocortisone-conditioned mice was not affected at all by the administration of heparin. Similarly, the administration of dextran sulfate, 1 mg/day/mouse, from Days 0 to 2, resulted in no improvement in the
survival of hydrocortisone-conditioned mice with Ehrlich hypotetraploid clone 1 tumor (the 1st injection of dextran sulfate on Day 0 was given 3 hr before tumor inoculation). The pharmacological actions of these agents in hydrocortisone-conditioned mice were revealed in the prolonged bleeding from the injection sites (the back and the tail), and many mice were excluded from the experiment because they died of blood loss within 48 hr after tumor inoculation. It might be argued that the observed enhancement of tumor metastasis was just an end result of the suppressed immune response of host under the influence of hydrocortisone. However, the administration of 6-mercaptopurine, 2 mg/day/mouse, from Days −4 to −1, resulted in no acceleration of tumor metastasis in mice inoculated with $5 \times 10^6$ tumor cells of Ehrlich hypotetraploid clone 1. Furthermore, the effects of 6-mercaptopurine and hydrocortisone on metastatic tumor take of the same cell line were compared with various levels of tumor inoculum. Table 4 summarizes the experimental conditions and results. The 50% tumor take doses for the hydrocortisone pretreatment group, 6-mercaptopurine pretreatment group, and control group were $5.0 \times 10^4$, $3.6 \times 10^4$, and $6.0 \times 10^4$ cells, respectively. Should the enhancing effect of hydrocortisone on tumor metastasis be involved in the immune mechanism, this hormone will have to be dissociated from 6-mercaptopurine regarding the action mechanism. Finally, the effects of 2 additional steroids were compared with that of hydrocortisone on the metastatic process of Ehrlich hypotetraploid clone 1 tumor and Ehrlich hyperdiploid stock tumor. As before, testosterone, estradiol, and hydrocortisone, 1 mg/day/mouse each, were administered for 4 consecutive days before tumor inoculation. In both tumor lines the survival curves for both the testosterone pretreatment group and estradiol pretreatment group were in good accordance with that of the control group, and no substantial change was detected in the distribution of metastatic tumors for the above sex steroid groups, as compared to the control. It is clear that the acceleration of tumor metastasis is specifically associated with the physiological action of hydrocortisone.

**Table 4**

<table>
<thead>
<tr>
<th>Pretreatmenta</th>
<th>Cell dose of the inoculumb</th>
<th>No. of tumor takes/total no. of micec</th>
<th>% tumor take, calculated by the Reed-Muench method</th>
</tr>
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<tr>
<td>Hydrocortisone</td>
<td>$5 \times 10^4$</td>
<td>0/20</td>
<td>0</td>
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<tr>
<td></td>
<td>$5 \times 10^5$</td>
<td>1/20</td>
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<td>$5 \times 10^6$</td>
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<tr>
<td></td>
<td>$5 \times 10^7$</td>
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<td>50.0</td>
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<td>$5 \times 10^8$</td>
<td>15/20</td>
<td>84.8</td>
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<tr>
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<td>$5 \times 10^9$</td>
<td>20/20</td>
<td>100.0</td>
</tr>
<tr>
<td>6-Mercaptopurine</td>
<td>$5 \times 10^4$</td>
<td>6/20</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>$5 \times 10^5$</td>
<td>9/20</td>
<td>55.6</td>
</tr>
<tr>
<td></td>
<td>$5 \times 10^6$</td>
<td>19/20</td>
<td>97.1</td>
</tr>
<tr>
<td>None</td>
<td>$5 \times 10^4$</td>
<td>2/20</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>$5 \times 10^5$</td>
<td>9/20</td>
<td>45.8</td>
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<tr>
<td></td>
<td>$5 \times 10^6$</td>
<td>18/20</td>
<td>93.5</td>
</tr>
</tbody>
</table>

* In the pretreatment groups, hydrocortisone acetate, 1 mg/day/mouse, or 6-mercaptopurine, 2 mg/day/mouse, were administered s.c. from Day −4 through Day −1.  
* The tumor inoculum was given on Day 0 via the tail vein.  
* The experiment was terminated on Day 60.

**Chart 4.** Relation between hydrocortisone dose and the survival of mice with metastatic Ehrlich hypotetraploid clone 1 tumor. ■, hydrocortisone, 1 injection of 1 mg/mouse; x, hydrocortisone, 4 injections of 1 mg/mouse; △, hydrocortisone, 4 injections of 0.5 mg/mouse; ●, no hormone treatment. Details of the experiment are given in the text.

**Chart 5.** Effect of hydrocortisone pretreatment on the retention of i.v. inoculated Ehrlich ascites tumor cells in the lung of a mouse. Mice in the hydrocortisone pretreatment group were given 4 hydrocortisone injections, 1 mg/day/mouse, and $5 \times 10^5$ ^125^I-labeled tumor cells were inoculated via the tail vein of hormone-treated and nontreated mice 24 hr after the last hormone injection. A total of 5 mice were sacrificed for each group and for each time interval, and the radioactivity was determined for each mouse and for each organ. Symbols represent the mean values of 5 mice each. ●, Ehrlich hypotetraploid clone 1; △, Ehrlich hypotetraploid stock; x, Ehrlich hyperdiploid stock; ---, nontreated group; - - - -, hydrocortisone pretreatment group.
less than 1% of the original inoculum 24 hr after tumor inoculation, whereas more than $1 \times 10^4$ tumor cells were arrested in the lung of a hydrocortisone-conditioned mouse at the same stage. The rate of tumor retention in the lung of a conditioned mouse was always higher for Ehrlich hypotetraploid clone 1 tumor than for the other 2 tumors during an observation period of 72 hr. By Student's $t$ test the difference between the hydrocortisone pretreatment and the control groups, as detected regarding the retention of clone 1 tumor cells in the lungs, was statistically significant from 1 through 72 hr after tumor inoculation. Also significant were the differences both between Ehrlich hypotetraploid clone 1 tumor and Ehrlich hypotetraploid stock tumor and between Ehrlich hypotetraploid clone 1 tumor and Ehrlich hyperdiploid stock tumor concerning the tumor retention in the lung of a hydrocortisone-conditioned mouse 72 hr after tumor inoculation. These results could be related to the predominant occurrence of lung metastasis in hydrocortisone-conditioned mice with Ehrlich hypotetraploid clone 1 tumor. Chart 6 indicates the tumor cell retention in the liver with and without hydrocortisone pretreatment. Contrary to the case of lung, the retention of labeled tumor cells in the liver 1 hr after tumor inoculation was significantly higher in a control mouse than in a hydrocortisone-conditioned mouse for all tumor lines tested. The situation was reversed at the 24th and 48th hr and the difference was more marked with Ehrlich hypotetraploid stock tumor and Ehrlich hyperdiploid stock tumor than with Ehrlich hypotetraploid clone 1 tumor, but the above dominance of hydrocortisone-conditioned mice over the control mice vanished at the 72nd hr. The increased retention of tumor cells in the liver of a hydrocortisone-conditioned mouse was observed only temporarily, and the observed difference was not so striking as that of lung retention. The question of whether the observed incidence of liver metastasis with Ehrlich hypotetraploid stock tumor and Ehrlich hyperdiploid stock tumor could be accounted for by the number of tumor cells retained in the liver was further explored in the next experiment. Ehrlich hyperdiploid stock tumor cells were labeled in vivo with $[^{125}\text{I}]]\text{IUDR}$, and an inoculum dose of $5 \times 10^6$ cells/mouse was given to a number of control and hydrocortisone-conditioned mice via the spleen. Five mice from each group were sacrificed at the 24th and 72nd hr after tumor inoculation for the estimation of labeled tumor cells in the spleen and liver. The remaining mice were used for the follow-up study of liver metastasis. The incidence of liver metastasis was 0 of 10 (0%) for the control group and 1 of 12 (83.3%) for the hydrocortisone pretreatment group; the difference between these groups, as checked by the $\chi^2$ test with Yates' correction, was statistically significant. The number of tumor cells retained in the liver at the 24th hr was $231,000 \pm 360,000$ (5 mice) for the control group and $1,450,000 \pm 830,000$ (5 mice) for the hydrocortisone pretreatment group; the difference was again statistically significant. It was shown that most of the inoculated tumor cells entered the blood circulation through the liver within 24 hr and that the hydrocortisone pretreatment increased the tumor retention in the liver (the difference at the 72nd hr was not statistically significant).

The tracer experiment revealed that the number of labeled tumor cells retained at the 72nd hr in the lung and liver of a hydrocortisone-conditioned mouse was not very high compared to the initial tumor inoculum. How much time is required, then, for an arrested tumor cell to establish a metastatic focus? The time sequence of tumor proliferation in the lung was followed histologically in mice inoculated with Ehrlich hypotetraploid clone 1 tumor. The lung of a hydrocortisone-conditioned mouse excised 1 and 24 hr after tumor inoculation contained many single-cell foci but no nest of tumor cells. After 48 hr, the number of single-cell foci decreased and a few multicellular nests appeared either within the alveolar network or on the pleural surface. After 72 hr, the number and size of distinct metastatic foci increased further. At the 120th hr, the portion of healthy lung tissue was reduced remarkably because of exuberant growth of tumor tissue. In the lung of a nonconditioned mouse, a few small tumor nests appeared at the 72nd hr and distinct metastatic foci increased in size and number at the 120th hr. It was concluded that metastatic foci in the lung were established between 48 and 72 hr following the entry of tumor cells into the blood circulation.

**Cell Size of Ehrlich Ascites Tumor.** The observation that there was a difference among the 3 tumor cell lines in the intrapulmonary retention of tumor cells, as well as in the distribution of metastatic tumors of hydrocortisone-conditioned mice, raised a question of whether the difference in tumor cell size is responsible for the differential distribution of metastatic tumors in mice. In the cell size distribution diagram of a given ascites lot, the mode was used as the representative value of that ascitic cell population. To minimize the influence of host environment on tumor cell size, the modes with more than 10 mice were determined separately for each mouse, and the mean and standard deviation for the above mode were used for the statistical analysis. Table 5 shows that the mean cell volume of Ehrlich hyperdiploid stock tumor is significantly larger than that of hypotetraploid tumors. However, there is essentially no difference in cell size between Ehrlich hypotetraploid clone 1 tumor and its ancestor Ehrlich hypotetraploid stock tumor. The low incidence of lung metastasis for Ehrlich hypotetraploid stock tumor is not to be explained in terms of size difference (Table 2).

**Effect of Cyclophosphamide Administration on Tumor Metastasis.** The merit of our hydrocortisone conditioning in
The administration of cyclophosphamide again prolonged tumors depends much on the time schedule and the route of tumor regression, the effectiveness of this drug in both this tumor. On the basis of the survival time and rate of the survival time of hydrocortisone-conditioned mice with tumor inoculation. An inoculum of $5 \times 10^6$ tumor cells was the production of experimental tumor metastasis could be metastasis was not reproduced either by sex steroids (testosterone and estradiol) or by an immunosuppressant (6-mercaptopurine). The administration of heparin and dextran sulfate had little effect on the progress of tumor metastasis in hydrocortisone-conditioned mice. These results indicate that the enhancing effect of hydrocortisone on tumor metastasis (1, 2, 7, 10, 13, 14, 16, 18). The lack of enhancing effect might have come from some unidentified characteristics of the host-tumor system (7), the interference of histocompatibility gene (10), or the dominance of tumor-suppressive effect over the metastasis-enhancing effect of cortisone (14). The literature is contradictory concerning the significance of thrombus formation in the course of the control of the above agents. The enhancement of tumor metastasis by hydrocortisone could be explained in part by an increased tumor cell arrest in a target tissue, which was confirmed in the tracer experiment with $^{125}$I-labeled tumor cells (Charts 5 and 6). However, it is plausible that metabolic changes of a target tissue after hormone injection may yield a new environment favorable for the establishment of metastatic tumor. The finding that extensive liver metastasis was induced by hydrocortisone pretreatment, in spite of a relatively small amount of tumor cell arrest in the liver, should be further explored from the metabolic standpoint.

There are some disputes about the effect of cortisone on tumor metastasis (1, 2, 7, 10, 13, 14, 16, 18). The lack of enhancing effect might have come from some unidentified characteristics of the host-tumor system (7), the interference of histocompatibility gene (10), or the dominance of tumor-suppressive effect over the metastasis-enhancing effect of cortisone (14). The literature is contradictory concerning the significance of thrombus formation in the course of

<table>
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<tr>
<th>Tumor strain</th>
<th>No. of mice used</th>
<th>Cell volume</th>
<th>$p$</th>
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<tr>
<td>Hypotetraploid clone 1</td>
<td>13</td>
<td>$1676 \pm 300^a (14.7)^b$</td>
<td>0.7 &lt; $p$ &lt; 0.8</td>
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<tr>
<td>Hypotetraploid stock</td>
<td>11</td>
<td>$1641 \pm 332 (14.6)$</td>
<td>&lt; 0.001</td>
</tr>
<tr>
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<td>13</td>
<td>$1053 \pm 211 (12.6)$</td>
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</table>

* Mean ± S.D. (cu mm)

* Each measurement represents the mode of the tumor population from 1 mouse, as determined by a Coulter counter type B. The number in parentheses indicates the mean diameter (mm) of a globular tumor cell. The above size distribution analysis was carried out with the ascites tumor cells of 4th inoculation day.

DISCUSSION

The enhancing effect of hydrocortisone on tumor metastasis was not reproduced either by sex steroids (testosterone and estradiol) or by an immunosuppressant (6-mercaptopurine). The administration of heparin and dextran sulfate had little effect on the progress of tumor metastasis in hydrocortisone-conditioned mice. These results indicate that the observed enhancement of tumor metastasis is specific for hydrocortisone, and it is in itself independent from the steps of immune reaction or blood coagulation, which are under
tumor metastasis (3, 6, 8, 9, 12, 17). The possible participation of blood coagulation in the enhancement of tumor metastasis by hydrocortisone was not substantiated in this study.

The i.v. inoculation of cancer cells in an experimental animal might be compared to the induction of showers of cancer cells during a surgical operation. It is anticipated that the level of hydrocortisone in the blood of the patient before, during, and after an operation is very high because of mental and physical stress. Our experimental system may serve as a simulation model for the study of metastasis prevention. It was reported that the effect of nitrogen mustard on tumor metastasis varied depending upon the time lag between tumor inoculation and administration of the drug; the earlier the start of treatment, the higher the effectiveness of the drug (4, 15). In this study, administration of cyclophosphamide resulted in a significant prolongation of survival time of mice and often complete prevention of tumor metastasis (Charts 7 and 8). Further studies along this line may render a clue for the prevention of metastasis in human neoplasia.

ACKNOWLEDGMENTS

We are grateful to Dr. H. Amô for help with the histological and autoradiographic analysis, and to Dr. T. Katô for help with the radioactivity measurements. We also thank T. Yokochi, K. Yamauchi, M. Ishikawa, and K. Naruse for excellent technical assistance and Dr. K. Ota for continued encouragement.

REFERENCES

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