Kinetics of Proliferation, Migration, and Death of L1210 Ascites Cells

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SUMMARY

L1210 leukemia cells were labeled with multiple injections of iododeoxyuridine\(^{125}\text{I}\) or tritiated thymidine and injected i.p. into new, nonradioactive hosts. The overall growth rate of the prelabeled L1210 cells was evaluated in terms of cell cycle length, growth fraction, and cell loss due to cell migration and cell death.

Following implantation into a new host, there was a 6- to 12-hr lag period before peritoneal L1210 cells started to divide. During the first 3 days of tumor growth, the average generation time remained constant at 8.5 to 9.5 hr. Then the length of the cell cycle gradually increased until it reached 32 to 36 hr shortly before the death of the mice. The growth fraction remained close to unity throughout tumor development.

Implantation of L1210 cells i.p. was followed by rapid migration of tumor cells from the injection site to other organs. Fractional L1210 metastasis was very high during early phases of tumor growth, and much slower in advanced tumors. L1210 cells injected at extraperitoneal sites did not invade the peritoneal cavity.

The rate of cell death depended on the location of tumor cells within the host. L1210 cells located outside the peritoneal cavity died at the fractional rate of 20%/day. Cell death among peritoneal L1210 cells was much lower (less than 5%/day) and did not significantly influence the overall growth of L1210 ascites populations.

INTRODUCTION

Numerous studies have shown that the growth rate of experimental tumors varies during the course of tumor development. As a rule, the increase in cell number is rapid during the early phases of tumor growth and then gradually slows as the tumor gets older. Recent studies on the relationship between the overall growth rate of tumors and individual cell proliferation parameters have indicated that during later phases of tumor development either an increasing fraction of tumor cells stops dividing (2, 18, 26) or the duration of the mitotic cycle increases (1, 3, 6, 8, 19, 28, 30, 31). Another factor contributing to the gradual deceleration of growth in advanced tumors could be increased “cell loss” due to either accelerated cell death (11, 13, 20) or increased migration of cells away from the site of tumor inoculation. Sometimes 2 or more of these factors combine to cause a progressive decrease in the growth rate (4, 17, 27).

\(^{125}\text{I}\)-labeling techniques in combination with autoradiography have contributed greatly to the evaluation of growth parameters at the cellular level. However, many aspects of tumor growth, particularly tumor cell migration and tumor cell death, cannot be directly investigated with autoradiographic techniques (16, 25). This report describes an alternate method for evaluating the growth parameters of experimental tumor populations. This method involves the labeling of tumor cells with IUDR\(^{125}\text{I}\), an analog of TdR, which is incorporated exclusively into the DNA of proliferating cells (5, 11, 12, 14, 21). After incorporation into the DNA, IUDR\(^{125}\text{I}\) remains bound within the cells until they die (5, 13, 14). When a labeled cell dies, rapid deiodination and excretion of the DNA breakdown products limit the reutilization of radioactivity by other cells (7, 13, 14). It thus becomes possible to estimate directly the rate of tumor cell death in vivo by continuously monitoring the rate of \(^{125}\text{I}\) excretion from individual live mice inoculated with IUDR\(^{125}\text{I}\)-labeled tumor cells (13).

In addition, the \(^{125}\text{I}\) activity of various organs can be measured at different time intervals after injection of prelabeled tumor cells into nonradioactive hosts, permitting a quantitative study of fractional tumor cell metastasis (11, 13). This should prove very useful in distinguishing between tumor cell metastasis and tumor cell death, the 2 basic modes of cell loss from the original site of inoculation.

The \(^{125}\text{I}\)-prelabeling procedure might also prove useful in evaluating the rate of tumor multiplication by allowing monitoring of the rate of decrease in DNA-specific \(^{125}\text{I}\) activity. In the case of peritoneal L1210 cells where the growth fraction appears to be close to 100% throughout the development of the tumor (30), the decline in \(^{125}\text{I}\) activity per cell should provide a direct measure of the mean generation time of the tumor. Ideally, the IUDR\(^{125}\text{I}\) prelabeling procedure could thus make it possible to evaluate simultaneously in 1 single experiment the rates of tumor cell proliferation, cell metastasis, and cell death, the 3 basic parameters of population growth.

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\(^{1}\) The abbreviations used are: IUDR, iododeoxyuridine; TdR, thymidine.
**Growth Parameters of L1210 Ascites Cells**

**MATERIALS AND METHODS**

**Labeling Techniques.** L1210 mouse lymphoid leukemia cells growing in the peritoneal cavity of 10- to 12-week-old C3H X DBA/2J F1 (hereafter called C3D2F1) female mice were prelabeled with IUDR-125I (specific activity, 10^2 to 10^3 Ci/m mole; source, New England Nuclear Corporation, Boston, Mass.). The total labeling dose was 1.2 µCi IUDR-125I/mouse, administered i.p. in 6 injections of 0.2 µCi IUDR-125I/mouse on Day 4 after i.p. implantation of 10^6 L1210 cells. The 6 injections were given at successive intervals of 2.5 hr; i.e., the total labeling time was 12.5 hr. This is more than double the time required to assure complete labeling of logarithmically growing peritoneal L1210 cells (30). Twelve hr after the completion of the IUDR-125I labeling course, the tumor cells were harvested, washed, and prepared for reinjection into nonradioactive hosts, essentially as described in earlier reports (10, 13).

For autoradiographic studies, L1210 cells were labeled with TdR-3H (specific activity, 19 Ci/m mole; source, Schwarz Bio-Research, Inc., Orangeburg, N.Y.). The TdR-labeling procedure was identical with the IUDR-125I-prelabeling schedule, except that the total TdR-3H dose was 12 µCi/mouse. For the preparation of autoradiographs, smears of ascites cells were made on clean slides. The slides were then dipped in Kodak NTB-2 emulsion, exposed for 2 to 8 weeks, and developed in Kodak D19 developer.

**Measurement of Tumor Growth Parameters.** For the study of the overall growth rate of L1210 ascites populations, groups of 5 mice were sacrificed in a chloroform chamber at various time intervals after i.p. implantation of 2.5 X 10^6 L1210 cells. Immediately after the death of the host, tumor cells were harvested by repeatedly rinsing the peritoneal cavity of each animal with 10 ml heparinized 0.9% NaCl solution. The total number of cells obtained from the peritoneum was determined by counting with a hemocytometer. Heavy infiltration of host leukocytes into the peritoneal cavity, which often amounted to 3 to 8 million cells, made it difficult to obtain reliable leukemia cell counts during the first 2 or 3 days of tumor development. Injection of TdR-3H-prelabeled L1210 cells (labeling index, 95% or higher), followed by autoradiographic evaluation of the cell mixtures recovered from the peritoneal cavities of recipient mice, made it possible to determine the actual number of L1210 ascites cells (total peritoneal cell count X labeling fraction-number of peritoneal L1210 cells). Although this procedure yielded results only during the first 2 days of tumor development, this was not a serious limitation, because the fractional contribution of host leukocytes to the total cell population was most important during such early phases of tumor growth. After Day 2, when the peritoneal tumor population became very large, correction for host cells was accomplished by determining the growth fraction and multiplying each experimentally determined cell count by this factor.

The growth fraction was estimated by determining the labeling index of the peritoneal tumor population after repeated TdR-3H injections, administered over a period of 12.5 hr (until Day 4) or 15 hr (Day 5 and 6). Two hr after the last TdR-3H injection, the tumor cells were harvested and autoradiographed, and the labeling index was used to provide an estimate of the growth fraction. This technique, like other methods used for measuring the growth fraction of tumor populations, gives rather poor resolution between cells with long cell cycle times and cells that are not proliferating at all (27). Nevertheless, since the labeling index after 12.5 or 15 hr of TdR-3H administration was always 90% or higher (Table 4), it can be assumed that the growth fraction of peritoneal L1210 cells remained close to unity throughout tumor development. These results confirm the observations of Yankee et al. (30), who found that even during very late stages of tumor growth virtually all peritoneal L1210 cells continue cell multiplication.

The techniques used for determining the rate of cell death and cell migration have been reported previously (13) and are essentially unchanged in this study. Briefly, whole-body 125I retention was monitored at daily intervals throughout tumor development by counting individual live mice in a well-type crystal scintillator. The rate of 125I excretion from mice bearing prelabeled L1210 cells was taken as an index of tumor cell death in vivo. For study of the rate of tumor cell migration, the anatomical distribution of radioactivity was determined on groups of 5 mice, which were killed in a chloroform chamber at various times after inoculation with prelabeled L1210 cells. The 125I activity in the DNA of the peritoneal tumor cells, the liver, the spleen, the intestine, the skin, and the rest of the body was measured after homogenization of all organs (except the skin), followed by precipitation of the DNA with 10% trichloroacetic acid to remove 125I activity not incorporated into the DNA (12, 14).

**RESULTS**

**Overall Tumor Growth.** The solid line in Chart 1 shows the increase in the number of cells recovered from the peritoneal cavity at various times after i.p. implantation of 2.5 X 10^6 TdR-3H-labeled L1210 cells. No significant increase in the total cell count was noted during the first 24 hr after implantation. After this initial delay, the number of cells obtained from the peritoneum increased rapidly until it reached a value of about 650 X 10^6 cells on Day 6 of tumor growth, shortly before the host mice died. However, during the course of tumor development the doubling time of the peritoneal cell population (L1210 cells plus host cells) gradually increased from 11 to 12 hr (2-day tumor) to 38 to 42 hr (6-day tumor).

During early phases of tumor growth, most of the cells obtained from the peritoneal cavity are host cells. Absolute cell counts are therefore meaningless in describing the behavior of tumor populations during the first 48 hr after implantation. The dashed line in Chart 1 shows the “corrected” number of peritoneal L1210 cells as obtained by autoradiographic evaluation of cell samples. From these results, it would appear that the number of L1210 cells present in the peritoneal cavity declined during the first 12 hr after inoculation. Thereafter, the number of L1210 ascites cells increased rapidly (doubling time in early tumors was 9.5 to 10.5 hr) until the 2 curves became virtually superimposable after Day 2 of tumor growth.
Chart 1. Total peritoneal cell count (solid curve) and autoradiographically determined number of L1210 ascites cells (dashed curve) at various times after i.p. injection of 2.5 x 10^6 TdR^3H-labeled L1210 cells. Each point represents the average of 5 mice ± S.D. when the presence of host leukocytes no longer contributed significantly to the total cell number.

Tumor Cell Metastasis. The initial decline in the number of peritoneal L1210 cells could be due to a variety of reasons, such as incomplete harvesting of peritoneal tumor cells, increased rates of cell death, or rapid migration of tumor cells out of the peritoneal cavity. For differentiation between these possibilities, groups of mice were inoculated i.p. with 2.5 x 10^6 IUDR-^{125}I-labeled L1210 cells, and the anatomical distribution of ^{125}I activity within the body of the host animals was determined on mice sacrificed at various intervals after tumor implantation. Almost 80% of the injected ^{125}I activity was recovered from the peritoneal cavity immediately after tumor implantation (Chart 2). Even 6 hr after transplantation, 50% of the injected radiiodide could be obtained from the peritoneum. However, the proportion of radioactivity present in the peritoneal cavity continued to decline until it reached 21% of the injected dose on Day 1 of tumor development. During the same time, extraperitoneal ^{125}I rose sharply from 14% to 57% of the injected dose. After Day 1 the amount of radioactivity associated with the peritoneal portion of the tumor population changed very little throughout the remainder of the experiment, while extraperitoneal ^{125}I retention declined at a rate of approximately 16 to 20%/day.

Table 1 lists the precise organ distribution of ^{125}I 1 and 5 days after injection of IUDR-^{125}I-labeled L1210 cells. Again, peritoneal ^{125}I retention declined very little during this time period, while the liver, the spleen, and the rest of the body lost more than half of the radioactivity present in these organs on Day 1 of tumor growth. Since the fractional retention of radioactivity in most organs (except intestine and skin) can be taken as an index of tumor cell migration (13), the results presented in Table 1 and Chart 2 indicate that large numbers of tumor cells left the peritoneal cavity during the first 24 hr of tumor development. After Day 1, the rate of tumor cell metastasis apparently decreased considerably.

The progressive decline in the rate of tumor migration from the peritoneal cavity to other parts of the body could be due to either limited capacity of migration pathways or overcrowding of extraperitoneal growth sites. For demonstration of possible limitations in the capacity of migration pathways, groups of mice were inoculated i.p. with 1, 2, 5, 10, 50, or 200 million labeled L1210 cells. The recipient mice were sacrificed and dissected 24 hr after tumor implantation. From the anatomical distribution of ^{125}I activity within these mice, it was apparent that the fractional rate of tumor cell migration decreased as the size of the inoculum increased. For example, only about 12% of the...
injected $^{125}$I activity could be recovered from the peritoneal cavity 24 hr after injection of 1 million labeled L1210 cells, whereas almost 70% of the total radioactivity was present at the site of inoculation in mice bearing 200 million cells (Table 2).

On the other hand, the number of extraperitoneal growth sites available for metastasizing tumor cells did not appear to be a limiting factor in determining the rate of tumor cell migration. For investigation of this possibility, mice were inoculated i.v. with $10^6$ unlabeled L1210 cells. Three days later, when the livers and spleens of the recipient mice were already considerably enlarged as a result of the rapid growth of i.v. injected L1210 cells in these organs (12, 29), the leukemic mice were inoculated i.p. with $2.5 \times 10^6$ IUDR-$^{125}$I-labeled tumor cells. Again the mice were killed 24 hr after the 2nd implant, and the anatomical distribution of radioactivity was determined. There was no difference in the rate of $^{125}$I transfer out of the peritoneal cavity between control animals and mice that had previously received i.v. inocula of unlabeled L1210 cells, i.e., both groups of animals showed the same anatomical distribution of $^{125}$I activity as is shown in the Day 1 (Living L1210) column of Table 1.

For determination of whether tumor cells that have migrated to extraperitoneal sites are able to return into the peritoneal cavity, living or heat-killed IUDR-$^{125}$I-labeled L1210 cells were injected i.v. into control mice and into mice that had received i.p. implants of unlabeled tumor cells 3 days before receiving the labeled inoculum. Since only trace amounts of radioactivity could be discovered within the peritoneal cavity (data not shown), it was concluded that tumor cells growing at extraperitoneal sites were unable to invade the peritoneal cavity.

**Tumor Cell Death.** The fractional rate of $^{125}$I excretion from the leukemic mice (Chart 2, whole-body curve) closely followed the pattern obtained in earlier experiments with the same tumor line (13). After i.p. injection of labeled L1210 cells, 10 to 15% of the $^{125}$I activity was lost from the recipient mice each day throughout tumor development. Most of this loss seemed to occur among tumor cells located outside the peritoneal cavity, where the rate of $^{125}$I disappearance after Day 1 of tumor growth was approximately 16 to 20%/day. This compares with a fractional loss of 5%/day from L1210 ascites cells. Since, after Day 1 of tumor development, two-thirds to three-fourths of the remaining tumor cells were located at extraperitoneal sites, their 16 to 20% death rate alone could have accounted for the composite 10 to 15% death rate observed in the whole body of the animals.

For determination of whether the presence of a large peritoneal tumor cell population during later stages of tumor growth might have caused the low rate of $^{125}$I loss from ascites cells (for instance, by inhibiting the removal of $^{125}$I-labeled breakdown products from the peritoneal cavity), the following experiment was carried out. Heat-killed $^{125}$I-labeled tumor cells were added to unlabeled peritoneal L1210 populations at various stages of tumor development. Twenty-four hr after receiving the labeled implants, the doubly inoculated mice were killed, and the amount of radioactivity retained in the peritoneal cavity was determined. The results (Table 3) showed that $^{125}$I removal from i.p. injected dead cells was very rapid throughout tumor development, although some slowing was observed in advanced tumors.

The low rate of $^{125}$I transfer out of the peritoneal cavity, therefore, can be taken as an index of low fractional cell loss from peritoneal L1210 populations. As stated above, most or all of the $^{125}$I loss from peritoneal L1210 cells represents tumor cell metastasis rather than cell death. Since such continuing migration would tend to partially replenish the $^{125}$I content at extraperitoneal sites, the actual rate of extraperitoneal cell death must have been more than 20%/day. This value would agree very well with the whole-body death rate observed after i.m. injection of L1210 cells (9, 13), when the entire tumor population was located outside the peritoneal cavity. Thus, although the available evidence does not constitute absolute proof that the rate of cell death among

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**Table 1**

*Anatomical distribution of $^{131}$I activity 24 and 120 hr after i.p. injection of $2.5 \times 10^6$ living or heat-killed labeled L1210 cells*

<table>
<thead>
<tr>
<th>Organ</th>
<th>Day 1 Living</th>
<th>Day 1 Dead</th>
<th>Day 5 Living</th>
<th>Day 5 Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body</td>
<td>85.1 ± 2.2</td>
<td>14.6 ± 1.7</td>
<td>49.1 ± 1.9</td>
<td>1.68 ± 0.26</td>
</tr>
<tr>
<td>Peritoneal L1210</td>
<td>21.2 ± 2.7</td>
<td>0.6 ± 0.2</td>
<td>16.4 ± 2.1</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td>Liver</td>
<td>6.8 ± 1.1</td>
<td>1.6 ± 0.4</td>
<td>2.9 ± 0.6</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.4 ± 0.4</td>
<td>0.3 ± 0.1</td>
<td>0.5 ± 0.2</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Intestine</td>
<td>1.6 ± 0.2</td>
<td>2.4 ± 0.3</td>
<td>1.2 ± 0.2</td>
<td>0.34 ± 0.08</td>
</tr>
<tr>
<td>Skin</td>
<td>5.1 ± 0.9</td>
<td>3.7 ± 0.8</td>
<td>2.8 ± 0.5</td>
<td>0.41 ± 0.18</td>
</tr>
<tr>
<td>Rest of body</td>
<td>42.7 ± 6.2</td>
<td>4.0 ± 1.1</td>
<td>19.4 ± 2.2</td>
<td>0.52 ± 0.17</td>
</tr>
</tbody>
</table>

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**Table 2**

*Anatomical distribution of $^{131}$I activity 24 hr after i.p. injection of 1, 2, 5, 10, 50, or 200 million labeled L1210 cells*

<table>
<thead>
<tr>
<th>Organ</th>
<th>1</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>50</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peritoneal L1210</td>
<td>12.3 ± 2.6</td>
<td>18.9 ± 2.2</td>
<td>24.5 ± 2.0</td>
<td>36.1 ± 2.8</td>
<td>49.6 ± 4.0</td>
<td>67.3 ± 5.1</td>
</tr>
<tr>
<td>Liver</td>
<td>7.4 ± 1.1</td>
<td>6.6 ± 1.2</td>
<td>4.9 ± 0.8</td>
<td>2.1 ± 0.4</td>
<td>1.8 ± 0.4</td>
<td>1.4 ± 0.3</td>
</tr>
</tbody>
</table>
L1210 ascites cells is zero, it must be so low as to be insignificant.

**Tumor Cell Proliferation.** Chart 3 shows the rate of decline of $^{125}$I activity/10^6 labeled L1210 cells throughout tumor development. The data presented in Chart 3 were obtained by dividing the total amount of $^{125}$I activity harvested from the peritoneal cavity of each mouse (Chart 2, intraperitoneal $^{125}$I).

**Table 3**

<table>
<thead>
<tr>
<th>Day of implantation</th>
<th>Peritoneal $^{125}$I retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>2</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>3</td>
<td>5.4 ± 0.7</td>
</tr>
<tr>
<td>4</td>
<td>7.6 ± 0.9</td>
</tr>
<tr>
<td>5</td>
<td>13.5 ± 1.6</td>
</tr>
</tbody>
</table>

Each value represents the average of 5 mice, expressed as percentage of the injected $^{125}$I counts ± S.D.

DISCUSSION

**Limitations of the IUDR-$^{125}$I Prelabeling Procedure.** The overall growth rate of tumor populations, like that of any other population, is the net result of cell proliferation, cell migration, and cell death. Since the growth fraction of L1210 ascites cells remains close to 100% throughout tumor growth (Table 4), the halving time in the $^{125}$I activity per million labeled cells directly reflects the average generation time of the peritoneal tumor population if (a) the $^{125}$I in the inoculum is associated exclusively with the DNA of the tumor cells and is not released from the DNA of the labeled cells while they are alive; (b) the radioactive label is distributed uniformly throughout the initial cell population; (c) the incorporated radioisotope does not affect the rate of division or death of labeled cells; and (d) radioactively labeled compounds are rapidly excreted following the death of labeled cells.

IUDR-$^{125}$I administered in small doses over relatively long periods of time appears to fulfill all of these requirements. In earlier studies, we have demonstrated that IUDR-$^{125}$I is incorporated into the DNA of L1210 cells (12) and does not leave these cells until they die (13). The question of radiation effects from the incorporated radioisotope also must be carefully considered, because intranuclear $^{125}$I appears to be extraordinarily radiotoxic (11, 13, 15). In the case of the L1210 tumor line, $^{125}$I incorporation must be kept below 0.03 μCi/million labeled cells to avoid adverse effects of $^{125}$I irradiation on the viability of transplanted tumor cells (13). In the studies discussed in this report, the level of $^{125}$I incorporation never exceeded 0.006 μCi/10^6 L1210 cells.

**Table 4**

<table>
<thead>
<tr>
<th>Time period after L1210 injection (hr)</th>
<th>Median doubling timea (hr)</th>
<th>Growth fraction (%)</th>
<th>Cell lossb (%/day)</th>
<th>Median cell cycle time (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–24</td>
<td>lag</td>
<td>79</td>
<td>lag</td>
<td></td>
</tr>
<tr>
<td>24–48</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>48–72</td>
<td>16</td>
<td>90–100</td>
<td>&lt;5</td>
<td>22</td>
</tr>
<tr>
<td>72–96</td>
<td>16</td>
<td>90–100</td>
<td>&lt;5</td>
<td>15</td>
</tr>
<tr>
<td>96–120</td>
<td>24</td>
<td>90–100</td>
<td>&lt;5</td>
<td>34</td>
</tr>
<tr>
<td>120–144</td>
<td>38</td>
<td>90–100</td>
<td>&lt;5</td>
<td></td>
</tr>
</tbody>
</table>

a Autoradiographically corrected L1210 number (Chart 1, dashed curve).
b Cell loss from peritoneal L1210 populations is almost entirely due to cell metastasis. Cell death is less than 5% or nonexistent.
Growth Parameters of L1210 Ascites Cells

Analysis of L1210 Growth Parameters. The results presented in Chart 3 can be interpreted as follows. Peritoneal L1210 cells start to divide within 12 hr after tumor implantation. Although Skipper et al. (23) have postulated a 48-hr lag period, neither our previously reported IUDR-125I incorporation data (12) nor the results presented in this communication show such a long phase. Both sets of data indicate that L1210 cells start to multiply within 12 hr after transplantation and continue to divide at gradually decreasing rates throughout the remainder of tumor growth. During the first 3 days of tumor growth, the average generation time remains constant at 8.5 to 9.5 hr. After that, the length of the cell cycle gradually increases until it reaches 32 to 36 hr during the 6th and last day of tumor development (Chart 3).

These results are within the range of findings reported in the literature. For instance, Skipper et al. (24) have shown that the in vivo doubling time of L1210 populations varies between 0.4 and 0.6 days (9.6 to 14.4 hr). Wodinsky et al. (29) observed even shorter doubling times (8.7 to 9.5 hr) for L1210 populations growing in the bone marrow and in the spleen. With consideration of the high death rate at extraverternal growth sites (Chart 2), the generation time of L1210 cells growing in these organs could have been as short as 8 hr. The experiments presented in this report indicate that the doubling time of peritoneal L1210 populations during the first 3 days of tumor growth is approximately 9.5 to 10.5 hr (Chart 1, dashed curve). Thus, a generation time of 8.5 to 9.5 hr during early stages of tumor development would appear to be a reasonable estimate. In fact, such a short generation time is required to explain the presence of more than 80 million L1210 ascites cells on Day 3 of tumor development. Since only 0.5 million (20%) of the injected 2.5 million L1210 cells remain in the peritoneal cavity, it takes approximately 7 doublings to produce 80 million ascites cells. With a 6- to 12-hr lag phase, the total time available for these 7 doublings would be 60 to 66 hr, i.e., the length of the generation time would have to be 9.5 hr or less.

After Day 3, the length of the cell cycle of peritoneal L1210 cells increases to 15 hr during the 4th day, 22 hr during the 5th day, and 34 hr during the 6th day post inoculation (Chart 3, Table 4). These observations agree well with the results of Yankee et al. (30), who found that the mean generation time of peritoneal L1210 cells increased from 11.7 hr on Day 6 of tumor growth to 21.0 hr on Day 7. Young et al. (31) observed an increase in the average generation time from 13.3 hr on the 6th to 18.1 hr on the 7th day of L1210 development. Since these authors used inocula of 10⁵ tumor cells, Days 6 and 7 in their experiments correspond approximately to Days 4 and 5 in this study (150 to 500 million peritoneal L1210 cells).

In early tumors, the overall growth pattern of the peritoneal L1210 population depends primarily on the rate of cell loss rather than on the rate of cell proliferation. As discussed earlier, virtually all of the 80% cell loss observed during the first 24 hr after transplantation is caused by tumor cell metastasis to extraverternal parts of the body (Chart 2, Table 1). Actual cell death among peritoneal L1210 cells could not have contributed very much because the fractional rate of cell death in the entire host animal was only 10 to 15%/day. After Day 1 of tumor growth, the fractional rate of 125I disappearance from the peritoneal cavity declines markedly and remains low throughout the remainder of tumor development (Chart 2). This indicates a marked decrease in the rate of tumor cell metastasis, probably due to limitations in the capacity of pathways available for tumor cell migration (Table 2).

The migration pattern derived from 125I distribution data may be compared with the findings of Skipper et al. (22, 23), who concluded that L1210 metastasis was relatively minor during early phases of tumor growth and became more substantial in advanced tumors. However, the experiments of Skipper et al. were designed to determine the total number of tumor cells present at extraverternal sites, whereas the 125I procedure indicates the fractional rate of cell migration. Thus, cell multiplication at extraverternal sites would strongly influence the results of Skipper et al., while 125I distribution data would not be affected by this factor. In addition, although the fractional rate of cell migration decreases during later stages of tumor development, the absolute number of tumor cells leaving the peritoneal cavity may actually increase. Examination of the data presented in Table 2 illustrates this point. The fractional rate of tumor cell metastasis as
determined 24 hr after tumor inoculation is more than 90% when 1 million labeled L1210 cells are injected and less than 30% with 200 million implants. Yet, expressed in absolute numbers, the actual number of migrating cells is much larger in the latter group.

In conclusion, the technique of IUDR$^{125}$I prelabeling of L1210 cells before transplantation into new hosts in combination with autoradiographic techniques based on TdR$^3$H permits simultaneous evaluation of cell proliferation, cell migration, and cell death of peritoneal L1210 populations. The results summarized in Table 4 demonstrate that the initial delay in tumor growth is caused partly by a 12-hr lag period before the onset of cell division and partly by a surprisingly high rate of cell metastasis during the early phases of tumor growth. The progressive increase in the doubling time of advanced L1210 populations is caused primarily by an increase in the length of the cell cycle after Day 3 of tumor growth. The growth fraction remains close to unity throughout tumor development, and the rate of peritoneal cell death is too small to influence importantly the overall growth rate of L1210 ascites populations.

REFERENCES

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