Cell Population Growth and Cell Loss in the MTG-B Mouse Mammary Carcinoma

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SUMMARY

Two radiobiochemical methods were used in studies of tumor cell population growth rate and cell loss during growth of grafts of C3H mouse mammary carcinoma MTG-B in BC3Fi mice. Six-hr uptake of orotic acid-5-3H into DNA and RNA was highest in small tumors (25 mg, Day 5 after grafting). RNA uptake fell to one-third maximum by Day 7 and DNA reached that point by Day 9, thereafter remaining constant until Day 16 (1200-mg tumors). Tumor DNA was prelabeled with multiple injections of either tritiated thymidine or 5-iodo-2'-deoxyuridine-125I given prior to the first day of sacrifice. The specific activity of tritiated thymidine-labeled DNA fell exponentially from Days 5 through 10 and then tended to plateau. 5-Iodo-2'-deoxyuridine-125I-labeled DNA specific activity decreased similarly until Day 13, and slowed thereafter. In all experiments, the growth rate of "cleaned" viable tumor tissue decreased as tumors became larger. It is postulated that: (a) the rate of decrease in 125I-labeled tumor DNA specific activity is the negative of the "potential" cell population growth rate exclusive of cell death; (b) the potential rate of cell population growth is constant in tumors weighing 22 to 1200 mg or more; (c) cell death rate disproportionately increases in large tumors; and (d) tritiated thymidine released from dying cells is reutilized more efficiently than 5-iodo-2'-deoxyuridine-125I. These data are consistent with the postulate that increased cell death and decreased nucleic acid synthetic activity result from the known decrease in functional vascular space relative to tumor size. Evidence for the constancy in rate of cell population increase during tumor latency is also presented.

INTRODUCTION

The importance of cell loss as a factor in the growth of solid tumors has recently been recognized (4, 8, 9, 13, 14, 19-21). Frindel et al. (8) and Frindel and Tubiana (9) reported that the mean cell cycle time of the NCTC tumor in the ascites form increased by 2.4 times from Days 4 to 12 after grafting, while the cell population doubling time increased nearly 7-fold. When the tumor was grown in solid form, the cell cycle time of the same tumor did not change with time, but the tumor growth rate decreased. It was concluded that cell loss, as well as a decrease in growth fraction, played an important part in the reduction in tumor growth rate.

Steel et al. (21) studied the growth rate, TdR-3H4 labeling index, and labeled mitoses of 2 experimental tumors which differed in volume doubling time by a factor of 8. The difference in growth rate was attributed both to a difference in growth fraction (95% in the fast-growing tumor, 30% in the slow) and to cell loss of about 20%/division in the slow-growing tumor. Mendelsohn and Dethlefsen (14) performed similar studies and estimated about 80% cell loss/division in a slow-growing tumor strain. Changes in cell loss rate at different times during tumor growth were not reported in these studies.

Tannock (24) found that the cell cycle time was unchanged but the growth fraction decreased with distance from the vascular supply of a tumor in which the viable cells were arranged in cords along capillaries. In other studies, the mean distance of living cells from the nearest capillary was greater in large tumors than in small tumors (26) and the mean distance of interphase cells from capillaries was greater than that of mitotic cells (25). The relative vascular space has been reported to decrease with tumor growth (23), and the function of capillaries in necrotic tumor areas has been found to be markedly impaired (25). Limitations in vascular growth and functional capacities may thus limit the rate of tumor growth.

In the current studies, the uptake of OA-3H into nucleic acids and the rate of dilution of IUdR-125I- and TdR-3H-labeled DNA have been studied in relationship to the increase in viable MTG-B tumor tissue with time after grafting.

MATERIALS AND METHODS

C3H/Wr mouse mammary adenocarcinoma MTG-B (11), which can be stored in a liquid nitrogen cell bank and resuscitated as desired, was used in the 40th, 27th, and 34th serial passages in Experiments 100, 110, and 111A, respectively. For transplantation, tumor suspen-
Tumor Growth and Cell Loss

Solutions containing 10, 1, 0.1, or 0.01% by volume of viable tumor material were prepared as previously described (1, 2). To ensure optimal growth conditions (2), a suspension of supralethally irradiated tumor was added to all viable tumor suspensions to yield a final concentration of 29 to 33% of the former before transplantation. Aliquots of 0.05 ml were injected subcutaneously in the medial aspect of each hind leg of young adult female (Experiments 100, 110, and 111A) or male (Experiment 111B) BC3F, Wr mice. The day of grafting is considered as Day 0 throughout.

Tumor latency was determined by daily palpation of each transplant site (1, 2). Estimates of tumor dimensions were made by palpation until tumors reached 3 to 4 mm in diameter. Thereafter, 2 tumor dimensions were determined daily by caliper measurements.

Solutions of OA-'H (13.8 Ci/m mole; 200 µCi/ml), TdR-'H (1.9 Ci/m mole; 20 µCi/ml), or IUdR-125I (Experiment 110, 2.57 mCi/mg; 4.7 µCi/ml; Experiment 111A, 15 mCi/mg; 6.2 µCi/ml) were injected i.p. in tumor-bearing mice according to the schedules indicated in the following section. In all cases, the animals to be autopsied for tumor harvest were chosen in groups of 5 at random following tumor palpation. After dissection and removal of grossly apparent necrotic and nontumor tissues, the remaining tumor tissue, presumably comprised primarily of living tumor cells, was weighed and quickly frozen until analyzed. Such tissue is referred to throughout as “cleaned.”

Ten % homogenates in chilled buffered medium were prepared from each frozen cleaned tumor with Teflon-glass homogenizers. An equal volume of chilled 1.0 M PCA was added to an aliquot containing 20 to 100 mg equivalent wet tissue weight. DNA hydrolysates were then prepared from the PCA-insoluble material by incubation in 0.5 M PCA at 96° for 45 min, after washing of the precipitate and alkaline hydrolysis of RNA according to the procedures of Wannemacher et al. (27). The DNA concentration of the extracts were calculated from the absorbance at 260 and 280 nm. The radioactivities of OA-'H and TdR-'H-labeled DNA hydrolysates were determined by liquid scintillation counting of aliquots in a dioxane-naphthalene based scintillation mix (27) and corrected for disintegrations/min according to counts from toluene-'H added as an internal standard. A well scintillation counter was used for radioactivity measurements of IUdR-125I-labeled samples. The data are expressed in terms of specific activity; i.e., as counts ('H)/min/mg DNA, corrected for decay as appropriate.

RESULTS

Tumor Growth. Plots of both sample measurements of external tumor dimensions in Experiment 111A (Chart 1) and the weights of cleaned tumor tissue in all 3 experiments (Chart 2) suggested that the rate of tumor growth decreased as tumor size increased. This conclusion was strengthened by statistical analyses (5, 16) of the regressions of log tumor volume and log cleaned tumor weight against time, which indicated that the data deviate significantly from a simple exponential growth model (Table 1).

Treatment with IUdR-125I in Experiment 111A did not affect the time interval from grafting until tumors at...
Table 1

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Data</th>
<th>Slope (95% limit)</th>
<th>Correlation coefficient</th>
<th>Lack of fit test</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Cleaned</td>
<td>0.160 (0.141-0.179)</td>
<td>0.828</td>
<td>5.963 9, 116</td>
</tr>
<tr>
<td>110, 111A</td>
<td>Cleaned</td>
<td>0.165 (0.152-0.178)</td>
<td>0.873</td>
<td>3.656 20, 183</td>
</tr>
<tr>
<td>110, 111A</td>
<td>125I-UdR</td>
<td>0.198 (0.177-0.219)</td>
<td>0.914</td>
<td>7.601 6, 63</td>
</tr>
<tr>
<td>110, 111A</td>
<td>0.9% NaCl solution volume</td>
<td>0.187 (0.160-0.214)</td>
<td>0.854</td>
<td>13.432 6, 65</td>
</tr>
</tbody>
</table>

* Probability that the deviations from an exponential growth model are due to chance is less than 0.01 in all cases.

* Degrees of freedom.

Nucleic Acid Precursor Incorporation and DNA Specific Activity Dilution. The radioactive label of OA-3H, a precursor of pyrimidines, is ultimately incorporated into both DNA and RNA. In Experiment 100, the uptake of radioactivity from OA-3H administered 6 and 3 hr before sacrifice was highest in the small tumors harvested on Day 5 (Chart 4). During the ensuing 2 to 4 days, the uptake of OA-3H into the nucleic acids fell to about one-third of the maximum and tended to plateau at that level through Day 16.

The specific activity of the DNA of tumors from animals that had previously received either TdR-3H (Experiment 110) or IUdR-125I (Experiments 110 and 111A) initially appeared to decrease exponentially (Chart 5). Following Day 10, the rate of decrease in the specific activity of the TdR-3H-labeled DNA slowed markedly. The rate of decrease in specific activity of the IUdR-125I-labeled DNA appeared to be exponential through Day 13, and to lessen thereafter. As the slopes of the specific activity data for IUdR-125I-labeled DNA were so similar

Table 2

<table>
<thead>
<tr>
<th>Host treatment</th>
<th>Tumor inoculum (%)</th>
<th>Day of autopsy</th>
<th>No. of tumors</th>
<th>Tumor weight (mg ± S.D.)</th>
<th>Probability of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl solution</td>
<td>10</td>
<td>17</td>
<td>9</td>
<td>2552 ± 745</td>
<td>0.3 &gt; p &gt; 0.2</td>
</tr>
<tr>
<td>IUdR-125I</td>
<td>10</td>
<td>17</td>
<td>10</td>
<td>2195 ± 649</td>
<td></td>
</tr>
<tr>
<td>0.9% NaCl solution</td>
<td>1</td>
<td>12</td>
<td>8</td>
<td>644 ± 377</td>
<td>0.3 &gt; p &gt; 0.2</td>
</tr>
<tr>
<td>IUdR-125I</td>
<td>1</td>
<td>12</td>
<td>10</td>
<td>475 ± 267</td>
<td></td>
</tr>
<tr>
<td>0.9% NaCl solution</td>
<td>0.1</td>
<td>12</td>
<td>10</td>
<td>407 ± 230</td>
<td>0.1 &gt; p &gt; 0.05</td>
</tr>
<tr>
<td>IUdR-125I</td>
<td>0.1</td>
<td>12</td>
<td>10</td>
<td>242 ± 138</td>
<td></td>
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<tr>
<td>0.9% NaCl solution</td>
<td>0.01</td>
<td>14</td>
<td>10</td>
<td>327 ± 202</td>
<td>0.9 &gt; p &gt; 0.8</td>
</tr>
<tr>
<td>IUdR-125I</td>
<td>0.01</td>
<td>14</td>
<td>10</td>
<td>346 ± 224</td>
<td></td>
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</table>

Table 3

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment group</th>
<th>Day of autopsy</th>
<th>No. of tumors</th>
<th>Tumor weight (mg ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>111B</td>
<td>125I-labeled</td>
<td>7</td>
<td>20</td>
<td>159 ± 81</td>
</tr>
<tr>
<td>111A</td>
<td>IUdR-125I</td>
<td>7</td>
<td>10</td>
<td>130 ± 93</td>
</tr>
<tr>
<td>110</td>
<td>IUdR-125I</td>
<td>7</td>
<td>10</td>
<td>162 ± 98</td>
</tr>
<tr>
<td>111B</td>
<td>125I-labeled</td>
<td>8</td>
<td>20</td>
<td>248 ± 154</td>
</tr>
<tr>
<td>110</td>
<td>TdR-3H</td>
<td>8</td>
<td>10</td>
<td>236 ± 153</td>
</tr>
<tr>
<td>111B</td>
<td>125I-labeled</td>
<td>9</td>
<td>20</td>
<td>302 ± 155</td>
</tr>
<tr>
<td>111A</td>
<td>IUdR-125I</td>
<td>9</td>
<td>10</td>
<td>393 ± 130</td>
</tr>
<tr>
<td>110</td>
<td>IUdR-125I</td>
<td>9</td>
<td>9</td>
<td>359 ± 193</td>
</tr>
</tbody>
</table>

Suspensions containing 10% tumor material were prepared according to standard procedures from a group of IUdR-125I-treated mice in Experiment 111A, Day 7 (see legend, Chart 2) and grafted in a 2nd group of mice. The latter were sacrificed 7 to 9 days after grafting and are designated as Experiment 111B.
in the 2 experiments, the data were normalized for analysis by (a) determining the ratio of the mean specific activities of Experiments 111A to 110 on each of Days 7, 9, 11, and 13; (b) deriving the mean ratio weighted according to the number of observations; and (c) adding the logarithm of the mean ratio to the logarithms of the specific activity data from Experiment 110.

Analyses of the regressions (5, 16) of log \(^3\)H specific activity against time over Days 5 to 14 and log \(^125\)I specific activity over Days 5 to 17 revealed significant deviation from linearity in both cases (Chart 5, Table 4). When only the initial slopes (Days 5 to 10 and 5 to 13 for \(^3\)H and \(^125\)I, respectively) were considered, however, deviations from linearity were insignificant.

The apparent reduction in rate of decrease in \(^125\)I-labeled DNA specific activity following Day 13 probably resulted in part from inadvertent inclusion of necrotic tissue with the cleaned tumor tissue samples. In another

![Chart 4](chart4.png)

Chart 4. Specific activities of tumor RNA and DNA after injection of OA-\(^3\)H at 6 and 3 hr before sacrifice of the host mice. Each point represents 11 to 12 observations on Days 6 to 16 and 4 observations on Day 5. Vertical bars, standard deviations.

![Chart 5](chart5.png)

Chart 5. Decrease in the specific activities of the DNA of tumors from mice treated with TdR-\(^3\)H or IUdR-\(^125\)I on Days 3 to 4 (Experiment 110) or IUdR-\(^125\)I on Days 1 to 6 (Experiment 111A) (see legend, Chart 2). Logarithms of the IUdR-\(^125\)I specific activities of Experiment 110 were normalized by addition of 0.442 to the mean values. Large symbols, 8 to 20 observations from tumors that developed from 10% inocula; vertical bars, standard deviations. Solid, dashed, and broken lines, least squares fits calculated for the 10% inocula data of the indicated days (Table 3). Small circle, Day 14, represents 2 observations from tumors that developed from 10% inocula, Experiment 110. Small X, Day 14, represents 10 observations from tumors that developed from 0.01% inocula, Experiment 111A. Superimposed small symbols, Day 12, represent 10 observations each on tumors that developed from 0.1% and 1.0% inocula, Experiment 111A, respectively, and 2 observations on tumors that developed from 10% inocula, Experiment 110.

Table 4

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Range of days</th>
<th>Slope</th>
<th>95% confidence limits</th>
<th>Correlation coefficient</th>
<th>Lack of fit test</th>
</tr>
</thead>
<tbody>
<tr>
<td>IUdR-(^125)I</td>
<td>5 to 13</td>
<td>-0.2155</td>
<td>-0.2082 to -0.2228</td>
<td>-0.985</td>
<td>2.309, 10, 97, &gt;0.05</td>
</tr>
<tr>
<td>IUdR-(^125)I</td>
<td>13 to 17</td>
<td>-0.1065</td>
<td>-0.0956 to -0.1274</td>
<td>-0.807</td>
<td>3.042, 3, 53, &gt;0.05</td>
</tr>
<tr>
<td>IUdR-(^125)I</td>
<td>5 to 17</td>
<td>-0.1888</td>
<td>-0.1821 to -0.1955</td>
<td>-0.979</td>
<td>11.716, 13, 122, &lt;0.01</td>
</tr>
<tr>
<td>TdR-(^3)H</td>
<td>5 to 10</td>
<td>-0.2309</td>
<td>-0.1978 to -0.2640</td>
<td>-0.919</td>
<td>0.321, 2, 35, &gt;0.05</td>
</tr>
<tr>
<td>TdR-(^3)H</td>
<td>10 to 14</td>
<td>-0.0529</td>
<td>-0.0142 to -0.0954</td>
<td>-0.431</td>
<td>1.032, 1, 33, &gt;0.05</td>
</tr>
<tr>
<td>TdR-(^3)H</td>
<td>5 to 14</td>
<td>-0.1496</td>
<td>-0.1338 to -0.1655</td>
<td>-0.922</td>
<td>12.106, 4, 59, &lt;0.05</td>
</tr>
</tbody>
</table>

* Degrees of freedom.
* Probability deviations from exponential model are due to chance.
experiment, 12 tumor-bearing mice were treated with IUdR\textsuperscript{125}I as in Experiment 111A. At autopsy 14 days after grafting, all tumors contained some fluid. Drained, grossly necrotic tissue comprised about 20% of the total weight recovered and contained about 60% of the recovered total radioactivity per tumor. The specific activity of the DNA in the necrotic tissue averaged 45% higher (0.02 > p > 0.01) than that of the cleaned tissue.

Only those data from tumors that developed from 10% inocula were used in the slope calculations. In Experiment 111A, the specific activities of the DNA of tumors which developed from 1, 0.1, and 0.01% inocula were also determined 12, 12, and 14 days after grafting, respectively. The mean specific activity values from these tumors were nearly identical with the values expected for tumors developing from 10% inocula if harvested on those days (Chart 5).

DISCUSSION

These studies were undertaken with the primary aim of obtaining information on the rate of DNA synthesis throughout the tumor growth period as a reflection of cell population growth changes, and in particular to relate these results to the changes in rate of increase in viable tumor tissue which are observed as tumors increase in size. The radiobiological techniques for measuring isotopic label uptake and/or dilution utilized in these studies yield data reflective of metabolism at the total cell population level. Interpretation of these data depends, however, on several assumptions. For example, the amount of OA\textsuperscript{3}H incorporation into DNA depends not only on the growth fraction, the mean duration of the DNA synthetic period, and the mean cell cycle time, but also on the availability of the isotopic precursor, and perhaps other metabolic elements, to the synthesizing cells. The latter, in turn, depends in part on the functional vascular supply. The measured incorporation of OA\textsuperscript{3}H into RNA depends on the precursor availability and also on the rate of catabolism of RNA as well as the synthetic rate. Thus the decrease in OA\textsuperscript{3}H incorporation into DNA observed as tumors increased in size in Experiment 100 could reflect a decrease in growth fraction or an increase in cell cycle time. In addition, the decreased uptake into both nucleic acids could reflect a decrease in precursor availability. Although pool sizes were not determined, the latter seemed to be an important possibility, in view both of the correlation observed between the changes in uptake into DNA and into RNA and of the reported decrease in relative functional vascular space as tumor volume increases (23, 25, 26).

Experiments 110 and 111A were thus designed to estimate the potential cell population doubling time exclusive of cell loss (the maximum potential tumor growth rate) from the rate of decrease in the specific activity of prelabeled DNA (6, 7, 12, 15, 17, 20, 23). The rate of dilution of prelabeled DNA is, of course, a function of both the growth fraction and the cycle time of the dividing cells. In addition, however, it is subject to modification by other events. For example, if cells bearing labeled DNA preferentially survive or preferentially die, the rate of decrease in specific activity will be less than or greater than the potential cell population growth rate respectively. If the presence of the labeled precursor in DNA modifies the percentage of dividing cells or the cell cycle time of the labeled cells, the rate of specific activity decrease will be modified accordingly and not yield a valid estimate of the potential growth rate of an unlabeled cell population. In Experiments 110 and 111A, labeled DNA precursors were administered at 6-hr intervals over 48 hr and 6 days, respectively. The duration of the DNA synthetic phase in similar tumor cells has been reported as greater than 6 hr (13), and previous observations indicate that the volume doubling time of small MTG-B tumors is less than 48 hr (1, 2, 22). The treatment schedules used would thus be expected to result in a high proportion of labeled cells. As noted above, however, there was no significant effect of the highest dose of IUdR\textsuperscript{125}I on the rate of tumor volume growth, on final cleaned tumor tissue weight, or on tumor growth following transplantation of IUdR\textsuperscript{125}I-labeled cells. It thus seems unlikely that the presence of this label markedly affected the rate of cell death, or other cell population growth parameters.

Finally, if cell death is a frequent event and the radioactively labeled compound released from dying cells is efficiently incorporated into the DNA of surviving cells, the rate of decrease in specific activity will be less than the potential cell population doubling time. Reutilization of TdR\textsuperscript{3}H has been shown in a variety of organs (3, 6, 7, 18, 24). Steel (18) found an increase in total radioactivity in tumors at a time following injection of TdR\textsuperscript{3}H which corresponded to release of the label with sloughing of the first of the labeled cells of the intestinal epithelium. Dethlefsen et al. (3) have reported data interpreted to indicate 52% reutilization of TdR\textsuperscript{3}H and 21% reutilization of IUdR\textsuperscript{131}I by intestinal epithelium. Half-times of mammary tumor DNA labeled with the 2 tracers were, however, insignificantly different (3).

The rate of reutilization of DNA precursor would be expected to correlate with the initial rate of incorporation. Hughes et al. (12) found that unincorporated radioactively labeled IUdR disappeared with a half-life of 5 min or less in intestine and thymus, and that 5 to 10% of the injected dose was retained in the bodies of mice. This is in contrast to the 40 to 50% retention reported for TdR in rats (7). The rate of disappearance of IUdR\textsuperscript{131}I-labeled DNA from the gut and other tissues corresponded well with other estimates of the rates of cell replacement (12). Hofer et al. (10) recently reported that 2.5 to 6.5% of the total radioactivity was retained in the bodies of mice 4 days after injection i.p. or i.m. of killed, IUdR\textsuperscript{131}I-labeled LI210 leukemia cells, and that about 1.5% remained at 6 days after i.p. injection. Feinendegen et al. (7) estimated 30 to 40% reutilization of TdR and 5% or less of IUdR in mouse marrow cells.
In experiments in these laboratories (M. B. Yatvin, D. T. Crouse, and K. H. Clifton, unpublished experiments), only 1.5 to 2.0% of the injected radioactivity was recovered 7 to 9 days after grafting in MTG-B tumors that developed from inocula containing 10 or 1% viable tumor suspension plus 32% lethally irradiated, IUdR-125I-labeled tumor suspension. There was thus minimal IUdR reutilization under conditions in which the dying labeled cells were intimately associated with the surviving cells.

On the basis of studies of the latency of tumors arising from serially diluted suspensions (1, 2, 22), we have concluded that the rate of tumor growth during the latent period is constant. In the current studies, the DNA specific activities of tumors arising from suspension containing 1.0, 0.1, and 0.01% viable cells were the same as those expected in tumors from 10% inocula on the same days of autopsy. This supports the conclusion that, over a 1000-fold inoculum dilution range, the DNA synthesis rate, the availability of the injected IUdR-125I, and, presumably, the cell population growth rates were constant in the prepalpable tumors during the 6-day isotope administration. It further indicates that the dilution of DNA and thus, presumably, the number of potential cell population doublings from the times of the last IUdR-125I injections until the times of sacrifice were essentially the same in tumors arising from all 4 concentrations of inocula. This was true despite marked differences in final cleaned tumor weights and the fact that by days 12 to 14, necrosis was common in tumors arising from the more concentrated inocula.

In Experiments 110 and 111A, as noted above, if only the initial slopes of the 3H- and 125I-labeled DNA specific activity regressions on time were considered, error due to lack of fit to a simple exponential model was markedly decreased (Table 4). Furthermore, the rate of decrease in specific activity of 3H-labeled tumor DNA thus calculated for Days 5 to 10 was nearly identical with that calculated for 125I-labeled DNA during Days 5 to 13 (Table 4). Specific activity half-times during these time intervals were 1.3 and 1.4 days for 3H- and 125I-labeled DNA, respectively. These results suggest either that the rates of reutilization of the 2 isotopically labeled precursors were similar during Days 5 to 10, or that deviations due to reutilization were below the level of detection by the current procedures.

As noted, analysis of the cleaned tumor weight data against time throughout the observation period revealed significant lack of fit to a simple exponential growth model (Table 1) and suggested that the rate of increase in weight progressively slowed throughout the tumor growth period, in accord with our freehand curve (Chart 3). This conclusion was strengthened by a plot of the day-to-day increment in tumor weight (Chart 6) which suggests that the absolute daily increase in viable tumor tissue reached a maximum of 200 to 300 mg. Furthermore, in larger tumors, cell death occurred to a significant extent as indicated by the presence of areas of necrosis which contained more than half of the total recoverable radioactivity, and in which the DNA specific activity was significantly higher than that of the non-necrotic tumor tissue. Thus, the potential rate of cell population growth in larger tumors must exceed the observed rate of increase in cleaned tumor tissue.

The 3 measurements, cleaned tumor weight, 3H-labeled DNA dilution, and 125I-labeled DNA dilution, are consistent with the following postulates: (a) the potential rate of tumor cell population growth, exclusive of cell death, was constant at least through Day 13, when the cleaned tumor tissue averaged in excess of 1200 mg; (b) cell death became a significant factor by Day 10, and the reduction in rate of 3H-labeled DNA dilution resulted from reutilization of TdR-3H from dying tumor and other tissue cells; and (c) the reduction in rate of 125I-labeled DNA dilution following Day 13 resulted from inadvertent inclusion of tissue of higher specific activity from the widespread necrosis with the cleaned tissue samples, as well as from any reutilization of IUdR-125I from dead cells. Thus the potential cell population growth rate during Days 13 to 17 was most probably faster than suggested by the rate of 125I-labeled DNA dilution during the terminal phase of growth. Finally, the ability of the cleaned tumor tissue to incorporate injected OA-3H into DNA appeared to reach its lower limit in tumors of about the size in which cell death became important.

In accord with this postulate, the maximum potential daily increase in viable tumor tissue was estimated as follows: the potential weight on Day n was calculated from the smoothed curve weight value on Day (n — 1) (Chart 5) multiplied by 10^0.2155 (reciprocal of the 5 to 13 day 125I specific activity regression, Table 4). The net differences between the potential weights, Day n, so calculated and the weights on Day (n — 1) are summarized in Chart 6. The calculated potential increases underestimated the smoothed curve values by 23 mg on
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Days 6 and 7 where any error stemming from an effort to obtain only viable tissue from the small tumors of Days 5 and 6 would have the greatest effect. By Day 12, the potential increase exceeded the smoothed curve value by over 200 mg, and by Day 17 the difference had increased to nearly 1 g. The daily cell loss in grafted MTG-B tumors thus appears to increase progressively as tumors increase in size, reaching 50%, and perhaps as many as 85%, of the potential new cells born.

In sum, these experiments illustrate a notable consistency in the potential rate of cell population growth, first in tumors yielding averages of 22 to 1200 or more of cleaned, presumably viable tissue, and secondly during tumor latency. Although the techniques used do not yield direct information on the cell cycle time or the growth fraction, the results indicate that changes in these studies may reflect the changes in relative functional vascular supply reported by others (23, 24, 26). Finally, as indicated by others (7, 12), labeled I UdR appears to be superior to TdR in longer term investigations of this nature.

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REFERENCES

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