Incorporation of I\textsuperscript{125}-labeled Iodo-deoxyuridine into the Deoxyribonucleic Acid of Murine and Human Tissues Following Therapeutic Doses*

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SUMMARY

The incorporation of I\textsuperscript{125}-labeled 5-iodo-2'-deoxyuridine (I UdR) into the deoxyribonucleic acid (DNA) of several mouse tissues (Adenocarcinoma 755, spleen, small intestine, liver) was measured at intervals up to 6 days after administration of the drug in a saline solution and in a depot form. The incorporation of I UdR into the DNA of human tissues (pancreatic carcinoma, skin, omentum, small intestine, muscle) was also determined after administration of I UdR by intravenous infusion. The drug dosage (50–200 mg/kg/injection in mice, 15 mg/kg in 24 hours in a patient) and regimens of administration were chosen in all experiments to approximate those used in radiosensitization experiments and clinical trials. The levels of I UdR incorporated into the DNA of the neoplasms studied (up to about 1 per cent of thymidine replaced by I UdR) approached those which would be expected to increase the radiation sensitivity of the cells. When measured during the first 24 hours after drug administration, the incorporation of I UdR into the DNA of mouse spleen and small intestine and of human skin and small intestine equaled or exceeded that in the DNA of the tumors. Six days after drug administration, however, I UdR incorporation into the DNA of the mouse tumor equaled or exceeded that into the DNA of the normal tissues. The incorporation of the drug into DNA of all tissues was proportional to the total dose administered, was 2.3–5.5 times greater after the administration of the drug in depot form as compared with administration in saline solution, and was 42–61 per cent higher after pretreatment with the thymidylate synthetase inhibitor, 5-fluoro-2'-deoxyuridine, than when given alone to mice. These results indicate that the mode and schedule of drug administration and the timing of radiotherapy may play an important, even decisive, role in the efficacy of combined therapy with such drugs.

5-Iodo-2'-deoxyuridine\textsuperscript{1} is incorporated into the deoxyribonucleic acid of human cells and markedly increases their sensitivity to x-rays (8, 10–12). These observations led to the possibility that I UdR could be utilized as a radiosensitizing agent in conjunction with radiation therapy for neoplastic disease (3, 5, 8, 10–13, 16, 29, 31). For this purpose, it would be desirable to have as much I UdR as possible incorporated into the DNA of the neoplasm, with as little as possible in the DNA of normal tissues at the time of radiotherapy (31).

The metabolism of I UdR \textit{in vivo} has been investigated, and the incorporation of I UdR into a variety of whole tissues and into DNA has been...
studied after administration of the labeled compound to laboratory animals and men (5, 9, 13, 16, 18, 24, 26, 31). Such studies have not, however, dealt with the development of optimum schedules of drug administration for the incorporation of IUDR into tumor DNA, or with changes in the relative quantities of IUDR in the DNA of normal and tumor tissues with time after such drug administration.

Experiments in these laboratories have been designed (a) to evaluate the incorporation of IUDR into the DNA of normal and neoplastic human and murine tissues and (b) to determine the effect of combined therapy with IUDR and radiation on experimental tumors. This communication deals with studies of the incorporation of IUDR into DNA in vivo following treatment regimens in which the dose and frequency of drug administration were designed to approximate those used in radiosensitization experiments or therapeutic trials, and of changes in the amount of IUDR in the DNA at intervals up to 6 days after drug administration.

MATERIALS AND METHODS

Tumors, transplantation procedures, and animals.—BDF1 mice were given injections in the medial aspect of the right hind leg of a suspension of mammary Adenocarcinoma 755 (1) prepared with the aid of a cytosieve (28). Thirteen to 25 days later, when the tumors averaged approximately 60 sq. mm. or more in cross-sectional area, the animals were assigned to groups and used in five experiments as outlined in Tables 1 and 2 and Charts 1 and 2.

Patients.—A 62-year-old white male with advanced cancer of the tonsil served as the subject for the comparison of IUDR clearance rates from the site of injection of the drug in saline solution or in depot form in Experiment 6. A 66-year-old white male with carcinoma originating in the head of the pancreas with metastases to the lungs, spine, and mesentery was the subject for Experiment 7. The latter patient was hospitalized for surgical removal of the mesenteric mass which threatened obstruction of the bowel.

IUDR injections.—I$^{125}$-labeled IUDR was prepared by the method of Prusoff (23). At the initiation of the experiments the specific activity of the labeled compound varied from 3 to 13 $\mu$c./mg. Unlabeled IUDR was added to adjust the total drug doses to levels approximating those used in therapeutic trials. For Experiments 1 and 2, labeled and carrier IUDR were dissolved in alkaline saline (23 parts 0.1 N NaOH in 177 parts physiological saline) at 95° C. at a final concentration of 10 mg total IUDR/ml. For Experiments 3, 4, and 5, alkaline saline solutions were prepared at room temperature at a concentration of 5 mg total IUDR/ml. The depot form of the compound was prepared by suspension of microcrystals in an aqueous gel containing aluminum monostearate at a concentration of 100 mg total IUDR/ml as described by Buckwalter and Dickison (4). In Experiment 1, the mice received 4–5.5 $\mu$c. total radioactivity (100 mg total IUDR/kg body weight); in Experiment 2, 10–16 $\mu$c. (200 mg/kg); in Experiment 3, about 5 $\mu$c. and 2.5 $\mu$c. (100 and 50 mg/kg, respectively); and in Experiments 4 and 5, about 2.5 $\mu$c. total radioactivity/injection/mouse (50 mg/kg/injection). With the counting procedure used in Experiments 4 and 5 this amounted to approximately 2.5 x $10^4$ total counts/min/mouse. Drug preparations used in Experiment 6 were the same as those used in Experiments 4 and 5.

The IUDR used for intravenous infusion in the clinical investigation in Experiment 7 was dissolved in alkaline saline at a concentration of 5 mg/ml as above, sterilized by filtration, and checked for the presence of bacteria and pyrogens. Two hundred ml. of the sterile preparation was diluted with 800 ml. of sterile saline for infusion. The patient received approximately 15 mg of IUDR/kg body weight (about 290 $\mu$c. $^{125}$I) during an infusion period of 23 hours. The tumor and normal tissues were removed at surgery 3–4 hours after the cessation of infusion.

Unless otherwise indicated all injections in mice were intraperitoneal. Injections in the patient in Experiment 6 were intramuscular, 2 ml. of the saline solution in the right upper arm and 0.1 ml. of the depot suspension in the left upper arm (2.4 $\mu$c./injection).

Chemical procedures and isotope measurement.—Mice were sacrificed, and the tumor, spleen, intestine from the pylorus to the ileocecal junction and, in Experiments 1–3, the livers were removed in a cold room at 4° C. Tissues were rinsed in physiological saline, and frozen and stored in liquid nitrogen until homogenized. In Experiments 1, 2, 4, and 5 each tissue was handled individually and was weighed before freezing. In Experiment 3 all tissues of a given type were frozen immediately after rinsing, pooled, shattered with a metal piston in a metal container, and the fragmented frozen tissues were divided into aliquots for storage. In Experiment 7, samples of the metastatic tumor, adjacent small intestine, omentum, muscle, and skin were removed and immediately cooled in a metal container, and the fragmented frozen tissues were divided into aliquots for storage.
beaker immersed in dry ice. They were then frozen in liquid nitrogen, wrapped in cheesecloth, and shattered with a hammer before homogenization.

Tissues in Experiments 1, 2, 4, 5, and 7 were homogenized for less than 3 minutes in cold distilled water, and in Experiments 2, 4, 5, and 7 aliquots were taken for determination of total tissue radioactivity. DNA extracts were then prepared according to the following procedure, which will be referred to as Method I. Equal volumes of cold 10 per cent TCA were then added, and the mixtures were stirred and centrifuged. The precipitates were thoroughly washed in sequence with cold 5 per cent TCA, 95 per cent ethanol, and 3:1 ethyl ether-ethanol. Finally, nucleic acids were extracted by heating for 15–20 minutes in 5 per cent TCA at 90°C, followed by centrifugation. Aliquots of the DNA extracts were taken for counting and for determination of DNA by Schneider’s modification (27) of the method of Dische.

The validity of the DNA isolation by Method I as outlined above was investigated in Experiment 3. The DNA of aliquots of each of the pooled fragmented tissues was isolated (a) by Method I, (b) by the method of Barnum et al. (Method II) (2), or (c) in a highly polymerized, RNA- and protein-free form by the method described by Szybalski and Mennigmann (Method III) (30). The DNA concentrations and specific activities were determined as in the other experiments.

Radioactivity measurements were made with a manual (Experiments 1 and 2) or automatic (Experiments 3, 4, 5, and 7) scintillation well counter with an efficiency of approximately 33 per cent. The sequence of the analyses of the mouse tissues was randomized so that inadvertent differences in procedure from day to day would be distributed throughout treatment groups. A series of injection standards was prepared at the start of each experiment and counted with each group of samples to test for reproducibility of the counting equipment. All figures were corrected for radioactive decay.

In Experiment 6, the rate of disappearance of the compound from the site of injection was measured with a scintillation counter placed over the injection sites on the arms. These counts were corrected according to a background count with the detector similarly placed over the uninjected thigh.

The incorporation data are expressed as \( \mu g \) IUdR per unit weight of DNA or tissue (specific activity). The counts/min in the experimental samples were divided by the counts/min/\( \mu g \) of the IUdR administered. When incorporation data are alluded to as \( \mu g \) IUdR/unit weight of tissue or DNA, it should be understood that the tissue or DNA extract contained radioactivity equivalent to the radioactivity in that amount of IUdR.

RESULTS

Maximal or near maximal incorporation of IUdR-I\(^{125}\) into the DNA of tumor and intestine (\( \mu g \) IUdR/mg DNA) was observed 1 hour after injection in Experiments 1 and 2 (Table 1). In Experiment 2, the percentage of the radioactivity of the entire tissue recovered in the DNA (100 \( \times \) the radioactivity in the DNA per gram tissue \( \div \) the total radioactivity per gram tissue) had reached or approached the maximal amount in tumor, spleen, and intestine by 8 hours after drug administration, but not until 24 hours in liver.

The maximal incorporation of label into the DNA of the spleen was observed 1 hour after injection in Experiment 1, and 8 hours after injection in Experiment 2. In both cases the maximal specific activities of the DNA extracts were 3–4 times greater than the second highest specific activities observed in the spleen DNA in the same experiments. In Experiment 1, the specific activity of the DNA extract of the liver was lower than that of the other tissues, but in Experiment 2 the mean value 1 hour after injection exceeded those of the other tissues from the same mice. As noted in the discussion, the inconsistency of the results with liver tissue may have been due to contamination of the DNA extracts with extraneous compounds containing I\(^{125}\).

Experiment 3 was performed to determine whether the specific activities of the DNA samples obtained from all four types of tissue by Method I as used in these studies were erroneously high because of such contamination. Specific activities of the DNA extracts prepared by Method I and by Method II (2) were in good agreement (Table 2). The specific activities of highly polymerized DNA prepared by Method III (30) were, however, somewhat higher than samples prepared from these tissues by the other two methods (Table 2). The highest specific activity values for liver in this experiment were considerably lower than for the other three tissues. These results indicate that the specific activity values obtained from DNA extracts of tumor, spleen, or intestine prepared by Method I 20 hours after the last injection of label are conservative—i.e., the major error is a slight overestimation of the DNA content, and the specific activities reported in the rest of these experiments should be considered in this light. Such overestimation probably resulted from the pres-
ence in the extracts of compounds other than DNA which reacted with the Dische reagent.

The relationship between the amount of IUdR incorporated into the DNA of tumor, spleen, and intestine and the number and frequency of IUdR injections was investigated in Experiment 4 (Chart 1). When the injections of IUdR were all given within a 24-hour period, incorporation into the DNA was directly proportional to the total amount of drug administered. Maximum uptake per mg. DNA was observed when four injections were given at 4-hour intervals in a single day (Group V, Chart 1), and the amount of IUdR incorporated was similar in all three tissues analyzed. When the total dose was reduced to one-half (two injections at 4-hour intervals, Group IV) the incorporation of IUdR into the DNA of all three tissues was reduced proportionately. After a single injection (Group VI), the specific activities of the DNA were about one-quarter of the respective maxima. Although the mean uptakes were somewhat lower when the injections were administered at 2-hour intervals (Groups I and II) rather than at 4-hour intervals, the differences were not statistically significant.

Lengthening the time interval between drug injections reduced the final concentrations of IUdR in the DNA of all three tissues—e.g., the

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tbody>
<tr>
<td>IUdR INCORPORATION INTO DNA OF ADENOCARCINOMA 755 AND HOST MOUSE TISSUES AFTER A SINGLE INJECTION OF THE DRUG IN SALINE SOLUTION</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exp.</th>
<th>IUdR dose (mg/kg body wt)</th>
<th>No. Mice</th>
<th>Incorporation time (hr.)</th>
<th>µg IUdR/mg DNA*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tumor</td>
<td>Spleen</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>3</td>
<td>1</td>
<td>1.20 ± 0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>8</td>
<td>0.60 ± 0.36†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>24</td>
<td>0.59 ± 0.06</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>5</td>
<td>1</td>
<td>0.71 ± 0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>8</td>
<td>(5.6 ± 2.9)‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>24</td>
<td>(19.2 ± 13.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>24</td>
<td>(20.0 ± 8.7)</td>
</tr>
</tbody>
</table>

* Mean values ± standard deviation.
† Two tissues analyzed.
‡ Values in parentheses are percentage of total tissue radioactivity recovered in the nucleic acid extract.

<table>
<thead>
<tr>
<th>TABLE 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFFECT OF THE METHOD OF PREPARATION OF THE DNA EXTRACT ON THE ESTIMATE OF THE IUdR INCORPORATED (EXPERIMENT 3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DNA ISOLATION METHOD</th>
<th>µg IUdR/mg DNA*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tumor</td>
</tr>
<tr>
<td>Method I</td>
<td>1.70 (1.49-1.95)†</td>
</tr>
<tr>
<td>Method II</td>
<td>1.49 (1.31-1.71)‡</td>
</tr>
<tr>
<td>(Ref. 30)</td>
<td>(7)</td>
</tr>
<tr>
<td>Method III</td>
<td>2.23 (2.06-2.40)</td>
</tr>
<tr>
<td>(Ref. 30)</td>
<td>(9)</td>
</tr>
</tbody>
</table>

* Determined by specific activity measurements. Mice had received one injection of 100 mg total IUdR/kg followed by two injections of 50 mg/kg 8 and 12 hours later. The animals were killed 20.5-21.5 hours after the last injection.
† Range.
‡ Number of determinations.
specific activities of the DNA samples after four injections in a single day (Group V, Chart 1) equaled or exceeded those observed after eight injections given over 4 days (Group XI). The effect of increasing the interval of time between injections was most marked in the spleen—i.e., the specific activities of the spleen DNA in groups receiving two injections per day for totals of two, four, and eight injections (Groups IX, X, and XI) were not statistically different, nor were those of groups receiving single daily injections over similar periods (Groups VI, VII, and VIII). In contrast, the correlation of the specific activity of the DNA of tumors with the total dose of IUdR received was marked, even with prolongation of the interval between injections. The effect of increasing the time between injections on the specific activity of the intestinal DNA was intermediate between the effects on the tumor and spleen. These results indicate that labeled DNA was lost or diluted during the longer intervals between injections—most rapidly in the spleen, somewhat less rapidly in the intestine, and least rapidly in tumor tissue.

When two injections of IUdR were preceded by two injections of a saline solution of FUdR (Group I, Chart 1), the mean uptake into DNA was increased by 42–61 per cent.

The extent of IUdR incorporation into the DNA and the persistence of the label in the DNA were compared after administration of the drug in saline solution and in depot form in Experiment 5. Twenty-four hours after a single injection of saline solution (day 2), the specific activities of the DNA were maximal in all three tissues and decreased in an exponential fashion thereafter (Chart 2). The amount of IUdR incor-
porated into the DNA 24 hours after administration of the drug in a single intraperitoneal injection in depot form was 2.3 times greater in tumor, 3.7 times greater in spleen, and 5.5 times greater in intestine than was found after drug administration in saline solution (Chart 2). The concentration of label in the DNA of spleen and intestine decreased relatively rapidly, and by 6 days after injection (day 7, Chart 2) the specific activity of the DNA of the tumor equaled or exceeded that in the normal tissues. After a single intramuscular injection of the depot form, the uptake of IUdR appeared to be higher in the DNA of the tumor and lower in the DNA of the spleen and intestine than was found after intraperitoneal injection.

When two intraperitoneal injections of IUdR in depot form were given, the specific activity of the DNA of the intestine initially exceeded that of the tumor and spleen (Chart 2). The specific activities of the DNA of the normal tissues again decreased rapidly, however, falling to levels equal to or less than that of the tumor by day 8.

The DNA recovered per gram of tissue was relatively constant in each tissue type (4.4 ± 1.1 and 4.3 ± 1.3 mg DNA/gm tumor tissue, 19.5 ± 6.1 and 21.7 ± 8.8 mg/gm spleen, and 6.6 ± 0.3 and 6.5 ± 1.2 mg/gm intestine in Experiments 4 and 5, respectively). The percentage of the radioactivity of the tissue homogenates recovered in the DNA varied between tissues, however, and the total tissue radioactivity, especially at short time intervals, did not serve as a reliable estimate of the incorporation of the drug into DNA. For example, in Experiment 4, the mean radioactivity in the DNA of the tumor was 57 per cent of the total tissue radioactivity; of the spleen, 50 per cent; and of the intestine, 47 per cent (ranges 31–46, 59–65, and 38–56 per cent, respectively).

The rate of absorption of labeled IUdR after administration in saline solution and in depot form was tested in a human in Experiment 6 (Chart 3). The counts/min recorded at the site of injection of the saline solution decreased exponentially, reaching about 4 per cent of the initial level by 3 hours after administration. Although measurements of the radioactivity at the site of injection of the drug in depot form were probably distorted by spreading of the very small inoculation volume, the count rate had decreased to only about 50 per cent in the same period of time. An interval 40 times longer (120 hours) was required for the count rate over the site of injection of the depot form of IUdR to fall to 4 per cent of the initial count rate.

In Experiment 7 the incorporation of IUdR into the DNA of a human pancreatic carcinoma following continuous intravenous infusion was found to be about equal to that in the DNA of normal skin and small intestine (Table 3). The radioactivity of the nucleic acid extract of the omentum was high

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. aliquots</th>
<th>IUdR/ mg DNA</th>
<th>DNA/ gm tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>9</td>
<td>1.27 ± 0.19</td>
<td>2.61</td>
</tr>
<tr>
<td>Small int.</td>
<td>6</td>
<td>1.32 ± 0.15</td>
<td>1.43</td>
</tr>
<tr>
<td>Omentum</td>
<td>3</td>
<td>1.58 ± 0.76</td>
<td>0.47</td>
</tr>
<tr>
<td>Skin</td>
<td>1</td>
<td>1.48</td>
<td>0.43</td>
</tr>
<tr>
<td>Muscle</td>
<td>1</td>
<td>0.70</td>
<td></td>
</tr>
</tbody>
</table>

* A total of 15 mg IUdR/kg body weight infused for 23 hours. Tissue samples obtained 3–4 hours after cessation of infusion.

lower in the DNA of the spleen and intestine than was found after intraperitoneal injection.

The counts/min recorded at the site of injection in a human after intramuscular administration of 126UdR dissolved in alkaline saline or suspended in a depot form (Experiment 6). Note difference in the scales of the abscissa.

Chart 3.—Disappearance of radioactivity from the injection site in a human after intramuscular administration of IUdR dissolved in alkaline saline or suspended in a depot form (Experiment 6). Note difference in the scales of the abscissa.

but variable in the aliquots analyzed. The specific activity of the muscle DNA was about one-third of that of the tumor. About 50 per cent of the injected radioactivity was recovered in the urine during the infusion period, and an additional 33 per cent was recovered during the following 3 days from the start of the experiment (Chart 4). The radioactivity of the serum reached its maximum by 12 hours after initiation of the infusion, and by 48 hours the radioactivity had fallen to about 31 per cent of the maximum. About 0.03 per cent of the total injected radioactivity was recovered in
the DNA of the 107 gm. of tissue analyzed (about 0.15 per cent of the body weight).

**DISCUSSION**

As the metabolic fate of most substances depends, at least in part, on their concentration, the doses of IUDR used in these studies were chosen to approximate those employed in tumor radiosensitization and, in several cases, approached the maximum tolerated doses. Under these conditions, the incorporation of IUDR into the DNA of the normal and neoplastic tissues investigated approached levels which would be expected to significantly increase their radiosensitivity. Erikson and Szybalski (10-12) found that radiosensitization of a human cell line in tissue culture was a function of the amount of IUDR incorporated as measured by the density of the DNA. Substitution of about 25 per cent of the TdR with IUDR (the maximum substitution achieved) resulted in a 3.2-fold increase in the radiosensitivity of the cells, whereas a 1.2-fold increase in sensitivity was achieved at a substitution level below that detectable with the methods used (i.e., less than 2 per cent substitution) (10-12). Other authors (7) reported a two-fold increase in radiosensitization of cells grown in vitro when 1 per cent of the thymidine was replaced by BUdR. Assuming that thymidylic acid comprises approximately 28 per cent of the weight of mammalian DNA (i.e., thymine comprises about 30 per cent of the bases on a molar basis [cf. 6]), the maximum mean incorporation of IUDR into the DNA of the tumors in Experiments 4, 5, and 7 is equivalent to about 0.7, 0.6, and 0.4 per cent replacement of TdR, respectively (i.e., at 2.5, 2.0, and 1.3 μg IUDR/mg DNA, respectively). A maximum upper limit of 2.2 per cent replacement of TdR in vivo was observed in an individual intestine sample after IUDR administration in depot form (Experiment 5). Results of Experiment 3 suggest that the figures for IUDR incorporation reported in this paper are conservative estimates.

The rapidity with which maximal incorporation of IUDR was reached after a single injection of the saline solution is in accord with the rapid absorption from the injection site seen in Experiment 6, and with the rapid degradation of tracer doses of the compound reported by others (5, 18, 18, 24). The relatively high and variable radioactivity of the nucleic acid extracts from mouse liver tissue in Experiments 1 and 2 and human omentum in Experiment 7 was most probably due to 129 contamination and not to IUDR that had been incorporated into the DNA of the cells (31). The liver rapidly dehalogenates BUdR (17) and probably is the site of breakdown of IUDR (5, 18, 31).

The effectiveness of FUdR in increasing the incorporation of IUDR into DNA was in line with expectations on the basis of results of others from studies of BUdR and IUDR incorporation in vitro into bacterial (21) and mammalian DNA (8). These results and similar conclusions reached in clinical chemotherapy trials (15, 31), or based on total tissue-retention experiments (13, 18, 26), are attributable to the inhibition of thymidylate synthetase by FUdR (14). In agreement with Kriss et al. (18) the correlation between the total tissue radioactivity and the specific activity of the DNA was poor, and estimates of the incorporation of IUDR into DNA made on this basis are subject to considerable error.

IUDR was absorbed much less rapidly when injected in the depot form than when given in saline solution and was more efficiently utilized for DNA synthesis in both normal and neoplastic tissues in agreement with the results of Hampton and Eidnoff (13). The specific activities of the DNA of the spleen and small intestine equaled or exceeded that of the tumor, however, during the first 24 hours after injection in all four experiments with mice. The specific activities of the DNA from the human skin and small intestine were likewise higher than that of the tumor when sampled a few hours after cessation of drug infusion. At that time, any increase in the radiosensitivity of the
tumor cells resulting from incorporation of the IUDR would presumably be accompanied by an equal or greater sensitization of the normal tissues (cf. 16, 81). It would be highly desirable to devise a treatment regimen in which the concentration of IUDR in the DNA of the neoplasm exceeded that in critical normal tissues at the time of radiotherapy.

If it can be assumed that the IUDR which is incorporated into DNA is stable, a decrease in the specific activity of the DNA would result from either or both (a) dilution with DNA synthesized without label or (b) loss of DNA from the tissue by sloughing or maturation and migration of cells. In growing tissue, such as tumors, the former process is most important, and the time required for the specific activity of the DNA to be reduced by one-half (the half-time) approximates the tumor cell generation time (20, 22). In adult spleen, the size and total DNA remain constant—i.e., production of new cells is apparently equaled by migration of matured blood cells. In the small intestine, production of new cells occurs almost exclusively in the epithelium of the crypts and is balanced by cell loss from the tips of the villi (19, 25). If it can be assumed that the DNA which contains labeled IUDR is randomly distributed between daughter cells at mitosis in these tissues, the specific activity of the DNA of successive cell generations would decrease exponentially. The half-time of the labeled DNA would again approximate the generation time of the labeled cells (20, 22). Generation times estimated from the rate of decrease in the specific activities of the DNA following a single injection of IUDR in saline solution in Experiment 5 were 1.6, 1.0, and 1.0 days for tumor, spleen, and small intestine, respectively. These figures are in fair agreement with those of Quastler and Sherman (25) for mouse ileum, and of Nygaard and Potter (22) for rat intestine and spleen. The high specific activities of the normal tissues 24 hours after drug administration are attributable to the short generation time.

As a result of the high cell turnover rate in the spleen and intestine, the specific activity of the DNA of the tumor equaled or exceeded that of those normal tissues 6 days after administration of a single dose of the drug. These results indicate that the mode and schedule of drug administration and the timing of radiotherapy may play an important, even decisive, role in the efficacy of combined therapy with such agents. Experiments aimed at increasing the ratio of the IUDR incorporation into the DNA of the tumor to that in the normal tissues by combined drug administration and/or temporary inhibition of tumor cell proliferation after exposure to the drug are planned.

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