Death and Metastatic Distribution of Tumor Cells in Mice Monitored With $^{125}$I-Iododeoxyuridine 1, 2

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SUMMARY—Tumor cells (L1210 and Ehrlich ascites) were labeled with $^{125}$I-iododeoxyuridine and inoculated into nonradioactive hosts. The fate of the labeled cells and their progeny was monitored at daily intervals by measuring the $^{125}$I retention of individual live mice in a well-type crystal scintillation counter. The excretion of $^{125}$I from the test animals was linked to in vivo death and breakdown of tumor cells. The fractional rate of tumor cell death depended on the route of implantation, i.e., on the location of tumor cells within the host. After intraperitoneal inoculation of labeled tumor cells, the rate of cell death was about 15% per day. Subcutaneous and intramuscular implantation produced much higher death rates (20-40%/day), and up to 55% of the cells died each day after intravenous inoculation. When prekilled tumor cells were inoculated into mice, $^{125}$I was rapidly excreted from the test animals. The anatomical distribution of tumor cells also varied with the route of their administration. After intraperitoneal implantation, large numbers of tumor cells migrated from the peritoneal cavity. Tumor cells inoculated at extraperitoneal sites did not invade the peritoneal cavity. Liver and spleen were major recipients of migrating tumor cells, especially after intravenous inoculation.—J Nat Cancer Inst 43: 763-773, 1969.

THE OVERALL GROWTH rate of tumor cell populations, like that of any other population, depends on cell proliferation, cell migration, and cell death. While the kinetics of tumor growth have been extensively studied in recent years (1-5), many aspects of cell migration remain speculative and obscure. Even less is known about tumor cell death in vivo. The lack of precise information on these questions is caused primarily by technical difficulties, since most of the procedures currently used for measuring cell death and cell migration yield reliable results only under very specialized conditions. For example, the equations presented by Quastler and Sherman (6) for estimating the

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average life expectancy of cells in the intestinal epithelium apply only to systems that are in a steady state. While the rate of cell death within a proliferating tumor population can be calculated if the total cell number, the generation time, and the rate of cell migration are known, accurate measurements of this nature are very complicated.

A direct measure of metastatic distribution and death of tumor cells should be possible by appropriately labeling tumor cells with a radioactive marker and following their fate and distribution after inoculation into new hosts. The label should be located intracellularly and should not leak out of the cells while they are alive. It also should not damage the cells and should be rapidly excreted from the host when the labeled cells die. Attempts to study the fate of tumor cells by labeling with $^{32}\text{P}$ (7) or $^{51}\text{Cr}$ (8-10) have been only partially successful, since a fraction of either isotope can exchange out of the living cell and because both isotopes are largely reutilized by surviving tumor cells or normal host cells after death and breakdown of labeled cells. This produces a progressively larger error when the fate and distribution of tumor cells are studied over extended periods (11).

Studies on normal tissues (12) suggested that the radioactive DNA precursor $^{125}\text{I}-\text{i}ododeoxyuridine (IUDR) might be more suitable for measuring death and migration of tumor cells. $^{125}\text{I}$UUDR injected intraperitoneally into mice bearing L1210 lymphoid leukemia is incorporated very efficiently into DNA of the peritoneal tumor cells, especially during the early phases of tumor development (13). After incorporation into the DNA, radioiodine ($^{125}\text{I}$) remains bound within the cells until they die (12, 14). When a labeled cell dies, rapid deiodination and excretion of the DNA breakdown products limit the reutilization of radioactivity by other cells (12). Since large amounts of $^{125}\text{I}$ activity can be incorporated into the DNA of L1210 cells, it should be feasible to inoculate these cells into new, nonradioactive mice and to monitor the fate of the labeled cells and their progeny within the new hosts. Since $^{125}\text{I}$ is a $\gamma$-ray-emitting isotope that can be detected in whole animals, it is possible to detect cell death by monitoring the loss of $^{125}\text{I}$ activity from individual mice throughout the development of the tumor without killing the test animals. In addition, the $^{125}\text{I}$ activity of various organs can be measured at different time intervals after inoculation of $^{125}$IUDR-labeled L1210 cells, permitting a quantitative study of the metastasis of L1210 cells. The following report describes this technique, indicates its limitations, and provides evidence for substantial rates of death and migration of some transplantable tumors. These results must be considered in any overall evaluation of tumor growth and may provide new avenues for tumor therapy.

**MATERIALS AND METHODS**

L1210 mouse lymphoid-leukemia cells were grown in the peritoneal cavity of 10- to 12-week-old (C3H X DBA/2J)F1 (C3D2F1J)4 female mice by weekly passage of $10^6$ cells. Four days after inoculation, the L1210 cells were labeled by two intraperitoneal injections of 0.5–25 $\mu$g $^{125}\text{I}$UUDR per mouse. Except for a few experiments, the usual labeling dose was 2.5 $\mu$g $^{125}$IUDR per injection per mouse. The two injections were given 6 hours apart to assure complete labeling of the tumor population. Two days after the administration of $^{125}$IUDR, the mice were killed in a chloroform chamber and the leukemia cells were harvested under sterile conditions by repeatedly flushing the peritoneal cavity with Earle’s balanced salt solution.

Tumor cells from 5-10 mice were pooled and twice washed by gentle centrifugation at 400 rpm for 10 minutes to remove associated radioiodide. After each centrifugation, the tumor cells were resuspended in fresh Earle’s balanced salt solution and filtered through a funnel lined with fine gauze to eliminate cell clumps from the suspension. During these procedures the cell suspension was kept continuously at 4°C. The final sample contained approximately 10% of the radioactivity originally injected into the mice and over 90% of this $^{125}$I activity was associated with the DNA of the leukemia cells (13).

To study the death and anatomical distribution of $^{125}$IUDR-labeled cells, 5 to $10 \times 10^6$ L1210 cells were inoculated intraperitoneally (ip), in-
tramuscularly (im, both hind legs), subcutaneously (sc, under the skin of the back), or intravenously (iv, tail vein) into C3D2F1 mice. To determine the excretion time of 125I-labeled breakdown products, a control group of mice received an inoculum of labeled cells that had been killed by repeated freezing and thawing or by heating in an 80°C water bath for 15 minutes. In some experiments, groups of mice were inoculated with free 125I or with a suspension of radioactive nucleoprotein, which had been extracted from dead 125IUDR-labeled tumor cells with a 1 M sodium chloride solution, clarified by centrifugation, and precipitated by dilution to isotonic salt concentration (16, 17).

One or two days before the mice were injected with 125IUDR-labeled tumor cells, their drinking water was supplemented with 0.1% sodium iodide to depress subsequent accumulation of 125I in the thyroid. Immediately after tumor inoculation, and at daily intervals thereafter, the whole-body 125I radioactivity of the mice was monitored by counting individual live mice in a well-type crystal scintillator (well diameter 35 mm). The mice were placed in plastic holders, which permitted efficient detection of the 30 keV X rays of 125I. Care had to be taken to avoid contamination of the well by urination. To study the anatomical distribution of radioactivity, some of the mice were killed in a chloroform chamber 4 days after inoculation of the 125IUDR-prelabeled cells. The leukemia cells in the peritoneal cavity were removed by repeated washings with saline. The 125I activity in the DNA of the tumor cells, the liver, spleen, intestine, skin, and the rest of the body was measured after homogenizing all organs (except the skin), followed by precipitation of the DNA with 10% trichloroacetic acid to remove 125I activity not incorporated into DNA (12).

Similar experiments were undertaken with Ehrlich ascites cells. The mouse strain used in these studies was SJL/J. Five µc of 125IUDR was injected ip on day 4 and again on day 5 after inoculation of 10⁶ Ehrlich ascites cells. The animals were killed on day 8 when enough tumor cells for transplantation had accumulated. Otherwise, the experiments with Ehrlich ascites tumor cells were carried out exactly as described for L1210 cells.

RESULTS

Loss of 125I Activity From Mice Inoculated With 125IUDR-Labeled Tumor Cells

Text-figure 1 shows the loss of 125I activity from groups of mice inoculated ip with 125IUDR-labeled L1210 cells, with radioactive nucleoprotein, or with radioiodide. The slope of the curve for living L1210 cells indicates that a significant fraction of the 125I activity was lost from the leukemic mice each day. The excretion rate in this particular experiment was about 15% per day. In other experiments it was as low as 12% or as high as 18%. During the later phases of tumor development, especially in the last 2 days before the animals died, the rate of 125I excretion seemed to level off. In early experiments, when cell suspensions were kept at 37°C and centrifuged 3 times at 1000 rpm, we frequently observed a rapid and variable loss of radioactivity the first 24 hours after inoculation. This drop was due to the damage associated with the original preparation technique. The subsequent rate of 125I excretion in such experiments, however, was comparable to that observed in later experiments with lower initial loss rates.

Text-figure 1.—Loss of 125I from mice inoculated ip with living 125IUDR-labeled L1210 cells, dead L1210 cells, radioactive nucleoprotein, or radioiodide.

When heat- or cold-treated 125IUDR-labeled L1210 cells were implanted into the peritoneal cavity of mice, more than 60% of the radioactivity was lost each day the first 2 or 3 days after inocula-
The loss of $^{125}$I activity decreased continuously throughout the experiment. None of the mice inoculated with heat-treated L1210 cells ever died of leukemia. $^{125}$I excretion from mice injected with radioactive nucleoprotein closely followed the pattern obtained with dead tumor cells. Free $^{125}$I, however, was much more rapidly excreted (text-fig. 1).

Text-figure 2 shows the loss of $^{125}$I activity from C3D2F1 mice following ip inoculation of L1210 cells labeled with 1, 2, 5, 10, or 50 $\mu$g $^{125}$IUDR. Labeling doses of up to 5 $\mu$g $^{125}$IUDR per mouse did not affect the rate of $^{125}$I excretion, while higher doses accelerated the loss of radioactivity from the usual 15% per day to 25–30%. On day 6, when the 1, 2, and 5 $\mu$g groups still retained 60% of the inoculated $^{125}$I activity, the 10 $\mu$g group retained only 41%, and the 50 $\mu$g group no more than 24%. The rate of $^{125}$I excretion from heat-killed L1210 cells did not vary with the different labeling doses and was exactly the same as shown in text-figure 1.

The loss of $^{125}$I from SJL/J mice inoculated ip with living $^{125}$IUDR-labeled Ehrlich ascites cells (text-fig. 4) was slightly higher than the loss of $^{125}$I from mice bearing labeled L1210 cells in the peritoneal cavity. Whether the higher rate of $^{125}$I loss was due to radiation damage from the larger labeling dose used in this experiment was not determined. $^{125}$I excretion again seemed to slow down shortly before the death of the mice. When heat-killed $^{125}$I-labeled Ehrlich ascites cells were injected ip, the rate of $^{125}$I loss appeared somewhat slower than in the case of dead labeled L1210 cells. However, the variation in $^{125}$I excretion between individual mice was higher in the experiments with labeled Ehrlich ascites cells than in experiments with L1210 cells.
Radioactive excretion as a measure of cell death.—Our most surprising observation was the rapid rate of excretion of radioactivity from mice bearing living 125I-labeled tumor cells. This loss of 125I activity can be used as an index of naturally occurring tumor cell death if: 1) the 125I in the inoculum is associated exclusively with the DNA of the tumor cells and is not released from the DNA of labeled cells while they are alive; 2) 125IUDR does not damage or kill the labeled tumor cells; and 3) 125I is rapidly excreted following the death of labeled cells.
Since earlier studies showed that iododeoxyuridine incorporated into the DNA of nonmalignant mouse cells did not leave these cells until they died (12, 14), and since IUDR was incorporated into the DNA of tumor cells in an analogous fashion during DNA synthesis, it appears unlikely that $^{125}$I is released from the DNA of living tumor cells. If DNA turnover (18) or deiodination of tumor cell DNA were responsible for the loss of radioactivity we observed, then equal amounts of $^{125}$I activity should be lost from living tumor cells in all parts of the body. However, L1210 cells inoculated iv lost radioactivity three times as fast as cells implanted ip. Moreover, this loss continues at an almost constant rate until most of the radioactivity is excreted, giving no evidence of loss by more than one mechanism. Therefore, we believe, this continuous excretion of radioactivity from leukemic mice indicates that a corresponding fraction of the labeled tumor population dies each day.

The death of tumor cells could be due to chemical effects resulting from the administration of the thymidine analogue iododeoxyuridine. IUDR may produce functional changes in the DNA by replacing part of the thymine component with iodouracil. However, except for the gradual increase in radiosensitivity that is observed when 1% or more of the thymine has been replaced (19-21), the chemical effects of incorporated IUDR appear to be minimal. Since the IUDR used in these studies was prepared without the addition of carrier iodide, its specific activity could be as high as 1 c/μmole, and thymidine substitution by IUDR should therefore be less than one part in 10,000. Consequently, chemical alterations of the tumor cell DNA must be insignificant. Other chemical effects on cell metabolism also appear unlikely because of the extreme dilution of IUDR and, in any event, these should have disappeared by the time of transplantation.

Radiation effects from the incorporated $^{125}$IUDR may also affect the viability of the labeled tumor cells. While much of the radiant energy of $^{125}$I is emitted as γ-rays and X rays, which largely escape not only the tumor but also the mouse, there is an important component of electron irradiation from the K-capture process which produces electrons with energies ranging from 34-3 kev and less (22). The electrons most frequently ejected in $^{125}$I disintegration have energies in the 3-4 kev range. These electrons largely expend their energy in the nucleus of the cell in which they originate. The more energetic electrons with energies between 22 and 34 kev usually escape the nucleus before they have lost half of their energy (range of 22 kev electrons in water is 8 μ). From the reported frequency of electrons within the various kev ranges (22), it can be calculated that the average radiation dose to the cell nucleus is about 8 kev per disintegration of $^{125}$I. This compares to a 6 kev irradiation from each tritium disintegration (d). From Marin and Bender's dose-survival curves for cultures of Chinese hamster cells exposed to tritiated thymidine (23), it can be estimated that one-half to three-quarter d/min of intranuclear
tritium was required to produce measurable cell death (20% loss in clone formation). Similar results should thus be obtained in their system with one-third to one-half \( d/\text{min} \) of intranuclear \( ^{125}\text{I} \), or 0.15-0.25 \( \mu\text{c}/10^6 \text{cells} \) (2.2 \( \times 10^5 \) \( d/\text{min} = 1 \mu\text{c} \)).

A similar degree of cell killing can be obtained in our experiments by exposing L1210 cells in vivo to 10 \( \mu\text{c} \) of \( ^{125}\text{IUDR} \). Since about 25% of the injected dose is incorporated into the 50 million L1210 cells present in the peritoneal cavity at the time of injection, these cells will contain initially 0.05 \( \mu\text{c} \) of \( ^{125}\text{I} \) per million cells; i.e., the average cell will receive radiation from 0.1 \( d \) \( ^{125}\text{I} \) per minute.\(^6\) Thus our system appears to be 3-5 times more radiosensitive than Marin and Bender's. However, Feinendegen and his associates (personal communication) estimate a much larger radiation dose from \( ^{125}\text{I} \) by including lower energy electrons apparently neglected by Myers. If we use Feinendegen's estimate we obtain a radiosensitivity with \( ^{125}\text{I} \) similar to Marin and Bender's with tritium.

On the other hand the difference in radiosensitivity may be larger than indicated, because some L1210 cells which do not release their \( ^{125}\text{I} \) activity may still be incapable of meeting Marin and Bender's criterion of reproductive potential. Furthermore, our experimental design minimizes the effects of \( ^{125}\text{I} \) toxicity on our results, since the labeled cells are permitted to grow for 2 days in the primary host before being transferred to secondary mice. This period allows time both for the removal of cells damaged by the injection of \( ^{125}\text{IUDR} \) and the dilution of the radioactivity among the surviving progeny. In any case, the differences in radiosensitivity between Marin and Bender's work and our studies are small enough so that we feel strongly that radiation effects are being observed at the higher doses of \( ^{125}\text{IUDR} \). Such adverse effects of \( ^{125}\text{I} \) irradiation on the viability of transplanted tumor cells can be avoided by use of sufficiently low doses of \( ^{125}\text{I} \).

In the case of L1210 cells labeled ip with \( ^{125}\text{I} \), a dose of less than 5 \( \mu\text{c} \) per mouse seems satisfactory. This should keep the level of incorporation below 0.03 \( \mu\text{c} \) per million tumor cells. We feel that safe dosage limits should be determined for each cell line studied and therefore would caution that our results for Ehrlich cells may include radiation effects, although 4 days were allowed to intervene between labeling and transplantation.

Mice inoculated with heat-killed labeled L1210 cells excrete about 90% of the \( ^{125}\text{I} \) activity within 2 days after implantation. When dead labeled tumor cells are added to unlabeled peritoneal L1210 populations at various stages of tumor development, the \( ^{125}\text{I} \) activity is also rapidly excreted (11). These observations indicate that only a small proportion of the radioactivity released from the dead tumor cells is reutilized. The high rate of \( ^{125}\text{I} \) excretion from dead tumor cells and the relatively inefficient reutilization of radioactive DNA breakdown products in new DNA synthesis combine to make the \( ^{125}\text{IUDR} \) technique a sensitive method for monitoring nonlogarithmic rates of tumor cell death in vivo, i.e., in the range of up to 95% cell death.

Since the radioactivity of dead tumor cells is not completely and instantaneously excreted from the leukemic mice, various steps involved in the breakdown of dead tumor cells have to be considered before the rate of \( ^{125}\text{I} \) excretion can be taken as a quantitative index of tumor cell death. The excretion of radioactivity from mice inoculated with dead tumor cells, or with radioactive nucleoprotein, is much slower than from mice given injections of radiodiode. This indicates that iodide excretion \textit{per se} is not the limiting factor in our experiments, but that the rates of autolysis or phagocytosis and of DNA breakdown are the significant factors affecting the shape of the curves after inoculation of inviable cells. Autolysis does not appear to be a major rate-controlling factor, since there is no significant difference in \( ^{125}\text{I} \) excretion between mice inoculated with heat-, freeze-, or irradiation-killed tumor cells. The rate of phagocytosis should depend on the location of the dead cells within the body. This may explain the extremely slow excretion of radioactivity from dead cells injected sc. Phagocytosis may also be a limiting factor in other injection sites. Reutilization of the label, although less important, also slows the \( ^{125}\text{I} \) excretion. This effect becomes most pronounced between 2 and 4 days after the

\(^6\) Feinendegen has observed radiation effects (characterized by a slowing of maturation) on rat bone marrow cells labeled with one-third of this amount of tritiated thymidine (29).
inoculation of dead cells, when most of the catabolic radioactivity has been excreted while the epithelial cells still retain a large fraction of the reutilized label (table 1). Retention of 125I in the skin (urinary contamination) is another factor slowing the excretion of radioactivity from mice inoculated with dead labeled tumor cells (table 1).

Factors affecting cell death.—Following ip or im inoculation, the fractional rate of 125I loss obtained with living inocula is much smaller than the rate observed with dead cells. This indicates that the rate of 125I excretion from mice bearing living labeled tumor cells is not limited by the rate of breakdown of dead cells, but by the rate of tumor cell death. Retention of radioactivity due to reutilization and urinary contamination is also not a major factor in mice inoculated with living labeled tumor cells (table 1). Most of the 125I activity remaining in such mice is associated with the DNA of the tumor cells, and the relatively small fraction retained elsewhere in the mouse does not significantly affect the initial slope of the 125I excretion curve. The rate and amount of 125I loss thus provide minimal estimates of cell death. This would appear to closely approach the actual rate of tumor cell death in vivo for ip or im administered cells.

Quantitative estimates for iv administration are less precise, since the cell death rate is so high that rates of catabolism and excretion may need to be considered, and these are not yet available for iv administered dead cells. After sc administration, counts on living mice are meaningless because the excretion rate was the same for living and dead inocula.

Continuous fractional death is not a peculiarity of the L1210 lymphoma. Ehrlich ascites cells (text-fig. 4) and WR-6 lymphocytic rat leukemia cells (unpublished observations) also show a high rate of cell death throughout the development of the tumor. Continuous in vivo death of tumor cells may therefore be characteristic of many transplantable tumor lines. The reasons for the high rate of cell death in logarithmically growing populations of tumor cells are not apparent. It may be that in each generation a certain percentage of daughter cells are nonviable as a result of mitotic alterations, which are frequently observed in neoplastic tissues (25). However, amethopterin, which slows the generation time of L1210 cells, does not decrease the death rate as might be expected from this hypothesis (26). Furthermore, reproductive cell death does not explain the different rates of cell death observed after different routes of inoculation. Therefore, local environmental factors within the host must play an important role in causing the death of tumor cells.

Since the cell death begins too early after transplantation to result from a conventional immune response of the host, other types of host-tumor interactions must be involved. Cell death appears to be slowest in the peritoneal cavity and more rapid in other parts of the body (cf. death rates after ip inoculation vs. other inoculation routes). We believe that the high rate of cell death after iv inoculation of L1210 cells is caused by mechanical stress on the tumor cells in the bloodstream. Inadequate nutrition due to lack of vascularization may also play a role, especially after sc inoculation. However, other mechanisms cannot be excluded, although they are still poorly understood. Electron microscopic studies on the invasion of skeletal and smooth muscle fibers by L1210 cells have shown that many leukemic cells growing in these organs undergo various degenerative changes (27). Partial or complete disappearance of the plasma membranes occurred in many leukemia cells, especially in those located in the host-tumor interzone. Aggregates of ribosomes and other cytoplasmic components, derived from the disintegrating leukemia cells, were frequently detected in the interstitial spaces between leukemic cells and muscle. The presence of this material initially seemed to exert a stimulatory effect on the muscle fibers (hyperplasia of cell organelles), but eventually caused the degradation and lysis of the normal structures. These observations suggest that tumor cell death may be a factor in the destruction of normal tissues in the course of tumor invasion. The death of tumor cells could therefore be the result of a host-tumor interaction. The absence of such a mechanism in the peritoneal cavity would account for the much smaller death rate of the peritoneal tumor cells.

Cell migration.—Besides offering a useful tool for monitoring the rate of tumor cell death throughout the development of the tumor, the 125IUDR-prelabeling technique makes it possible to quantitatively study the migration and metastatic spread
of tumor cells. In most organs the amount of radioactivity can be taken as an index of tumor cell migration if proper corrections are made for reutilization and for residual catabolic radioiodide. For instance, the skin and the intestine retain as much radioactivity in mice inoculated with dead labeled tumor cells as in mice receiving living inocula (table 1), and, therefore, the data provide no evidence of metastasis to these organs. While the skin may contain some metastatic cells, it also contains large and variable amounts of excreted radioactivity that is difficult to remove. Therefore, this organ was not analyzed further. Since cell renewal in the intestine normally accounts for almost half of the DNA synthesis in the mouse (12), most of the activity in the intestine can be readily attributed to radioactive IUDR released from dead labeled tumor cells and reutilized by the rapidly proliferating cells of the intestinal epithelium. Similarly, an appreciable fraction of the activity in the spleen must result from reutilization. These artifacts could be eliminated and more detailed information obtained by autoradiographic analysis, which could differentiate weakly labeled cells (reutilization) from those of the original inoculum. Most of the radioactivity in the liver seems to be attributable to metastatic tumor cells, and since the amount of $^{125}$I in this organ depends on the route of implantation, some information as to the mechanisms of tumor migration can be obtained.

The factors responsible for the metastatic spread of tumor cells are complex. The formation of metastases in distant parts of the body depends on the ability of the tumor cells to invade adjacent normal tissues and gain entrance to the vascular system. Two different concepts have been advanced to explain the distribution of blood-borne metastases. Some investigators believe that purely anatomical or mechanical factors of the blood flow are sufficient to explain the organ distribution of tumor cells (28), whereas others maintain that various tissues of the body have different metabolic or biologic properties which encourage or inhibit the growth of migrating tumor cells (29–31). Recent studies on the behavior of different tumor lines in the same strain of animals indicate that intrinsic factors within the tumor cell itself may also influence the pattern of metastatic spread (10). In the present studies the differences between L1210 and the Ehrlich tumor in terms of cell migration are truly impressive. In fact, our data show no evidence of measurable migration of the Ehrlich tumor from the peritoneal cavity. This may explain the longer lifespan of mice carrying this tumor.

With iv injected L1210 cells, the liver and especially the spleen accumulate $^{125}$I activity disproportionate to their contribution to the body mass (table 1). If the tumor cells were distributed uniformly throughout the body of the mouse, the liver should retain no more than 10% and the spleen less than 1% of the radioactivity present at the time the animals are killed. Actually, the liver contains up to 50% and the spleen almost 7% of the radioactivity associated with tumor cells 4 days after iv inoculation (the $^{125}$I content of skin and intestine are disregarded in this calculation because their radioactivity does not represent tumor cells). L1210 cells inoculated im also migrate selectively, though in smaller numbers to the liver (20%) and spleen (5%) (the radioactivity remaining at the site of inoculation, the leg muscles, is not included in this calculation). A similar situation exists when L1210 cells are implanted sc. The data for ip inoculation do not indicate preferential accumulation of L1210 cells in the liver and spleen. However, this is probably because the values given in table 1 for the “rest of the body” are not corrected for local metastases of tumor cells within the peritoneal cavity. Our results, therefore, indicate that the anatomical distribution of L1210 cells which have migrated from the site of inoculation is determined primarily by the organ factor (preferential accumulation of L1210 cells in liver and spleen), although the route of inoculation and therefore the transport factor also influence the pattern of tumor spread.

CONCLUSIONS

The technique of $^{125}$IUDR labeling of tumor cells permits a quantitative study of tumor cell migration and tumor cell death in vivo. Care must be taken to avoid damaging cells by the labeling procedure or by radiation from excessive amounts of incorporated radioisotopes. The procedure is most valuable in determining early cell death during logarithmic growth of the tumor and has already been used to distinguish between cytocidal and cytostatic effects.
of antimetabolites (26, 32). It becomes insensitive when most of the labeled cells have died since, under these circumstances, variations in the residual load remaining from earlier cell death destroy the precision of measurement. The site of implantation also affects the precision of the results, and sc implantation has proved unsuccessful, since mice inoculated subcutaneously lose radioactivity at the same rate whether the cells are living or dead. Recent reports have also appeared in which IUdR is used in studies on cell death in solid tumors (33-35).

REFERENCES


