Reutilization of Thymidine and Iododeoxyuridine by Mouse Mammary Carcinoma Strain MTG-B[1] (37195)

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Isotopically labeled thymidine (TdR) and TdR analogues are commonly employed in studies of the DNA metabolism and cell population kinetics of tumors. Of the compounds commonly used, TdR itself is most efficiently incorporated following administration (1–4). However, autoradiography or liquid scintillation-counting techniques are required for detection and quantitation of its most common isotopic labels, ^{3}H and ^{14}C. The analogue 5-iodo-2′deoxyuridine (IUdR) labeled with radioiodine is less efficiently incorporated following injection (1–4). It has the advantage, however, of easy detection and quantitation by crystal scintillation counting and, when labeled with ^{125}I, is suitable for autoradiography (1, 5).

The use of TdR and IUdR frequently depends not only on the specificity of their incorporation into DNA, but also on the rapidity with which any tracer molecules which are not incorporated are catabolized and thus removed from the DNA precursor pool. DNA synthesizing cells can, however, incorporate ("reutilize") labeled TdR or its analogues which have been released from dying labeled cells (1, 3), and this may become important in longer-term studies. For example, Bryant (6) injected Ehrlich ascites tumor cells intraperitoneally at intervals after intravenous administration of ^{3}H-TdR, and withdrew tumor cell samples for autoradiographic analyses. He found an early period (4–6 hr after tracer injection) during which tumor cells placed in the peritoneal cavity did not become labeled. Tumor cell samples placed in the body cavity 6 or more hours after ^{3}H-TdR injection did, however, take up label, as did cells enclosed in dialysis chambers which were left in the peritoneal cavity for 18 hr beginning 24 hr after isotope administration. The latter indicates that the reutilized molecule was either ^{3}H-TdR or a dialysable TdR anabolite. Following a single ^{3}H-TdR injection, Steel (7) found that the total radioactivity in grafted August rat mammary tumors remained constant for 48 hr, and increased thereafter. He concluded that these results correlated "remarkably well" with previous observations on the time of loss of ^{3}H-TdR from eight normal renewal tissues of the rat, and noted that the small intestine discharged 5% of the injected dose during the third through fifth days after ^{3}H-TdR injection. No detectable increase in radioactivity was observed, however, in grafted Marshall rat fibrosarcoma or C^{+} mouse mammary carcinoma.

In contrast, Hughes *et al.* (1), Commerford (2) and Feinendegen *et al.* (3) reported that reutilization of labeled IUdR was considerably less than that of TdR. Hofer *et al.* (8) reported retention of 1.5% of the radioactivity in the bodies of mice 6 days after the injection of killed L1210 leukemia cells labeled with ^{125}I-UdR. In a series of detailed studies, Dethlefsen (4, 9) compared the reutilization of these two compounds, and analyzed the applicability of labeled IUdR to studies of tumor-cell loss. He concluded that approximately 22% of the ^{3}H-TdR released from dying cells was reincorporated, whereas reutilization of IUdR was on the

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order of a few percent.

We employed ^{3}H-TdR and ^{125}I-UdR in studies of the potential cell-population growth rate and cell loss from C3H/Wr mouse mammary carcinoma MTG-B, and reported indirect evidence of greater reutilization of ^{3}H-TdR than of ^{125}I-UdR by this tumor strain (10).

The current experiments were designed to compare the uptake of ^{3}H-TdR and ^{125}I-UdR into MTG-B carcinoma cells under conditions in which all or most of the labeled compound which reached the tumor cells had first been incorporated into normal host tissues. The small intestine is both a major utilizer of injected TdR analogues and releaser of significant quantities of label as its epithelial cells mature and are sluffed. The uptake and persistence of radioactivity from the two TdR analogues was thus investigated concurrently in the small intestine both as a reference to reflect the degree of initial labeling and as an example of a rapidly renewing tissue.

Any TdR or IUdR which is reabsorbed from sluffed intestinal epithelial cells would traverse the hepatic portal circulation before reaching tumor cells grafted subcutaneously. The liver is thought to be the primary site of halogenated pyrimidine catabolism (11, 12). Therefore, the incorporation of ^{3}H-TdR and ^{125}I-UdR into tumor and intestinal tissues were studied following intraperitoneal injection and after injection into the lumen of the small intestine.

Material and Methods. The MTG-B mammary carcinoma (13) was used throughout these experiments. Tumor suspensions were prepared for transplantation with the aid of a cytosieve and injected subcutaneously in both hind legs of recipient mice as previously described (14). Young adult BC3fF_1/Wr female mice served as recipients in Experiments 126 and 128, the young adult C3H/HeJax females in Experiment 141. The concentrations of the suspensions were 10% or 1% by volume of centrifically packed tumor material. The suspensions of lethally irradiated tumor material which were added to some inocula were similarly prepared from tumors which had been exposed to 11,000 or 18,000 rads ^{137}Cs gamma rays (10, 14). When employed, the lethally irradiated material was added to a final concentration of 32% by volume.

In Experiments 126 and 128, ^{3}H-TdR and ^{125}I-UdR were administered intraperitoneally in nine equal doses given at six-hour intervals over a period of 48 hr. ^{3}H-TdR was administered at a dose of 0.5 μCi/g body weight (specific activity 1.9 Ci/mmole), and ^{125}I-UdR at 0.08 μCi/g body weight (400 or 580 mCi/mmole) in Experiments 126 and 128, respectively. Tumor suspensions were injected subcutaneously in both hind legs 4–6 hr after the last isotope injection. In Experiment 128, some groups received ^{125}I-UdR solutions containing nonradioactive KI at a concentration of 500 mg/100 ml and a dose of 0.05 mg KI/g body weight. In addition, some groups received drinking water containing 1 g nonradioactive KI per liter, 2.5 g nonradioactive TdR per liter, or both. Stock TdR drinking solutions were prepared in sterilized glass-distilled water and stored in the refrigerator until use. Sterile drinking containers were changed daily and the water consumption was recorded. The experimental groups and treatment schedules of Experiments 126 and 128 are summarized in Tables I and II.

In Experiment 141, ^{3}H-TdR (1.9 Ci/mmole; 1 μCi/g body weight) or ^{125}I-UdR (3.5 Ci/mmole; 0.2 μCi/g body weight) was administered during surgery when tumors were palpable 7 days after grafting. Food was withdrawn from the cages eighteen hours before, and water was removed 1 hr before, surgery and isotope injection. Food and water were returned to the cages *ad libitum* 5–6 hr after surgery and isotope administration. For isotope injection, the mice were anesthetized with ether, and the pyloric junction was approached through a midventral incision. Either saline placebo or a solution of one of the labeled compounds was injected into the lumen of the small intestine with a 27-gauge needle. In those animals receiving labeled compound in the intestinal lumen, an equal amount of saline placebo was injected directly into the abdominal cavity, and in those which received tracer in the abdominal cavity, an equal volume of saline placebo was injected into the intestinal lumen.

TABLE I. Design and Tissue Weights, Experiment 126 (See Also Fig. 1).

Group	Tracer[a]	Inoculum[b]	Day of Sample	Small intestine wt		Tumor wt	
				n	mg ± SD	n	mg ± SD
A	^{3}H	—	0	7	212 ± 16	—	—
	^{125}I	—		7	187 ± 24	—	—
B	^{3}H	10% LC	6	7	211 ± 20	14	24 ± 15
	^{125}I			7	190 ± 23	12	17 ± 9
C	^{3}H	10% LC + DC	6	7	207 ± 28	14	27 ± 19
	^{125}I			6	187 ± 26	11	40 ± 27
D	^{3}H	1% LC	8	7	191 ± 20	12	12 ± 5
	^{125}I			7	210 ± 20	12	7 ± 6
E	^{3}H	1% LC + DC	8	7	184 ± 40	14	37 ± 25
	^{125}I			7	218 ± 22	14	31 ± 24

[a] "^{3}H" indicates subgroup treated with ^{3}H-TdR; "^{125}I" indicates subgroup treated with ^{125}I-UdR.
[b] "% LC" indicates percentage by volume of sedimentable material in a transplant inoculum suspension prepared from living tumor. "+ DC" indicates that the tumor inoculum also contained material from lethally irradiated tumors at a final concentration of 32% by volume.

The incisions were closed with sutures and skin clips. The animals were killed 24 hr after surgery and isotope administration.

In all experiments, the mice were killed by cervical dislocation. Approximately 5 cm of the small intestine beginning immediately distal to the pyloric junction was rapidly removed, slit, and rinsed in physiologic saline. If tumors were present, they were also removed and dissected as free as possible of nonmalignant host tissue, cysts, hemorrhagic foci, and necrotic areas. All tissues were rapidly weighed and frozen on dry ice. They were held frozen until radioactivity determinations were performed and DNA was extracted by a modified perchloric acid precipitation procedure as previously described (10). Estimation of ^{125}I radioactivity of either whole frozen tissues or DNA extracts were performed with the aid of a well scintillation counter equipped with a pulse height analyzer. For quantitation of ^{3}H radioactivity, aliquots of whole tissue homogenates or DNA extracts were hydrolized and then counted

TABLE II. Design and Tissue Weights, Experiment 128 (See Also Figs. 2 and 3).

Group	Tracer[a]	Drinking water	Day of sample	Small intestines		Tumors	
				n	mg ± SD	n	mg ± SD
A	^{3}H	plain	0	9	207 ± 16	—	—
	^{125}I		0	9	209 ± 17	—	—
B	^{125}I	KI[b]	0	10	206 ± 14	—	—
C	^{3}H	plain	7	9	214 ± 19	17	70 ± 37
	^{125}I		7	9	197 ± 26	16	78 ± 33
D	^{125}I	KI[b]	7	10	207 ± 11	20	77 ± 31
E	^{3}H	TdR[c]	7	9	211 ± 25	16	56 ± 20
	^{125}I	KI + TdR[d]	7	7	213 ± 22	14	75 ± 42

[a] "^{3}H" indicates subgroup given ^{3}H-TdR, "^{125}I" indicates subgroup given ^{125}I-UdR. Groups C, D, and E received bilateral grafts of 10% MTG-B tumor suspension on Day 0.
[b] Water contained 1 g nonradioactive KI per liter, Day 5 to day of death. Tracer solution contained 5 mg unlabelled KI per ml.
[c] Water contained 2.5 g unlabelled TdR per liter (10 mM) from Day 0 to Day 7.
[d] Water and tracer solution contained KI and TdR combined as under *b* and *c* above.

with the aid of a liquid scintillation counter. The DNA concentrations of the perchloric acid extracts were estimated spectrophotometrically as previously (10). All data are presented with Standard Deviations.

Results. Experiments 126 and 128 were designed to compare the incorporation of ^{3}H-TdR and ^{125}I-UdR into tumors grafted into prelabeled hosts treated as described in Tables I and II. In both experiments, the day of last isotope injection and of tumor transplantation is taken as Day 0. As a reference, the concentration of the respective isotopes in samples of small intestine was determined in groups of control animals on Day 0.

In Experiment 126, tissue specific activity was measured in tumors which developed from inocula containing 10% or 1% "living" suspensions with or without 32% lethally irradiated tumor suspension. The growth of tumors from cell injections is stimulated by the presence of lethally irradiated cells, and this effect is particularly marked when the living cell number of the inoculum is small (see Ref. 14). Although the inclusion of lethally irradiated cells in the 1% tumor inocula increased the final tumor weights (group E vs group D, Table I), there was no significant effect on incorporation of tracers into tumor DNA (Fig. 1).

The sp act of the intestinal tissues of ^{3}H-TdR-treated mice on days 6 and 8 were 31% and 18–19% of the Day 0 controls, respectively (Fig. 1). In contrast, the intestinal sp act were 6% and 3% of the Day 0 values in the comparable ^{125}I-UdR-treated groups. Similarly, relative to the Day 0 intestine values, the tissue sp act in tumors which developed from 10% inocula in mice which had received ^{3}H-TdR were 13% and 17%, while those which developed from 1% inocula were 7% and 11%. The comparable figures for tumors which grew in animals which had received ^{125}I-UdR were 2–4% (Fig. 1).

In Experiment 128 (Table II) the administration of KI both in the drinking water and with the labeled compound injection, had no effect on the incorporation of ^{125}I-UdR into whole intestine or intestinal DNA in samples taken on Day 0 (Group B,

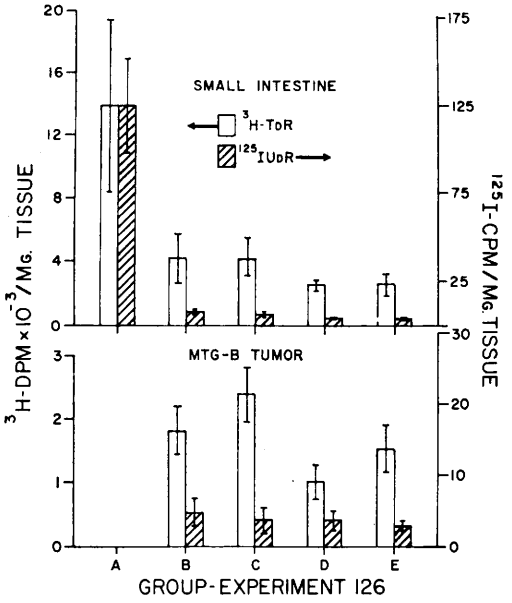

FIG. 1. Persistence of radioactivity in small intestine and uptake into MTG-B tumors grafted after treatment of the host mice with ^{3}H-TdR (open bars) or ^{125}I-UdR (hatched bars). Scales normalized to Day 0 small intestine activities (Group A). Vertical lines are standard deviations. Groups are as described in Table I.

Figs. 2 and 3). The administration of nonradioactive KI or of nonradioactive TdR and KI appeared to modestly reduce the tissue specific activities in both the intestinal samples and the tumors taken from the animals on Day 7 (Groups D and E vs Group C, Fig. 2), but the DNA specific activities in both tissues in these three groups were virtually identical (Fig. 3). As in Experiment 126, relative to the Day 0 values, the persistence of ^{3}H-TdR radioactivity in the whole intestinal tissues and in the intestinal DNA was considerably greater than in the ^{125}I-UdR-treated animals 7 days after the last isotope administration and tumor transplantation (Figs. 2 and 3). The relative concentrations of ^{3}H-TdR radioactivity in whole tumor tissue and in tumor DNA were two- to threefold that in comparable tissue and DNA samples from ^{125}I-UdR-treated animals (Figs. 2 and 3). The inclusion of unlabeled TdR in the drinking water did not significantly affect ^{3}H-TdR uptake or incorporation. The daily water intake of the animals which re-

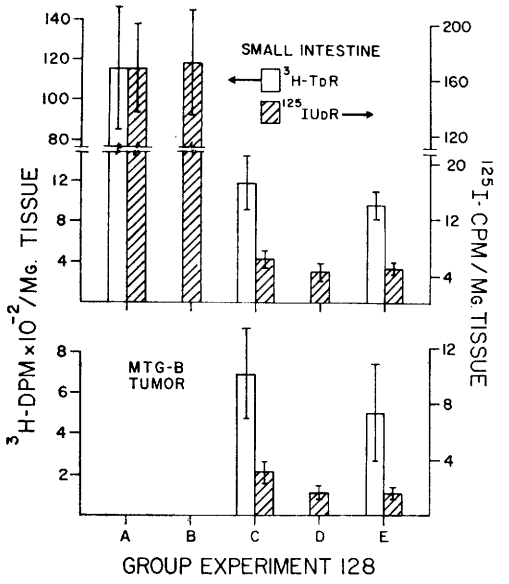

FIG. 2. Persistence of radioactivity in the small intestine and uptake into MTG-B tumors in mice grafted after treatment with ^{3}H-TdR (open bars) or ^{125}I-UdR (hatched bars). Scales normalized to Day 0 small intestine activities (Group A). Note the change in upper scales. Groups as in Table II.

ceived unlabeled TdR during the seven-day tumor growth period averaged approximately 4.1 ml per mouse for a mean TdR intake of approximately 10 mg per day.

In Experiment 141, when ^{3}H-TdR was administered into the lumen of the small intestine, the uptake into the DNA of the intestine and of the tumor was about 50% of that incorporated when the isotope was administered intraperitoneally (Table III, Fig. 4). In contrast, when ^{125}I-UdR was administered into the intestinal lumen, incorporation into tumor and intestinal DNA was 14–20% of that following intraperitoneal injection.

Discussion. These results confirm the previously reported (1–4) reutilization of both ^{3}H-TdR and ^{125}I-UdR. Assuming a transit time on the order of 48 hr from the origin of the intestinal epithelial cells in the crypts to the sluffing of these cells from the tips of the villi and a DNA synthesis period of 6 hr or more (see 4), the schedule of ^{3}H-TdR and ^{125}I-UdR administration in Experiments 126 and 128 would be expected to result in labeling of virtually all of these cells. Most or all of the relatively heavily labeled epithelial cells would be sluffed during the ensuing 48 hr, and would presumably release their labeled precursors into the intestinal lumen. If reutilization did not occur, the remaining epithelial cell radioactivity would be expected to be released in an exponentially decreasing fashion thereafter with a half-time approximating the epithelial-cell generation time. In previous studies in these laboratories (12), the incorporation of ^{125}I-UdR into the DNA of normal and neoplastic tissues after injection of the drug in a depot carrier from which it was slowly released was markedly more efficient than when the drug was administered in aqueous solution. The absorption of TdR analogues from the sluffing intestinal epithelium in particular, and other cell renewal tissues in general, may produce a situation similar to administration of the drugs in a depot carrier, *i.e.,* a relatively constant low blood level of the tracer compounds might be expected to obtain with continual death of labeled cells. This is in contrast to the high and acute blood levels which follow injection of aqueous solutions. Despite the catabolism of TdR in

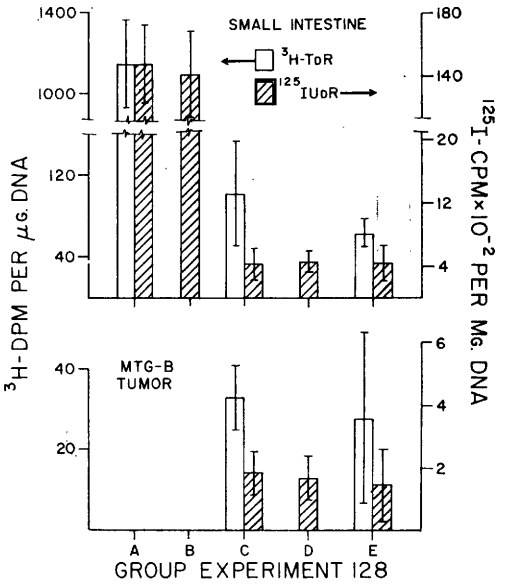

FIG. 3. Persistence of radioactivity in the small intestinal DNA and incorporation into MTG-B tumor DNA in mice grafted after treatment with ^{3}H-TdR or ^{125}I-UdR. Groups, tissues, treatments and data presentation as in Fig. 2 and Table II.

TABLE III. Design and Tissue Weights Experiment 141 (See Also Fig. 4).

Tracer administration		Small intestines		Tumors	
Compound	Site	n	mg ± SD	n	mg ± SD
^{3}H-TdR	peritoneal cavity	8	264 ± 16	15	192 ± 112
^{3}H-TdR	intestinal lumen	9	218 ± 15	17	231 ± 75
^{125}I-UdR	peritoneal cavity	8[a]	223 ± 34	18	217 ± 163
^{125}I-UdR	intestinal lumen	8	236 ± 16	16	176 ± 133

[a] One sample lost.

the liver indicated by the results of Experiment 141, the blood levels of unlabeled TdR would be expected to be high immediately after ingestion of drinking water containing 2.5 mg TdR/ml. The biological half-life of TdR is, however, less than a half hour (2), and mice drink sporadically. These factors probably account for the lack of detectable effect of unlabeled TdR ingestion on reutilization of ^{3}H-TdR and ^{125}I-UdR. Dethlefsen found that significant competitive inhibition of injected labeled IUdR incorporation could be observed only when injections of unlabeled TdR were given during the interval from 10 min before to 15 min after the labeled compound (4), or when the TdR was given in the drinking water at 2–4 times the concentration used in our experiments (9).

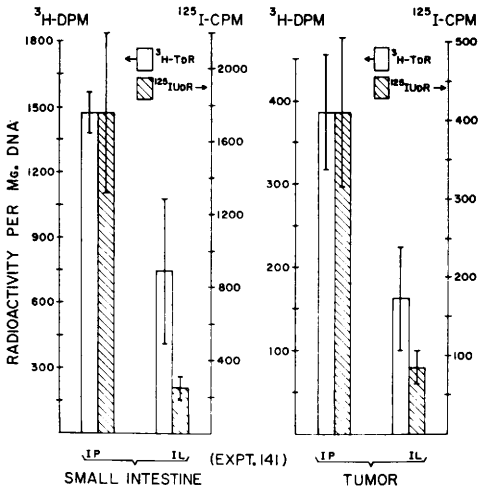

FIG. 4. DNA specific activities in the small intestine and in MTG-B tumors 24 hr after intraperitoneal (IP) injection or injection into the intestinal lumen (IL) of either ^{3}H-TdR (open bars) or ^{125}I-UdR (closed bars). Scales normalized to data from IP-injected animals. Numbers of observations as in Table III.

The mechanism by which inclusion of lethally irradiated cells in tumor inocula increases the growth of living tumor cells is as yet unclear. It has been suggested, however, that the lethally irradiated cells, which die slowly, release macromolecular metabolites which serve as nutrients for surviving cells (15). It is of interest, therefore, that the presence of killed cells in the inocula did not significantly affect the incorporation of either TdR or IUdR, although it caused greater final tumor weights.

The modest effect of administration of nonradioactive KI on tissue specific activity, and its lack of detectable effect on DNA specific activity in ^{125}I-UdR-treated mice is in accord with expectation (4, 12), and illustrates that catabolites of ^{125}I-UdR may contribute to radioactivity measurements performed on whole tissues.

Our results indicate that the greater portion of the ^{3}H-TdR and ^{125}I-UdR which are incorporated into the intestinal epithelium after administration into the intestinal lumen must first have traversed the general circulation. If the stem cells of the crypts absorbed significant quantities of these precursors directly from the lumen, one would expect the efficiency of incorporation to be as high or higher after injection into the intestinal lumen as after intraperitoneal administration. Furthermore, if direct utilization from the lumen was significant, one would also expect the incorporation into the intestinal epithelium relative to that into the tumor after injection into the lumen to be greater than after intraperitoneal injection. Neither situation obtained. The incorporation of both tracer compounds was reduced by intralumenal administration, and for a given compound the relative reduction was about the

same in the tumors as in the intestines.

Finally, the results support the conclusion that IUdR is either more readily catabolized by the liver than is TdR, or is less readily absorbed from the intestinal lumen. It does not seem likely that the more marked decrease in IUdR incorporation after intralumenal administration is the result of chemical inactivation in the lumen before absorption. IUdR would be expected to be chemically stable in the alkaline environment of the small intestine (11).

In accord with the conclusion of others (1–4, 9), these studies illustrate that labeled TdR and IUdR released from dying cells is reutilized by proliferating tissues. Reutilization of TdR is significantly greater than that of IUdR, and the efficiency of the latter appears to be but a few percent. It is probable that the small intestinal epithelium is a major source of reutilizable TdR and IUdR (7), but that both must traverse the general circulation before reincorporation into the intestinal epithelial cells.

As others have noted (1, 9), labeled IUdR is the current compound of choice for cell kinetic studies, but reutilization should be considered in interpretation of results. For example, our previously reported values of the potential MTG-B tumor cell population growth rate and of the cell loss rate (10) are both probably modest underestimates as a result of reutilization.

Summary. The incorporation of ^{3}H-TdR and ^{125}I-UdR into MTG-B tumor tissue and DNA, and the persistence of radioactivity from these precursors in the small intestine, were studied in mice treated with these drugs before tumor grafting. The results confirm the results of others that DNA synthesizing cells can reutilize precursors released by dying cells, and that TdR is more readily reutilized than IUdR. Neither the administration of drinking water containing 2.5 mg unlabeled TdR/ml, nor the inclusion of lethally irradiated cells in the tumor inocula, significantly affected reutilization of either precursor. When the drugs were injected into the intestinal lumen rather than the peritoneal cavity of tumor-bearing animals, the incorporation of ^{3}H-TdR was reduced to 50%, and of ^{125}I-UdR, to 14–20%, in both small intestinal and tumor DNA. These findings suggest that labeled precursors from sluffing intestinal epithelium entered the general circulation before reutilization by either intestinal or tumor cells. In accord with the conclusions of others, radiolabeled IUdR appears to be the DNA precursor of choice in studies of tumor cell population kinetics, with the reservation that cell population growth and cell loss rates will be moderately underestimated as a result of reutilization of the labeled compound.

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