

Fate of Intraintestinal Thymocytes Labeled with ^{125}I iododeoxyuridine or Tritiated Thymidine^{1, 2} (39375)

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Migration of blood lymphocytes into the lumen of the small intestine has been reported (1, 2). The origin of these lymphocytes remains unknown; however, the thymus has been proposed as a possible source (3). Following local thymic labeling with a radioactive DNA precursor, a slight accumulation of radioactivity has been detected in the intestine and its contents (4). If migrants are not catabolized in the intestine, the enteric loss of these labeled thymocytes might be quantitated by measuring fecal radioactivity. To our knowledge, quantitative data on the kinetics of breakdown in the intestinal lumen of cells labeled with radioactive DNA precursors are not available. The present experiments were designed to evaluate the fate of donor thymocytes labeled with either ^{125}I iododeoxyuridine ($^{125}\text{IUdR}$) or tritiated thymidine ($^3\text{HTdR}$) and injected into the small intestine of recipient mice.

Materials and methods. Random bred, female Swiss albino mice of the Hale-Stoner strain, 8 to 12 weeks old, were used as donors and recipients. Two days before intrajejunal (ij) injection of cells labeled with $^{125}\text{IUdR}$, KI (0.5 mg/ml) was added to the drinking water of recipients (4). The intestinal flora of one group of conventional mice was removed by adding high doses of non-absorbable antibiotics to the drinking water and housing under sterile conditions for 1 week as reported by van Bekkum and co-workers (5).

$5\text{-}^{125}\text{I}$ odo-2'-deoxyuridine (New England

Nuclear, Boston, Mass.; sp act > 200 Ci/mmole; 203 to 480 $\mu\text{Ci/ml}$) was diluted with normal sterile saline (9 mg/ml) to contain 100 $\mu\text{Ci/ml}$. Donor mice were labeled by two to four iv or ip injections at 6- to 12-hr intervals (total dose per mouse 20, 40, or 70 μCi). Thymidine (methyl- ^3H ; Schwarz/Mann, Orangeburg, N.Y.; sp act 1.9 Ci/mmole; 1 mCi/ml; diluted with normal saline to contain 200 $\mu\text{Ci/ml}$) was injected ip into donor mice at 8-hr intervals (eight injections, total dose per mouse 160 μCi). All donors were killed by ether overdose 24 hr after the last injection. Donor thymi were minced in Hanks' balanced salt solution (HBSS, without Ca and Mg; Gibco, Grand Island, N.Y.) to which 4 mg/ml of Na_2EDTA had been added. The cell suspensions were forced through 22-gauge needles, filtered with nylon mesh, washed twice in 50 ml of HBSS and concentrated to contain about 1 to 9×10^7 cells per dose. This corresponded to approximately 3000 and 6000 cpm for ^{125}I and ^3H , respectively. Trypan blue-positive cells were enumerated (6). In a control experiment, the suspended cells were killed by repeated freezing and thawing. Mice were anesthetized by ip sodium pentobarbital (4). The proximal end of the recipient jejunum was exposed through a small (5 mm) incision, and 0.05 ml of the cell suspension was injected ij using a 30-gauge atraumatic needle. The wound was closed and the animals placed in individual plastic cages (4). Recipients were killed at the time indicated in the figures. The intestine (pylorus to anus) and its contents, carcass, feces, urine, and in some instances spleen and thymus were collected and kept at 4° prior to counting ^{125}I -activity in a well-type scintillation counter (Nuclear Chicago, 1185 series). Aliquots of thymocytes labeled with $^3\text{HTdR}$ and tissues of recipient animals were subjected to formalin

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extraction of acid-soluble radioactivity (ASR) (4). Total tritium activity of tissues and formalin extracts was determined by scintillation counting following oxidation (TriCarb oxidizer, Packard).

Results. Thymocyte suspensions were essentially monodispersed and contained less than 10% trypan blue-positive cells. More than 90% of the radioactivity of the suspension was cell-bound and not extractable with formalin. In the control experiment, more than 95% of the morphologically recognizable cells were stained by trypan blue.

Following injection of thymocytes labeled with ^{125}I UdR, the decline of radioactivity of the recipient intestine and its contents could be described by two exponential components, with a high rate of activity loss during the first 5 hr (Fig. 1). Viability of the cells prior to injection did not significantly affect the rate of ^{125}I -activity loss, i.e., 35.6, 9.9, and 4.5% of the intestinal activity present at 0 hr remained 1, 3, and 5 hr following injection of thymocytes killed by freezing and thawing. The bulk of the injected ^{125}I -activity lost from the intestine was recovered in the urine, the ^{125}I -activity curve of the latter essentially mirroring the intestinal activity. Less than 3% of the initial activity appeared in the feces.

During the first 4 to 5 hr following ij

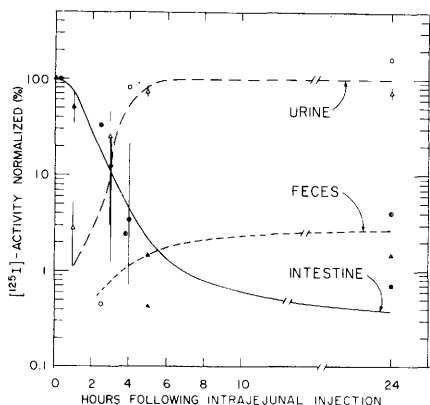


FIG. 1. Normalized ^{125}I -activity (percentage of intestinal activity at 0 hr, \pm SD) versus time (0, 1, 2.5, 3, 4, 5, 10, and 24 hr) following ij injection of thymocytes labeled with ^{125}I UdR. Triangles (average of three mice) and circles (average of two mice) represent data from two separate experiments. The curves were fitted by eye. $\bullet\blacktriangle$ = Intestine and contents; $\circ\triangle$ = cumulative urinary activity; $\bullet\blacktriangle$ = cumulative fecal activity.

injection, intestinal loss of ^{125}I -activity in recipients pretreated with antibiotics (Fig. 2) was nearly as rapid as that observed in conventional mice. Thereafter, a slower rate of loss was observed; however, values at 24 hr postinjection were not significantly different from those seen in conventional mice. The proportion of cumulative ^{125}I -activity in the feces was somewhat higher than in conventional animals. The sum of activities recovered in urine, feces, intestine, and the remainder of the body during the first 24 hr postinjection accounted for the near-totality of the initial radioactivity in both groups.

Tritium activity was lost from the intestine initially as rapidly as ^{125}I -activity; 3 hr after ij injection approximately 10% of the total initial ^3H -activity remained in the intestine (Fig. 3). Thereafter, the loss of ^3H -activity was slow. The relative activity of the intestine including ASR 24 hr postinjection was about 15 times higher than the corresponding values for ^{125}I .

Discussion. Thymocytes labeled with ^3H TdR or ^{125}I UdR appeared to be rapidly catabolized in the small intestine. Elimination of ^{125}I -activity in the feces was, however, minimal. It was observed earlier that the fecal radioactivity following systemic labeling with ^{125}I UdR remained low even though the major part of activity was lost from the intestine presumably through sloughing of labeled epithelial cells into the lumen. The bulk of the label was apparently

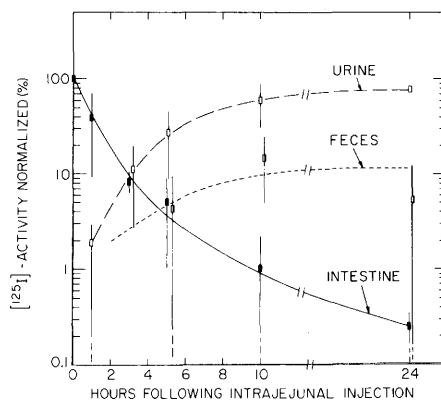


FIG. 2. Recipients were pretreated with antibiotics. ^{125}I -activity (percentage of intestinal activity at 0 hr, \pm SD) versus time following ij injection of thymocytes labeled with ^{125}I UdR. Symbols analogous to those used in Fig. 1.

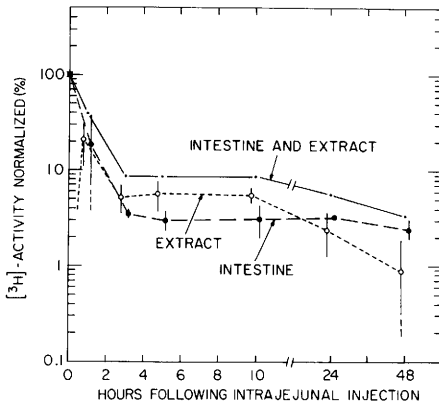


FIG. 3. Normalized ^3H -activity (percentage of intestinal activity at 0 hr, average of five mice, $\pm\text{SD}$) of intestine and contents = \bullet , formalin extracts = \circ , and sum of activities of intestine and formalin extracts = \circ , versus time (0, 1, 3, 5, 10, 24, and 48 hr) following ij injection of thymocytes labeled with $^3\text{HTdR}$.

reabsorbed and could be recovered in the urine (4).

It is unlikely that damage due to radiotoxicity to thymocytes prior to ij injection would significantly alter the interpretation of the results, since the proportion of ASR as well as the number of trypan blue-positive cells in the suspensions remained low. Presumably, the thymocytes were killed very rapidly, since the breakdown of dead cells occurred essentially at the same rate as that of viable counterparts.

Catabolism of viable or of dead cells and excretion of breakdown products was initially very rapid. Similarly, Kotani and co-workers (1) found a rapid increase in ^3H -activity of portal blood and thoracic duct lymph following injection of lymphocytes labeled with $^3\text{HTdR}$ into the small intestine. In portal vein serum, ^3H -activity was associated with thymine, thymidine, and thymidic acid (1).

Selective removal of ^{125}I from intact DNA labeled with $^{125}\text{IUdR}$ has not been demonstrated *in vivo* (7). The marked similarity of the initial phase of activity loss following ij injection of cells labeled with $^{125}\text{IUdR}$ or $^3\text{HTdR}$ is compatible with this observation, since cell death followed by degradation of DNA labeled with $^3\text{HTdR}$ is considered the only way in which ^3H could be lost from a tagged cell (8). Ingested nucleic acids are degraded in the small intestine by intestinal

and pancreatic nucleolytic enzymes to yield absorbable nucleosides, free bases and inorganic phosphate (9–11), and DNA, normally present in the food, has not been listed among the normal constituents of human feces (10).

Catabolites of DNA from $^3\text{HTdR}$ -labeled lymphocytes injected either intraintrastinally (1) or intravenously (12) are locally and systemically reutilized by cell renewal systems in the body. In the present experiment, thymus and spleen of recipient mice showed very low, but definite ^3H -activity 24 hr following ij injection of labeled thymocytes, whereas no ^{125}I -activity could be detected in these organs. Local intestinal utilization, and hence reutilization, may be less than systemic utilization: the intestinal uptake of $^3\text{HTdR}$ and $^{125}\text{IUdR}$, following intraintrastinal injection is about 50 and 85%, respectively, less than following ip administration of the same isotope dose (13). This difference may be due to a poorer intestinal uptake of $^{125}\text{IUdR}$ and/or more active catabolization in the intestine or liver (7, 13).

Whereas substantial ^3H -activity not associated with DNA persists for long periods of time following labeling with $^3\text{HTdR}$ (14, 15), ^{131}I not incorporated into DNA is rapidly lost following iv injection of $^{131}\text{IUdR}$ (7). Therefore, the slower phase of activity loss following ij injection of $^3\text{HTdR}$ -labeled as compared to $^{125}\text{IUdR}$ -labeled thymocytes may indicate a slower loss of ^3H -activity not associated with DNA and/or a greater reutilization.

The intestinal flora did not appear to play a significant role in the rapid catabolism of labeled cells, because no marked difference was observed between conventional animals and mice pretreated with antibiotics that effectively sterilized the gut. Similarly, the rapid intestinal hydrolysis of nucleotides *in vitro* was not altered by addition of antibiotics to the system (16).

It is concluded that cells labeled with radioactive DNA precursors may be detected in the intestinal lumen only for a short time; additional techniques such as intestinal perfusion may be required to disclose the presence of labeled migrants in the gut.

Summary. A suspension of thymocytes labeled with $^{125}\text{IUdR}$ or $^3\text{HTdR}$ was injected into the jejunum of mice. The bulk of the

radioactivity disappeared within few hours from the intestine and was recovered principally in the urine. This indicated a very rapid breakdown of labeled thymic cells, reabsorption and subsequent elimination of the tracer in the kidney. In mice injected with cells labeled with $^3\text{HTdR}$, the initial rapid loss of radioactivity was of shorter duration, and slower during the second phase, presumably due to more extensive reutilization and/or prolonged persistence of acid-soluble radioactivity. Pretreatment of the recipients with antibiotics did not significantly reduce the rate of radioactivity loss.

1. Kotani, M., Yamashita, A., Rai, F., Seiki, K., and Horii, I., *Blood* **29**, 616 (1967).
2. Ambrus, C. M., and Ambrus, J. L., *Ann. N. Y. Acad. Sci.* **77**, 445 (1959).
3. Joel, D. D., Hess, M. W., and Cottier, H., *J. Exp. Med.* **135**, 907 (1972).
4. Laissue, J. A., Chanana, A. D., Cottier, H., Cronkite, E. P., and Joel, D. D., *Blood* **47**, 21 (1976).
5. Van Bekkum, D. W., Roodenburg, J., Heidt, P. J., and Van Der Waaij, D., *J. Nat. Cancer Inst.* **52**, 401 (1974).
6. Laissue, J., Marx, W., Grieder, A., and Schindler, R., *Exp. Cell Res.* **69**, 57 (1971).
7. Hughes, W. L., Commerford, S. L., Gitlin, D., Krueger, R. C., Schultze, B., Shah, V., and Reilly, P., *Fed. Proc.* **23**, 640 (1964).
8. Cleaver, J. E., in "Thymidine Metabolism and Cell Kinetics," North-Holland Research Monographs Frontiers of Biology No. 6. North-Holland, Amsterdam (1967).
9. White, A., Handler, P., and Smith, E. L., in "Principles of Biochemistry." McGraw-Hill, New York, and Kôgakusha, Tokyo (1968).
10. Altman, P. L., and Dittmer, D. S., eds., in "Biology Data Book," (2nd Ed.), Vol. 3, Fed. Amer. Soc. Exp. Biol., Bethesda, Md. (1974).
11. London, E. S., and Schitterling, A., Hoppe-Seylers *Z. Physiol. Chem.* **41**, 10 (1910).
12. Bryant, B. J., *J. Cell Biol.* **18**, 515 (1963).
13. Clifton, K. H., and Cooper, J. M., *Proc. Soc. Exp. Biol. Med.* **142**, 1145 (1973).
14. Chang, L. O., and Looney, W. B., *Cancer Res.* **25**, 1817 (1965).
15. Rubini, J. R., Cronkite, E. P., Bond, V. P., and Flidner, T. M., *J. Clin. Invest.* **39**, 909 (1960).
16. Wilson, H. T., and Wilson, W. W., *J. Biol. Chem.* **233**, 1544 (1958).

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