Reutilization of Thymidine in Various Groups of Rat Bone Marrow Cells

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Thymidine reutilization was studied in single cells of the rat bone marrow. Using \(^3\)H-TdR in parallel with \(^{125}\)I-UdR in conjunction with the autoradiographic technique, cells of the erythrocytic series, the megakaryocytic group, and the lymphoid cells were analyzed. Reutilization of thymidine was observed only in those cells known to synthesize DNA. An estimate of the amounts of the thymidine reutilized by the salvage pathway indicated that approximately 40–60 per cent of the thymidine in the blast cells is supplied from DNA of dead cells. This value is similar to that reported previously for whole bone marrow cell populations, suggesting the presence of a common thymidine pool within the bone marrow.

The interpretation of DNA renewal using labeled thymidine (TdT) should take into account that this precursor may be reutilized.\(^1\)\(^4\) The salvage of TdR following catabolism of DNA of dead cells requires thymidine kinases, which are known to be present in DNA-synthesizing cells.\(^5\)\(^6\) The high specificity of thymidine for DNA and the small pool size of thymidine and thymidylic acids in mammalian cells\(^7\) are complemented by a rapid incorporation of about 50 per cent of a tracer dose injected into rats and mice,\(^8\)\(^9\) whereas the rest is rapidly catabolized, mainly by the liver.\(^9\)\(^11\)

The thymidine analogue 5-iodo-2'-deoxyuridine (IUdR) is also specific for DNA, yet only 5 per cent of an injected tracer dose is incorporated into DNA in mice.\(^12\)\(^13\) as compared to 50 per cent for thymidine. The difference between the two precursors is explained partly by an enhanced catabolism of IUdR\(^13\) and probably by a discrimination by TdR kinases against IUdR\(^14\) since this TdR analogue is incorporated inefficiently via the salvage pathway, provided that a path utilizing larger fragments of DNA (oligonucleotides) does not exist, and that a bypass of the thymidine compartment in the precursor pool does not occur. Indeed, IUdR was shown to be one of the catabolites of DNA labeled with IUdR excreted in the urine.\(^14\)

IUdR therefore is a useful DNA precursor that allows determining in whole organs or in single cells DNA renewal with little contribution from reutilization, whereas when thymidine is the precursor, the salvage pathway should also be taken into account.
Using both precursors in parallel, the thymidine salvage pathway can thus be quantitatively assayed. Previous data on whole organs in mice and in rats showed that the salvage pathway is largely confined to the organs, where TdR is liberated from dead cells. In the present study, autoradiographic data on single bone marrow cells of the rat were obtained in order to determine the involvement of the thymidine salvage pathway in different cell groups within the heterogeneous bone marrow cell population. It is shown that reutilization of TdR is recognized only in those cells expected to synthesize DNA. Quantitative estimates of the salvage pathway yielded comparable data for blast cells, irrespective of whether they belong to the erythrocytic precursors, to the megakaryocytic cell line, or to the lymphoid cell group.

Materials and Methods

Female Sprague-Dawley rats, weighing approximately 200 Gm., were divided into two groups: one group received intravenously 1.5 µCi of 125I-UdR per gram body weight (0.017 µg. UdR/Gm., specific activity approx. 30 Ci/mmole). 125I was chosen as the suitable nuclide for analysis by both external gamma counting and by autoradiography. The labeled precursor was kindly prepared by Dr. S. L. Commerford, Brookhaven National Laboratory, Upton, N.Y., as previously described. The second group of rats received intravenously 1 µCi per gram body weight of 3H-TdR (0.13 µg. TdR/Gm. spec. act. 1.9 Ci/mmole).

Two days before starting the experiments, all animals were put on drinking water, containing 0.1 per cent of sodium iodide. The animals labeled with 125I-UdR were killed by ether at 1, 24, and 72 hours after precursor injection; after 3H-TdR labeling rats were sacrificed at 1, 4, 8, 12, 24, 48, and 72 hours.

Bone marrow cell smears were made and processed for autoradiography, as described previously. The autoradiographic exposure time was 21 days for 125I-preparations. The Giemsa-stained autoradiograms were evaluated for grain count and labeling indices, including proper background correction, for the following cells:

(1) erythrocytic cells: basophilic normoblasts dividing
polychromatic normoblasts dividing
orthochromatic normoblasts not dividing
(2) lymphoid cells: large lymphoblasts dividing
medium-sized lymphocytes dividing
small lymphocytes not dividing
(3) megakaryocytic cells: megakaryoblasts (stage I) dividing
megakaryocytes not dividing

Between 60 and 100 cells were counted per cell type; there were two animals per time interval after UdR labeling.

The product of grain counts and labeling indices yielded for each type the total activity per 100 cells (labeled plus unlabeled). The data were normalized to the highest value found in each cell line ( = 100%).

Results

Labeling Index

Labeling indices of the various rat bone marrow cell types for 125I-UdR and 3H-TdR are shown in Fig. 1. Whereas the initial labeling indices at 1 hour are similar for the two precursors, they are quite different at 24 and 72 hours for those cells known to pass through DNA synthesis. In those cells,
Fig. 1.—Labeling indices as a function of time following bone marrow cell labeling with either $^3$H-TdR (●) or $^{125}$I-UdR (○). The data are obtained from two animals per experimental point.

which are characterized by absence of DNA synthesis, such as orthochromatic normoblasts, megakaryocytes and small lymphocytes, the labeling indices are also similar at 24 hours.
Following labeling with tritiated thymidine, the labeling indices rise steeply towards a plateau in all cells studied, and it is noted that the maxima are reached first in the immature blast cells, then they appear successively in the transitional cells (polychromatic normoblasts and medium-sized lymphocytes) and in the nondividing cells (orthochromatic normoblasts, small lymphocytes and megakaryocytes).

The indices of the IUdR-labeled cells steeply drop in the immature blasts, such as basophilic normoblasts, and large lymphoblasts, whereas they show a temporary slight rise to 24 hours in the transitional cells, such as polychromatic normoblasts and medium-sized lymphocytes. It is interesting that this pattern is also seen in the megakaryoblasts. In those cells that are considered the progeny of the transitional cells, the temporary rise to the maximum at 24 hours parallels the steep rise observed after labeling with 3H-thymidine.

**Mean Grain Count**

The autoradiographic exposure times for the two different labels were chosen so that the initial labeling intensities in terms of grain counts were comparable (Fig. 2). In blast cells, within the first 12 hours, 3H-TdR counts remain close to their initial value. Subsequent slopes of the two tracers are parallel, but 3H-TdR curves remain consistently at a higher level. In those cells that do not synthesize DNA and are considered the progeny of the transitional cells, the grain counts initially rise and then decline at a rate similar to that seen in the corresponding precursor cells.

**Total Activity per Cell Group**

The total radioactivity per 100 cells (labeled plus unlabeled) is represented in Fig. 3 as the percentage of the highest value observed. The renewal curves for 125I-UdR-labeled cells are steeper than those after thymidine labeling, in all cell groups. The differences between the two curves are initially most prominent in the immature blast cells; They are, for the first 24 hours, consecutively less striking in the transitional cells and in the "end cell" group. In fact, in the latter cell group the difference becomes noticeable only after 24 hours. The radioactivity in these initially nonlabeled cells is determined by the inflow of label coming from the corresponding parental cell groups.

**Discussion**

The differences in results obtained as a function of time after labeling the cells with 3H-TdR or with 125I-UdR might be explained by the existence of a thymidine pool constantly feeding into cells synthesizing DNA. Yet there is no evidence for the existence of such a pool over a period of several cell generations. In addition, it must be considered, whether the accelerated rate of decline after 125I-UdR labeling results from instability of iodine or from pharmacological or radiological toxicity to the labeled cells. The stability of IUdR in DNA has been demonstrated.15,16,17 Pharmacological effects are indeed unlikely with low tracer doses.18,19 However, at doses more than 30 times higher than that used in this experiment, prolongation of the cell cycle in gastrointestinal tract epithelium and spleen was observed.20 Parallel experi-
Fig. 2.—Mean grain count as a function of time following bone marrow cell labeling with either ³H-TdR (●—●) or ¹²⁵I-UdR (o----o).

ments in this laboratory²¹ and elsewhere²² indicated that ¹²⁵I is more toxic than expected on the basis of radiation dosimetry alone. An additional inherent quality factor resulted in a toxicity as much as 20 times more than that of tritium. Nevertheless, radiotoxicity of the incorporated ¹²⁵I could not be demon-
ACTIVITY PER 100 CELLS

A. ERYTHROPOIETIC CELLS

B. LYMPHOID CELLS

C. MEGAKARYOCYTES

Fig. 3.—The total number of grains per 100 cells (labeled and unlabeled), equivalent to specific activity, is listed as a function of time after labeling the bone marrow cells with either $^3$H-TdR (---) or $^{125}$I-UdR (— — — —).

strated here. The mitotic indices in these preparations did not alter, and no change in the pycnotic indices, from 1 to 72 hours was noted in this study. In addition, the labeling index curves (Fig. 1) and the total activity curves
(Fig. 3), especially for the lymphoid cells, reveal that there has been no induced cell death or loss of label from viable cells due to $^{125}$I-decay.

In order to explain properly the difference in turnover rates in the various cell groups, reutilization of thymidine via the salvage pathway of the nucleoside must be considered, since intercellular exchange of polynucleotide chains should not lead to discrimination between the two precursors.

The source of the thymidine in the salvage pathway is the DNA of dead cells. A component derived from so-called labile DNA is unlikely because various organs of the mouse or rat showed the onset of reutilization to coincide with the time of death of the first labeled cells.15,23

Reutilization of TdR is most prominent in the immature blast cells. Here, after $^{3}$H-TdR injection, practically 100 per cent of the cells become labeled already during the first 24 hours, during which time the labeling after $^{125}$I-UdR injection drops rapidly. On the other hand, reutilization is absent in non-dividing and differentiated cells, such as orthochromatic normoblasts, small lymphocytes and megakaryocytes, since the two curves for total activity rise practically in parallel for 24 hours. Thus, the phenomenon pertains only to those cells known to synthesize DNA.

The rise in the labeling indices, after injection of $^{125}$I-UdR, in the groups of transitional cells, such as polychromatic normoblasts, medium-sized lymphocytes, and probably also megakaryoblasts, must be viewed as an indication of influx of labeled cells from the corresponding precursor groups, the situation being similar to that for the "end cell" groups.

In the group of megakaryoblasts (stage I) the difference in the initial labeling indices at first may suggest a special situation for this cell group with respect to precursor uptake. However, grain counting with proper background correction imposes an error particularly large in megakaryocytes.16 In addition the present data in megakaryocytes (stage III) show a parallel rise of the labeling indices and indicate no principal deviation from the data in the other "end cell" groups.

In an attempt to estimate the relative amounts of thymidine reutilized in the blast cells, the curves in Fig. 3 were analyzed as described in detail elsewhere.15,24 Thus assigning to the renewal rate obtained after labeling with $^{3}$H-TdR the regression coefficient from the formula:

$$A_t (TdR) = A_0 (TdR) \cdot e^{at}$$

and, likewise, to the renewal rate obtained after $^{125}$I-UdR labeling, the regression coefficient from the formula:

$$A_t (IUdR) = A_0 (IUdR) \cdot e^{bt}$$

the difference in the regression coefficients ($\beta - \alpha$) divided by $\beta$, yields:

$$\frac{\beta - \alpha}{\beta} = \text{TdR supplied by reutilization}/100.$$
Table 1.—Pycnotic Index

<table>
<thead>
<tr>
<th>Hours After Precursor Injection</th>
<th>Per Cent Pycnotic Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>^H-TdR</td>
</tr>
<tr>
<td>1</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>24</td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td>72</td>
<td>1.9 ± 0.8</td>
</tr>
</tbody>
</table>

*The relative number of pycnotic cells found at various times after bone marrow cell labeling with either ^H-TdR (1 µCi per Gm. body weight) or with ^125-I-UdR (1.5 µCi per Gm. body weight); 10,000 cells were counted per time interval.

Similar values are obtained when the calculation is based on the slopes between 24 and 72 hours for the groups of transitional cells and for the "end cell" groups. The reproducibility of these data in the various cell categories gives significance to the accuracy of the initial estimates.

Previous biochemical analyses on the whole bone marrow cell population indicated that in rats, as in mice, approximately 40 per cent of the thymidine is supplied by reutilization. Moreover, it was emphasized that the amount of thymidine reutilization also in the bone marrow is largely controlled by conditions specific for single organs. Therefore the bone marrow should be viewed as a nearly closed system with respect to the thymidine reutilization pathway. The fact that reutilization in bone marrow can be recognized within 24 hours after injection of the tracers, agrees well with the notion that labeled nuclei extruded from orthochromatic normoblasts and phagocytized in the bone marrow are the major source of TdR being reutilized by the bone marrow cells.

It appears therefore that in the bone marrow exists a common TdR-pool, supplying all cells capable of DNA-synthesis within the heterogeneous cell population. Since the amount of thymidine supplied by reutilization to the bone marrow cells seems to be rather similar without specificity for a certain cell group, it can be argued that a specific effect of changes of the extracellular thymidine level on cell kinetics is unlikely.

REFERENCES

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