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REUTILIZATION OF DNA-THYMINE, AND CONVERSION OF RNA-PYRIMIDINES FOR DNA-THYMINE, IN NORMAL RAT BONE MARROW, STUDIES WITH TRITIATED NUCLEOSIDES

by

L.E. FEINENDEGEN (EURATOM)
V.P. BOND, E.P. CRONKITE and W.L. HUGHES (BNL)

1964

Work prepared at Brookhaven National Laboratory
Medical Research Center, Upton L.I. New York - USA

Paper presented at the Symposium
on the Use of Tritium in Hematological Research
Lisbon, Portugal - August 26-31, 1963

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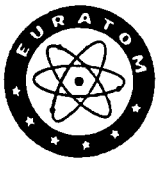

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If one injects into an animal H^3-thymidine, 50% of it is incorporated into deoxyribonucleic acid (DNA), within approximately 30 to 45 minutes, while the rest is catabolized(1). A storage of H^3-thymidine for later incorporation into DNA does not occur, on the basis of available evidence. Once incorporated, the label remains bound to DNA until cell death and no unequivocal evidence has as yet been presented to indicate metabolic renewal or intracellular turnover of the DNA molecule. The loss of labeled DNA from the bone marrow is therefore directly influenced by the rate of proliferation of the various cell types with release of mature cells into the peripheral blood.

The turnover of ribonucleic acid (RNA) within the total bone marrow - on the other hand - depends on the rate of renewal, within the individual cells, of the various molecular entities in which RNA occurs. Turnover measurements of RNA are further influenced by reutilization of RNA breakdown products within the single cell as well within the total cell population. In addition, the renewal of RNA within a total tissue appears to conform to some degree to the rate of cell proliferation of the tissue examined.(2)

The data here presented are limited to the regression of labeled thymine or its analogue, in DNA of rat bone marrow following a single intravenous injection of various labeled precursors, namely: 1) H^3-thymidine, as specific DNA precursor; 2) 5-I-deoxyuridine, labeled with I^{131}, a thymidine analogue, and 3) H^3-cytidine, a precursor for the pyrimidines in RNA as well for thymine and cytosine in DNA.

Groups of female Sprague-Dawley rats received the following doses in a single injection into the tail vein: H^3-thymidine: 0.5 µc/GM weight, specific activity 1.9 C/mM, or H^3-cytidine: 1 µc/GM weight, specific activity 1 C/mM, or 5-I^{131}-deoxyuridine: 0.18 µc/GM weight, specific activity of more than 100 C/mM.

At various times after injection, to 9 or 11 days, the animals were killed with ether, and the marrow of femur and tibia was examined for the regression of the specific activity of the labeled DNA-thymine, or its analogue.

Eidinoff[3] and co-workers have shown that 5-I-deoxyuridine is a specific, but less efficient precursor for DNA than thymidine. From the work of Commerford[(4)] and Krueger[(5)] it was suggested that the labeling of DNA with 5-I^{131}-deoxyuridine allows the observation of true DNA turnover by minimizing reutilization. Upon degradation of the DNA the label dissociated from its carrier, but in the native form of DNA the label remained bound, parallel to H^3-thymidine. This is, for example, observed in a rapidly growing culture of HeLa-S_3-cells, demonstrated in Figure 1. In this culture system cell death and hence reutilization of DNA-nucleotides from dead cells is, if at all, minimal.

Figure 2 shows the data obtained from rat bone marrow. All curves are normalized to their value at 12 hours after injection.

The two lower dotted lines give the specific activities of DNA labeled with 5-I-^{131}-deoxyuridine. The lower one of the two reflect the measurements of the total bones including the marrow. The upper one of the two gives the specific activities of the isolated marrow after extraction of the acid soluble fraction and of the lipids. These two curves are practically identical from day 2 on. They are the expression

of the true DNA turnover in the marrow, showing a 50% regression within 1.5 days (regression co-efficient: 0.462), over the observed period of time.

The solid line gives the specific activities of DNA labeled with H^3-thymidine, at various times after a single injection. For the first 24 hours, DNA labeled with 5-I^{131}-deoxyuridine behaves rather parallel to DNA labeled with H^3-thymidine. Then the two values separate, and H^3-thymidine labeled DNA regresses to 50% within 2.8 days (regression co-efficient: 0.248). The difference in the slopes indicates that approximately 50% of the loss of label with the DNA was compensated by continuous influx of H^3-thymine. The source of this delayed precursor may be within or outside the marrow from other parts of the organism. Since DNA is lost when mature cells enter the circulation or, within the marrow itself, when the most mature normoblasts lose their nuclei to become red cells[15], it is conceivable that the DNA of these cells could be a source of precursor for new DNA synthesis.

Following injection of H^3-cytidine, the specific activity of H^3-thymine rises for the first 12 hours, parallel to H^3-cytosine, as shown elsewhere[6]. The initial rise probably is due to continuous incorporation from the acid soluble pool, which falls proportionally at the same time. From 12 hours on, the specific activity of the H^3-thymine declines slowly with a regression to 50% within approximately 7 days (regression co-efficient: 0.099). This means that approximately 80% of the loss of label with the DNA from the marrow was compensated by influx of labeled precursors. The acid soluble H^3 declines already within the first 12 hours, and followed in parallel to the overall RNA specific activity,

as shown elsewhere[6]. It is therefore unlikely that the late precursor comes from the initial acid soluble fraction. Approximately 50% of the lost label was already accounted for by reutilization of DNA-thymine, therefore an additional 30% is supplied from the pyrimidines of RNA, nearly continuously over the observed period of 9 days. Indeed S. Cohen[7] has shown that conversion of cytosine nucleotides from RNA to cytosine-deoxyribose-diphosphate to DNA occurs in phage infected E. coli.

The given values of label reutilization, and conversion from RNA to DNA do not allow a statement as to the total amount of nucleotides actually involved in these processes. The specific activities of the reutilized or converted nucleotides are not known; however, it is expected that the specific activities fall progressively with time after injection. It is obvious that the data present therefore minimum values.

These findings confirm in general for the bone marrow the hypothesis and the reports of reutilization of DNA-thymine given by Hill and Drasil[8], Krueger[5], Fichtelius[9], Bryant[10], Rieke[11], and Robinson and Brecher[12], whose paper is presented in this session. The data furthermore demonstrate a continuous contribution also of RNA pyrimidines to DNA-thymine in the normal bone marrow cell population. It is even feasible that the de-novo synthesis of thymidine occurs totally via the RNA. The presence of thymidine kinase and thymidylate kinases in rapidly dividing cells[13], and the work of Potter[14] let one assume that thymidine is not an unphysiological precursor for DNA, but that it lies on the salvage pathway and is probably a major intermediate in DNA-catabolism.

The results warn to caution in the interpretation of data on long term studies on cellular kinetics using H^3-thymidine.

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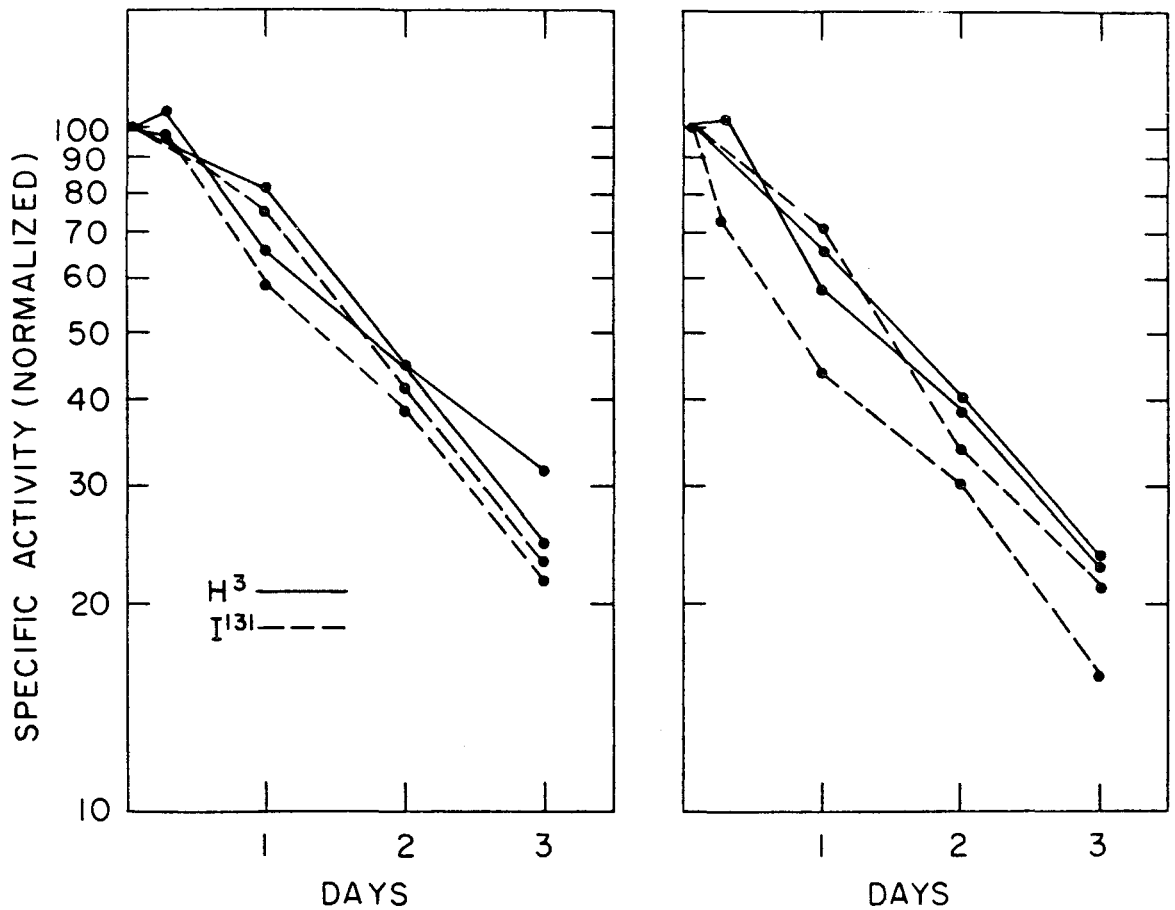
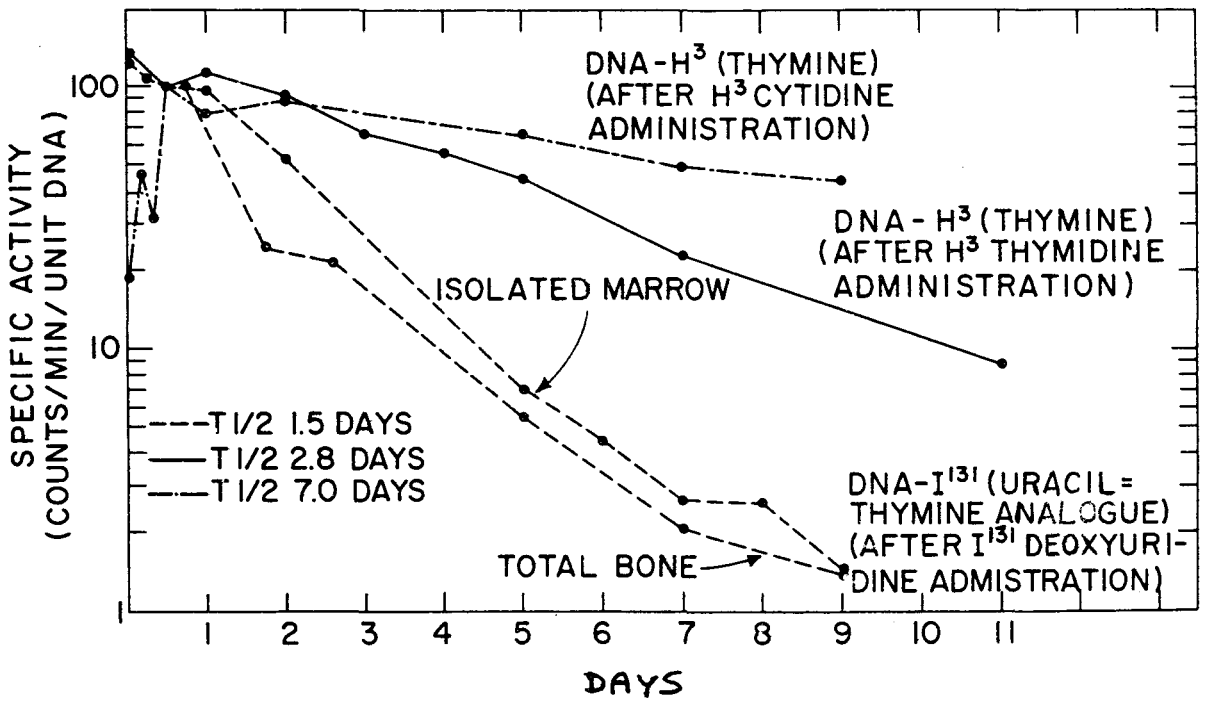
FIGURE 1



HeLa-S_3 cells in culture were exposed for 45 minutes to H^3-thymidine or 5-I^{131}-deoxyuridine. The specific activities of DNA (left), or total cells (right) were examined following the short-term labeling at various times to 3 days.

FIGURE 2



The specific activity, in normal rat bone marrow, of DNA-thymine or its analogue 5-I-uracil was examined following a single intravenous injection of H^3-thymidine, H^3-cytidine, or 5-I^{131}-deoxyuridine.