

RELATIVE EFFECTS OF RADIATION ON DE NOVO DNA BASE SYNTHESIS AND THYMIDINE INCORPORATION INTO DNA

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It is generally accepted that radiation diminishes cell synthesis of DNA. Whether this effect is related to specific radiosensitive steps in the biosynthesis of DNA or whether it represents nonspecific diminution in DNA synthesis has been a subject of much speculation. The present work was undertaken to

determine the relative radiosensitivity of the processes responsible for monocarbon fragment incorporation into DNA thymine and adenine and those responsible for incorporation of preformed thymidine into DNA.

MATERIALS AND METHODS

Within ½ hr after aspiration, dog bone marrow cells were exposed to an x-ray dose in the range of 50–3,200 R (100 kvp, 15 ma, 0.5 cm aluminum filter). Within ½ hr after the termination of the irradiation, the dog bone marrow cells were subjected to short-term incubation in a balanced salt solution containing ^{14}C -formate (Cal Biochem 16.6 mCi/mM-final conc. 0.075 mM) or serine labeled with ^{14}C in the number 3 position (Nuclear-Chicago 20.4 mCi/mM-final conc. 0.061 mM) and thymidine labeled with tritium (New England Nuclear 2.02 Ci/M-final conc. 1.23 μM). After 1½ hr of incubation at 37°C in a Dubnoff metabolic shaker, culture tubes were immediately placed on ice and the cells washed with iced culture media. DNA was isolated using a modification of the technique of Kay *et al* (1). The washed cells were disrupted by freeze-thawing in acetone-dry ice, washed in saline and the nucleic acids were dissolved using sodium dodecyl sulfate (final concentration 0.4%). RNA was precipitated by adjusting the salt concentration to 1 M sodium chloride. DNA was precipitated from the supernate by adding alcohol, dried and hydrolyzed in formic acid at 175°C for 35 min in a sealed container. The hydrolyzed DNA bases were then separated by paper chromatography [(isopropyl alcohol: HCl:H₂O, (2)]. Adenine and thymine were eluted from the chromatogram using 0.01 N HCl and the concentration of adenine and thymine within the

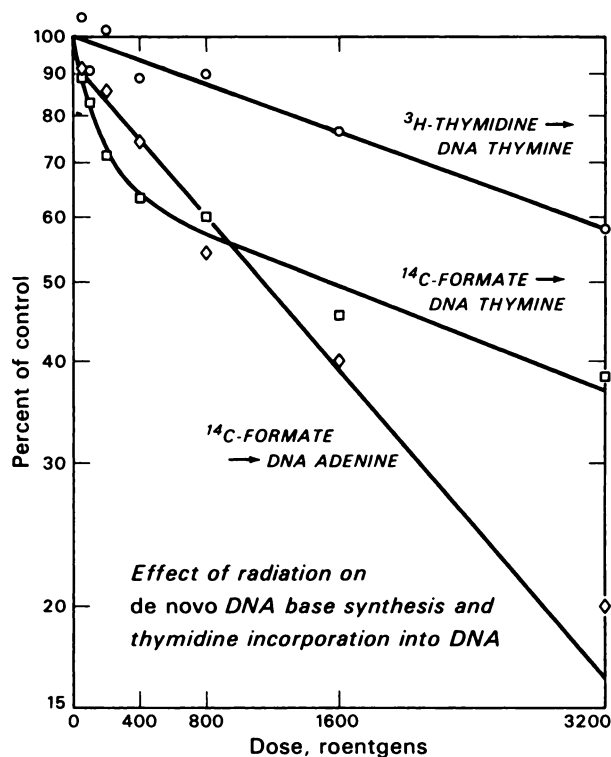


FIG. 1. Effects of radiation on de novo base synthesis and thymidine incorporation into DNA. DNA base specific activity expressed as percent of control values is plotted on ordinate, and exposure dose in roentgens is plotted on abscissa. Circles represent incorporation of tritiated thymidine into DNA thymine and squares represent incorporation of ^{14}C from formate into DNA thymine and diamonds represent incorporation of ^{14}C into DNA adenine. Data were fitted to exponential functions using digital-computer program designed to minimize squares of difference between data and fitting function. ^3H -incorporation into DNA thymine was fit by function $100 e^{-1.7 \times 10^{-4} D}$. Incorporation of ^{14}C from formate into DNA thymine was fit by function $66 e^{-1.8 \times 10^{-4} D} + 34 e^{-6.9 \times 10^{-3} D}$ while incorporation of ^{14}C from formate into adenine was fit by function $93 e^{-5.4 \times 10^{-4} D} + 7 e^{-4.9 \times 10^{-1} D}$ (where D is expressed as dose in roentgens).

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FIG. 2. Schematic representation of incorporation into DNA of ^{14}C from formic acid or serine, tritium from tritiated thymidine and ^{32}P from $^{32}\text{PO}_4$ added to culture medium. Clearly $^{32}\text{PO}_4$ will be incorporated into DNA associated with all nucleic acid bases regardless of whether these bases arise from *de novo* synthesis or from reutilization pathways. Tritiated thymidine would be expected to mix homogeneously in extracellular fluid with preformed thymine arising from catabolism of DNA. It is not known whether such labeled thymidine mixes homogeneously with entire intracellular thymidine pool. Incorporation of ^{14}C into DNA bases in this scheme is measure of both *de novo* synthesis of these bases as well as of processes required for their incorporation into DNA.

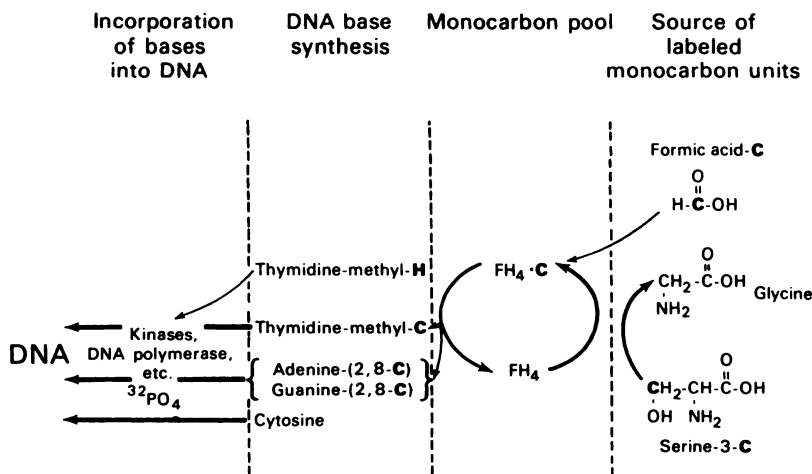


TABLE 1. RESULTS OF INCORPORATION OF ^{14}C FROM FORMATE OR SERINE-3- ^{14}C INTO DNA THYMINE AND ADENINE AND INCORPORATION OF TRITIATED THYMIDINE INTO DNA THYMINE FOLLOWING EXPOSURE OF DOG BONE MARROW CELLS TO X-IRRADIATION*

Exp. no.	^{14}C - substrate	Dose (R)	% of control values		
			^{14}C - thymine	^{14}C - adenine	H^3 - thymine
2	formate	50	89.11	91.42	105.93
2	serine	50	92.06	—	103.03
2	formate	100	83.06	—	91.23
1	serine	100	74.04	72.00	96.89
2	formate	200	71.37	85.71	102.28
2	serine	200	69.05	—	104.26
2	formate	400	63.30	74.28	88.80
1	serine	400	61.07	56.00	86.20
2	formate	800	59.87	54.28	90.16
2	serine	800	59.52	—	72.89
1	serine	800	61.83	52.00	83.23
2	formate	1,600	45.76	40.00	76.68
2	formate	3,200	38.30	20.00	58.26

* All values listed are presented as DNA base specific activity expressed as % of control value.

eluate determined by UV absorption spectroscopy (263 and 264 $m\mu$, respectively); the samples were counted for tritium and ^{14}C activity using liquid scintillation counting.

RESULTS

Figure 1 and Table 1 summarize results of changes in incorporation of tritiated thymidine and ^{14}C from formate into DNA bases in dog bone marrow cells after exposure to x-rays. In Fig. 1 the abscissa represents radiation dose and the ordinate specific activity of label in DNA bases expressed as percent of control values. With doses of radiation below 100 R there appears to be little effect on the incorporation of tritiated thymidine into DNA. Indeed at these low

doses there appears to be a modest increase in tritiated thymidine incorporation into DNA (Table 1). At higher doses of radiation, however, we note a progressive decrease in the incorporation of tritiated thymidine into DNA. In contrast to the rather minimal effect of radiation on tritiated thymidine incorporation into DNA at low radiation doses, *de novo* synthesis of DNA thymine and adenine measured by incorporation of ^{14}C from formate into these bases seems to be markedly suppressed at low radiation doses.

DISCUSSION

Figure 2 gives schematically the biochemical process involved in incorporating the tritium or ^{14}C label into DNA bases. The ^{14}C from formate or from labeled serine enters the monocarbon pool attached to tetrahydrofolic acid where it is used in the methylation of deoxyuridine to form thymidine and in synthesis of purines being incorporated at the number 2 and 8 position. Thus in the present system the incorporation of ^{14}C into DNA thymine or DNA purines is a measure of *de novo* synthesis of DNA bases as well as the incorporation of such bases into DNA. On the other hand, the incorporation of the tritium label into DNA when tritiated thymidine is used does not depend on integrity of the monocarbon transport system. If exogenously added tritiated thymidine mixes completely with the intracellular thymidine pool, then the incorporation of tritiated thymidine into DNA is a measure of DNA synthetic rate. If, however, it does not come into mixing equilibrium with the intracellular thymidine pool, then the incorporation of tritiated thymidine into DNA may not reflect overall DNA synthesis. When one measures the incorporation of ^{32}P -phosphate into DNA, one presumably has an overall measure of DNA synthesis regardless of whether the bases

were synthesized immediately before their use by *de novo* pathways or whether the bases arose from reutilization sites.

Previously published results (3) showed significant depression of ^{32}P incorporation into DNA following low doses of radiation (< 100 R). Such results contrast with the results presented here and those discussed previously by Rasmussen and Painter (4) in which ^3H -thymidine incorporation into DNA was normal or increased after exposure to low doses of radiation.

If we accept the premise that the incorporation of ^{32}P -phosphate into DNA is the least ambiguous method available for measuring overall DNA synthesis, then we must conclude that the incorporation of tritiated thymidine into DNA under the present circumstances is a poor measure of the overall rate of DNA synthesis. If, as claimed by Rasmussen and Painter (4), the incorporation of labeled thymidine into DNA after radiation represents in large part DNA repair processes, then such processes would have to involve replacement of DNA bases alone, leaving the pentose phosphate backbone of the nucleic acid molecule intact. If replacement of an entire segment of the DNA molecule including the pentose phosphate backbone occurred during DNA repair of radiation damage, one would see identical results using ^{32}P -phosphate and tritiated thymidine. An alternate explanation for the results noted above is suggested by prior work from this laboratory on the effects of methotrexate, a folic acid reductase inhibitor, on the incorporation of ^{14}C label from 3- ^{14}C -serine and the ^3H label from ^3H -thymidine into DNA thymine (5). In those experiments methotrexate inhibited synthesis of ^{14}C -thymine while enhancing incorporation of the ^3H label into DNA thymine. These results with methotrexate are therefore remarkably similar to those obtained in the present experiment following exposure of bone marrow cells to x-rays. Since there is no evidence at present to suggest that methotrexate causes direct damage to DNA molecules, it is difficult to ascribe the results with that agent to use of tritiated thymidine in DNA repair processes such as has been suggested after irradiation.

Our results are consistent with the possibility that tritiated thymidine added to the cell culture media does not mix homogeneously with the intracellular thymidine pool and its incorporation into DNA is not an accurate measure of overall DNA synthesis. However, it would be expected to mix with the thymidine released during DNA catabolism and made available for reuse. Accordingly the incorporation of tritiated thymidine into DNA thymine may be in large part a measure of the reuse of preformed thymine

into DNA. On the other hand the incorporation of ^{14}C -labeled monocarbon fragments from formate or serine should primarily be a measure of *de novo* synthesis of DNA thymine. Since in our experiments both the *de novo* synthesis of the pyrimidine, thymine, as well as the purine, adenine, were markedly suppressed after radiation, we may postulate that the common metabolic pathway requiring monocarbon transport required for synthesis of both these bases may be markedly radiosensitive. Further, this step is much more radiosensitive than the metabolic steps required for the incorporation of DNA bases into the DNA molecule. We may further speculate that the critical radiosensitive step in this process involves radiation inactivation of tetrahydrofolic acid or the processes required for its production. Tetrahydrofolic acid is known to be quite labile, and since it is readily oxidized on exposure to room air, one would anticipate that it would be inactivated by the peroxides produced after radiation exposure. Indirect evidence for this postulate has been previously presented by Ngo and Winchell (6) in their studies using ^{14}C -labeled histidine. We may further postulate that when *de novo* synthesis of DNA thymine is suppressed either by methotrexate or radiation, there is an increase in the fraction of thymine incorporated into DNA which originates from reutilization sources as opposed to *de novo* synthesis sources and thus one may actually see an increase in the incorporation of tritiated thymidine into DNA, even though overall DNA synthesis may be markedly reduced.

CONCLUSIONS

In the present work the synthesis of DNA thymine and adenine using monocarbon units from ^{14}C labeled formate or 3- ^{14}C labeled serine is found to be more radiosensitive than the incorporation of tritiated thymidine into DNA thymine. Since both the *de novo* synthesis of the pyrimidine, thymine, as well as the purine, adenine, were markedly suppressed after radiation, we postulate that the common metabolic pathway requiring monocarbon transport needed in the synthesis of both these bases may be markedly radiosensitive. Furthermore we postulate that this step is more radiosensitive than metabolic steps required for the incorporation of DNA bases into the DNA molecule. Our results suggest that after irradiation the incorporation of tritiated thymidine into DNA is not a good measure of overall DNA synthesis but may rather be a measure of the incorporation of preformed thymidine, from reutilization pathways, into DNA.

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