Incorporation of Glycine-2-C^{14} into Purines of Pentose Nucleic Acid and Desoxyribose Nucleic Acid.* (18524)

CHARLES HEIDELBERGER AND G. A. LEPAGE. (Introduced by V. R. Potter)

From the McArdle Memorial Laboratory, The Medical School, University of Wisconsin.

The concept of the biochemical stability of desoxyribose nucleic acid (DNA) in non-growing tissues has gained wide acceptance. This concept has been supported by many studies involving chemical analysis, which have been admirably summarized by Davidson and Leslie(1). Moreover, Brown and co-workers(2) have reported that labeled adenine is not incorporated into DNA purines. Hevesy (3) has reviewed the work with P^{32}, which is incorporated to only a very slight extent into the DNA of resting tissues. In striking contrast to these results, we have reported (4,5) the extensive incorporation of glycine-2-C^{14} into the DNA purines, even in the case of adult rat liver, which is characterized by its low rate of mitosis. A considerable incorporation of small-molecule precursors into DNA purines has been reported independently by Elwyn and Sprinson(6) with glycine-2-C^{14} and with serine-3-C^{14} and by Totter *et al.*(7) with labeled formate. These workers, however, isolated their DNA from mixed tissues, some of which have a higher growth rate than liver. It has been demonstrated in birds by Sonne, Buchanan, and Delluva(8) that glycine is incorporated into positions 4, 5, and 7 of uric acid, and formate, in carbons 2 and 8. Karlsson and Barker(9) have shown specifically that carbon-2 of glycine becomes carbon-5 in uric acid. That these conclusions also applied to the rat was demonstrated by Heinrich and Wilson(10) with adenine and guanine isolated from mixed nucleic acids.

Since it is well known that glycine is converted into formate, also a purine precursor, it appeared to be of importance to determine whether the incorporation observed in our experiments(4,5) was that due to formate, which might be thought to exchange more readily with the two ureide carbons, or to glycine itself. Accordingly, the amount of radioactivity in carbon-5 of adenine and guanine derived from the PNA and DNA of liver and tumor was determined.

Experimental. Rats, bearing multiple transplants of Flexner-Jobling carcinoma, were given a single dose of glycine-2-C^{14} by stomach tube. After the appropriate time, the animals were sacrificed and the nucleic acids were isolated as the barium salts as described elsewhere(5). The DNA samples were contaminated with less than 10% of PNA. The nucleic acids were hydrolyzed and the purines isolated and purified by a modification of the ion-exchange chromatography method of Cohn(11). The specific radioactivity of the pure bases was determined, and they were hydrolyzed to glycine (12). The formaldehyde that was liberated

* This work was supported in part by a grant from the Wisconsin Section of the American Cancer Society, recommended by the Committee on Growth of the National Research Council, and in part by a grant-in-aid of the National Cancer Institute.

1. Davidson, J. N., and Leslie, J., *Cancer Research,* 1950, v10, 587.

2. Furst, S. S., Roll, P. M., and Brown, G. B., *J. Biol. Chem.,* 1950, v183, 251.

3. Hevesy, G., *Adv. in Biol. Med. Physics,* 1948, v1, 409.

4. LePage, G. A., and Heidelberger, C., *Fed. Proc.,* 1950, v9, 195.

5. LePage, G. A., and Heidelberger, C., *J. Biol. Chem.,* 1951, v188, 593.

6. Elwyn, D., and Sprinson, D., *J. Am. Chem. Soc.,* 1950, v72, 3317.

7. Totter, J. R., Volkin, E., and Carter, C. E., Abstracts of Am. Chem. Soc. Meeting, Chicago, Sept. 1950, p. 55C.

8. Sonne, J. C., Buchanan, J. M., and Delluva, A. M., *J. Biol. Chem.,* 1946, v166, 395.

9. Karlsson, J. L., and Barker, H. A., *J. Biol. Chem.,* 1949, v177, 597.

10. Heinrich, M. R., and Wilson, D. W., *J. Biol. Chem.,* 1950, v186, 447.

11. Cohn, W. E., *Science,* 1949, v109, 377.

12. Cavalieri, L. F., Tinker, J. F., and Brown, G. B., *J. Am. Chem. Soc.,* 1949, v71, 3973.

TABLE I. Distribution of C^{14} Radioactivity in the 5-carbon of Purines from Rat Nucleic Acids. Specific activity in cpm per μg.

Animal	Sample	Sp. act. purine	Sp. act.* CH_2O calc'd	Sp. act. CH_2O found	% in carbon-5
G 24, 25	Liver PNA, guanine	1.1	5.1	3.1	60
G 24, 25	" DNA, "	0.3	1.4	1.1	75
G 6, 7	" PNA, "	1.9	8.3	4.5	54
G 6, 7	" DNA, "	0.77	3.5	2.2	63
G 6, 7	Tumor PNA, "	6.1	27.0	16.0	59
G 6, 7	" DNA, "	4.8	22.0	15.0	68
G 26	Liver PNA, "	2.1	9.3	3.4	36
G 26	" DNA, "	1.2	5.4	2.6	48
G 26	" PNA, adenine	4.7	21.0	8.0	38
G 26	" DNA, "	10.1†	45.0	13.0	29

Specific activity of the administered glycine was 10,000 cpm per μg.

* This would be the specific activity of the formaldehyde if the entire radioactivity of the purine were in carbon-5.

† Since this DNA adenine had a higher specific activity than that of the corresponding PNA adenine, it was subjected to further chromatography. No significant change in the specific activity was observed.

G 24, 25 were 2 female 220 g rats, sacrificed 48 hr after 1.87 mg of glycine was given to each. The livers were pooled.

G 6, 7 were 2 female 166 g rats bearing 8-day implants of Flexner-Jobling carcinoma. They were sacrificed 18 hr after administration of 1.49 mg of glycine to each and the tissues were pooled.

G 26 was a 250 g male rat, sacrificed 18 hr after administration of 2.75 mg of glycine.

from the glycine by ninhydrin is derived from carbon-5 of the purine base. The specific activity of the formaldehyde was determined by colorimetric analysis of an aliquot with chromotropic acid, and by direct counting of the dimedon precipitate (carrier added). The specific activity of the formaldehyde was compared with that of the purine, and the results are shown in Table I.

Results and discussion. The data clearly show that a very appreciable per cent of the C^{14} in the purine bases was derived from glycine itself. Presumably of the remainder, most was incorporated as formate into the 2 and 8 carbons. The significant fact is that the results are essentially the same for the guanine and adenine derived from the DNA and PNA, and for both tumor and liver. Thus it is clear that even in the DNA of resting tissue the incorporation of glycine into the purines represents not a simple exchange of ureide carbons, but that exchange or synthesis of the main carbon skeleton of the purines has taken place.

Thus, it must be concluded that at least two pathways of DNA synthesis exist. One involves the incorporation of pre-formed adenine together with desoxyribose and phosphorus(2,3) into the nucleic acid. The other, and perhaps preponderant route, involves the incorporation of small-molecule precursors of purines into a skeleton to which the desoxyribose and phosphorus have already been affixed. This incorporation must take place in such a way that the total quantity of DNA in resting tissue remains essentially static(1). A possible intermediate in this process might be 4(5)-Amino-5(4)-imidazole carboxamide (13-15).

Summary. The extensive incorporation of glycine-2-C^{14} into the purines of PNA and DNA has been shown in both cases to take place largely by an actual incorporation of the glycine molecule, and not by a simple exchange of ureide carbons. The main implication of this finding is that at least two pathways of DNA synthesis must exist.

13. Shive, W., Ackermann, W. W., Gordon, M., Getzendaner, M. E., and Eakin, R. E., *J. Am. Chem. Soc.*, 1947, v69, 725.

14. Ravel, J. M., Eakin, R. E., and Shive, W., *J. Biol. Chem.*, 1948, v172, 67.

15. Miller, C. S., Gurin, S., and Wilson, D. W., *Science*, 1950, v112, 654.

Received February 5, 1951. P.S.E.B.M., 1951, v76.