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ISOTOPIC TRACER STUDIES ON THE BIOSYNTHESIS OF DEOXYRIBOSE IN THE RAT

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SUMMARY

The biosynthesis of deoxyribose, *in vivo*, was studied with $NaH^{14}CO_3$ as a tracer, in normal adult, starved adult, normal newborn, "thiamine-deficient" adult and "tumor bearing" rats and in Yoshida ascites-tumor cells. Comparisons of the tracer patterns in ribose and deoxyribose isolated from the nucleic acids of skin and liver suggest that in young tissues the formation of deoxyribose may occur by direct reduction from ribose or via an intermediate common to ribose. The data obtained from the livers of young adults do not support this concept unless the pattern of ^{14}C in the ribose which is the precursor of deoxyribose, is different from that of the ribose isolated from the RNA. The presence of an ascitic tumor in the young adult seems to influence pentose metabolism in the host liver.

INTRODUCTION

Two possible mechanisms for the biosynthesis of D-2-deoxyribose have been demonstrated *in vitro*. RACKER[1] first described deoxyribose phosphate aldolase which catalyzes the condensation of glyceraldehyde 3-phosphate and acetaldehyde to form deoxyribose 5-phosphate. Although this enzyme has been detected in various bacterial[2] and animal tissues[3,4] no evidence favoring its synthetic importance, *in vivo*, has been obtained. The primary metabolic role of this enzyme may be in the degradation of deoxyribose. The second pathway, which involves a direct conversion of ribose to deoxyribose while the former is in ribosidic linkage, has been supported by data from experiments carried out *in vivo*[5–8] as well as *in vitro*[9,10].

Results from tracer studies with growing *Escherichia coli*[11–13] and with cultures of this organism infected with phage [14,15] have been interpreted as indicating that in this organism ribose is directly reduced to deoxyribose or that both pentoses have a common precursor. A similar conclusion can be drawn from tracer experiments with *Torula utilis*[16,17]. However, it appears that the biosynthetic pathway for deoxyribose in animal cells is less certain. For example, SHREEVE[18] reported that in regenerating

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rat liver there were differences in the patterns of labeling in ribose and deoxyribose from [^{14}C]glycine suggesting that at least some of the deoxyribose may not arise directly from ribose or from a precursor of the latter. HORECKER *et al.*[19] in comparable experiments on regenerating rat liver using [1-^{14}C]glucose and [2-^{14}C]glucose, also found some differences between the labeling patterns in the two pentoses. In addition, LALAND *et al.*[20] reported that the ratio of incorporation of ^{14}C from [1-^{14}C]acetate and [2-^{14}C]acetate was different in ribose and deoxyribose when rat-liver slices were separately incubated with these two substrates.

This paper reports the results of a tracer study, *in vivo*, of the biosynthesis of deoxyribose in the liver and skin of the rat. The patterns of ^{14}C in deoxyribose and ribose, isolated from the nucleic acids after administration of $NaH^{14}CO_3$, were compared to gain information on the mechanism of this biosynthetic process. Although the data obtained with newborn animals support the hypothesis that deoxyribose is synthesized directly from ribose or that both pentoses arise from a common precursor, the results of studies with young adult rats suggest that presently known mechanisms may not entirely account for the formation of deoxypentose in the liver of animals at this age.

MATERIALS AND METHODS

White rats of the Sprague–Dawley strain were used in each of the experiments reported. All rats received $NaH^{14}CO_3$, intraperitoneally. Experimental details are shown in Table I.

For the experiments involving thiamine deficiency, rats were fed on a "thiamine-free" synthetic diet over a period of 3 weeks prior to the administration of the tracer. The deficient condition was indicated by abnormally high levels of pyruvate in the blood as measured by the method of FRIEDEMANN AND HAUGEN[21].

In order to study the synthesis of pentose in tumor tissue, rats were inoculated intraperitoneally with 0.1 ml of a 10 % suspension in saline of the Yoshida hepatoma tumor in the ascitic form obtained from Dr. P. MARKS, Columbia University, New York, N. Y. (U.S.A.). After 6 days each rat was given $NaH^{14}CO_3$ subcutaneously as shown in Table I. 45 min after the last injection, the tumor cells were removed by as-

TABLE I

EXPERIMENTAL DETAILS OF TRACER EXPERIMENTS

Experiment No.	*Age of animals*	*Average weight per animal (g)*	*No. of rats per experiment*	*Conditions*	*Concentration of $NaH^{14}CO_3$ (mC/ml)*	*Dose (mC per animal)*	*No. of doses per animal*	*Time interval between each dose (h)*	*Length of experiment (h)*
1	Young adult	200	3	Normal, fed	2.7	0.32	4	4	24
2	Young adult	200	3	Normal, starved*	1.0	0.25	4	0.5	2.5
3	Young adult	200	4	Normal, starved*	0.63	0.25	4	0.5	2.5
4	Young adult	200	3	Thiamine deficient	2.7	0.32	4	4	24
5	5 days old	9.5	10	Normal, fed	2.7	0.03	5	0.5	5.5
6	Young adult	200	3	Bearing Yoshida ascitic tumor	1.3	0.15	4	0.5	6

* Normal rats were starved for 36 h and each was given 160 mg of glucose, as a 40 % aqueous solution, by stomach tube before $NaH^{14}CO_3$ was administered.

piration through a No. 18 needle. The rats were then sacrificed and the livers removed and washed free of tumor cells and fluid.

Extraction of RNA and DNA from skin and liver

The combined sodium nucleates were isolated from pulverized skin or liver by extraction with 10 % NaCl subsequent to extraction with cold $HClO_4$ and defatting (*cf.* ref. 22). The sodium nucleates were precipitated from the salt solution by the addition of 2.1 vol. of 95 % alcohol.

Isolation of RNA and DNA from ascites-tumor cells

The ascitic cells were removed from the ascitic fluid by centrifugation at 10 000 $\times g$ at 4° for 15 min and ground in a mortar with 1 g of Pyrex glass powder (10 mesh) per 30 g (wet wt.) of cells. DNA and RNA were then extracted as described above.

Isolation of ribose and the deoxyribonucleosides from the sodium nucleates

The RNA was separated from DNA by incubation in NaOH, to degrade the RNA into its constituent nucleotides, followed by precipitation of the undegraded DNA upon acidification (*cf.* refs. 12, 22). Ribose was obtained from the purine ribonucleotides in the supernatant solution by refluxing in 0.5 N H_2SO_4. The pentose was isolated by paper chromatography in two solvent systems[23].

The deoxyribonucleosides were obtained from DNA by enzymic hydrolysis using deoxyribonuclease and an extract of the venom of *Crotalus adamantus* containing phosphodiesterase and nucleotidase. The deoxyribonucleosides were then purified by ion-exchange chromatography on columns of Dowex-1 (formate) using the gradient-elution technique of HURLBERT *et al.*[24] as previously described[12]. The elution was begun with 100 ml of 0.02 M ammonium formate (pH 10.6) in the mixing flask and 500 ml of 0.2 M ammonium formate (pH 10.6) in the reservoir.

After isolation, the ribose and the deoxyribonucleosides were diluted 30–50 fold with the respective unlabeled compounds prior to being submitted to isotopic analysis.

Determination of radioactivity

The distribution of ^{14}C in ribose was determined by fermentation with *Lactobacillus pentosus*, Strain 124-2 (ATCC 8041), also termed *Lactobacillus plantarum*, followed by chemical degradation of the resulting acetic and lactic acids[25]. The specific activity of the ribose was determined by preparation[26] and combustion of potassium ribonate. Total combustion was carried out by the method of VAN SLYKE AND FOLCH[27]. In all cases, the total ^{14}C in ribose as determined by fermentation and by total combustion agreed to within 5 %. The distribution of ^{14}C in the deoxyribose of deoxyribonucleosides was determined by fermentation with *Escherichia coli* (ATTC 9773) and subsequent chemical degradation of the fermentation products[28]. This organism converts deoxynucleosides to the free base, ethanol, CO_2, acetic acid and H_2. Total combustion of deoxyribonucleosides was not done since the base

contained more activity than did the pentose. However, the results of duplicate degradations, carried out as a check on the validity of the fermentation results, agreed to within 5 %. Radioactivity was determined in a gas-phase counter[29] to a standard counting error of 5 % or less. The efficiency of this instrumentation was 72±2 % as determined by counting a standard sample of $NaH^{14}CO_3$ obtained from the National Bureau of Standards, Washington, D. C. (U.S.A.).

Preparation of $NaH^{14}CO_3$

$Ba^{14}CO_3$, obtained from the Oak Ridge National Laboratory, Oak Ridge, Tenn. (U.S.A.) was converted to $NaH^{14}CO_3$ by liberating the $^{14}CO_2$ with 7 % $HClO_4$ in an evacuated, closed system and trapping the gas in an equivalent amount of CO_2-free, 2N NaOH.

RESULTS

The labeling patterns of ^{14}C in ribose and deoxyribose isolated from the skin and liver after injection of $NaH^{14}CO_3$ are shown in Table II. Data are shown for normal young adult, starved young adult, normal newborn, thiamine-deficient and "tumor bearing" young adult rats and for Yoshida ascites-tumor cells. The figures in Table II indicate the relative specific activities of the various carbons of ribose and deoxyribose. As C-3 showed the highest specific activity, it was given an arbitrary value of 100 in each case.

TABLE II

DISTRIBUTION OF ^{14}C IN RIBOSE OF RNA AND DEOXYRIBOSE OF DNA IN RAT TISSUES AFTER ADMINISTRATION OF $NaH^{14}CO$*.

Experiment No.	*Condition*	*Tissue*	*Pentose*	*Relative specific activity* **					*Counts/min/mmole of C-3 equal to 100****	*Dilution factor*
				C-1	*C-2*	*C-3*	*C-4*	*C-5*		
1	Normal young adult rats (fed)	Liver	Ribose	22	48	100	2	1	1317	50
			Deoxyribose	36	9	100	4	2	250	45
2	Normal young adult rats (starved)	Liver	Ribose	22	49	100	4	2	450	50
			Deoxyribose	55	0	100	0	0	197	35
3	Normal young adult rats (starved)	Liver §	Ribose	25	55	100	5	3	440	40
			Deoxyribose	39	17	100	0	0	220	35
4	Thiamine-deficient young adult rats	Liver	Ribose	9	65	100	4	6	530	50
			Deoxyribose	21	7	100	0	2	220	50
5	Newborn rats (fed)	Liver	Ribose	37	75	100	3	3	1147	50
			Deoxyribose	33	67	100	3	0	283	50
5	Newborn rats (fed)	Skin	Ribose	33	73	100	2	2	3366	50
			Deoxyribose	32	60	100	4	6	311	40
6	Young adult rats bearing Yoshida tumor	Liver	Ribose	36	50	100	0	0	398	65
			Deoxyribose	40	53	100	0	0	227	30
6	Ascitic tumor	Cells	Ribose	20	61	100	1	2	3420	50
			Deoxyribose	20	37	100	5	12	1200	40

* For details of the procedures see text.

** C-3 for each pentose arbitrarily given a value of 100 in each experiment.

*** The specific activity of C-3 as found in the tissues is equal to the dilution factor multiplied by the counts/min/mmole equal to 100.

§ The incorporation of radioactivity in the skin was too low to measure significantly in this experiment.

DISCUSSION

Administration of $NaH^{14}CO_3$ to rats results in glycogen (and presumably the "glucose pool") predominantly labeled in C-3 and C-4 (see ref. 30). In starved animals, C-3 and C-4 have nearly equal radioactivities whereas in fed animals the labeling is apparently asymmetric[31]. If it is assumed that the distribution of ^{14}C in the glycogen indicates the labeling of the glucose 6-phosphate, then in the starved rat, ribose formed by the "hexose monophosphate oxidative" pathway should be labeled equally in C-2 and C-3 when $NaH^{14}CO_3$ is administered (Fig. 1). On the other hand,

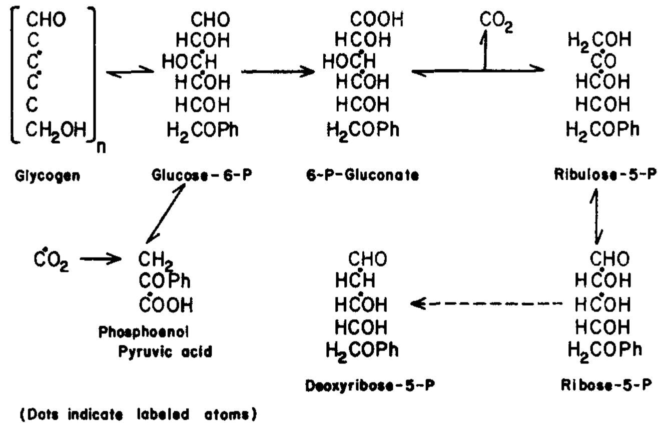


Fig. 1. "Hexosemonophosphate oxidative" pathway for the synthesis of pentoses.

formation of this pentose by the "transketolase–transaldolase" mechanism would lead to labeling in C-1, C-2 and C-3 from this precursor (Fig. 2). Assuming that 2 molecules of hexose and 1 of triose are converted to 3 molecules of pentose as shown

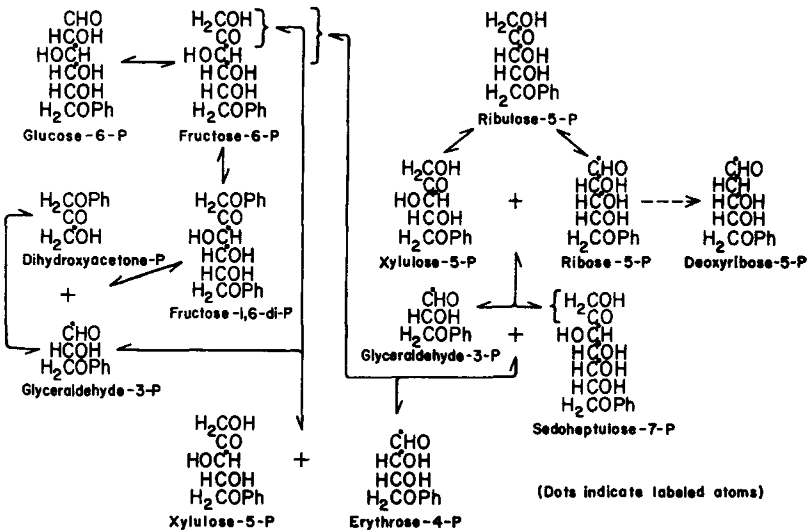
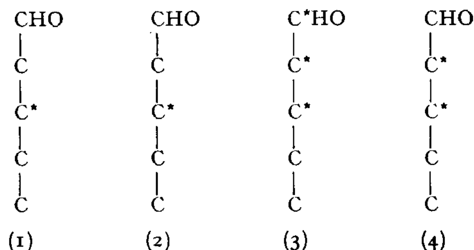


Fig. 2. "Transaldolase–transketolase" pathways for pentose synthesis.

in Fig. 2, C-3 would have 3 times the radioactivity in either C-1 or C-2. The ^{14}C in the latter two carbons would be equal. Experimentally, administration of $NaH^{14}CO_3$ to starved rats produces ribose labeled most highly in C-3. C-2 has approx. one half the ^{14}C found in C-3 and C-1 has about one quarter the radioactivity of C-3(ref. 32) (*cf.* Table II). Administration of [3,4-$^{14}C_2$]glucose to a starved rat produces a pattern of labeling in ribose similar to that obtained with $NaH^{14}CO_3$[33]. This distribution of ^{14}C in ribose would occur if three-fourths of the molecules of the pentose arose by the "transketolase–transaldolase" mechanism as shown in Fig. 2 and one-fourth, by the "hexose monophosphate oxidative" system. If this suggestion were true, molecules of ribose with the following patterns of ^{14}C would be produced



As shown in Table II, ribose from the livers of young adults had this distribution of ^{14}C whether or not they were starved prior to the administration of the tracer. The thiamine-deficient animals, however, showed a pattern of labeling consistent with a much greater contribution by the "hexose monophosphate oxidative" pathway relative to the "transketolase–transaldolase" mechanism. As compared with the pattern of tracer in the normal, C-1 had less activity and C-2 more. Presumably this change is a result of a decrease in the activity of transketolase which requires thiamine pyrophosphate as a cofactor[34]. These results confirm the report of HIATT[35] who, using labeled glucose in thiamine-deficient rats, found that thiamine deficiency decreased the importance of the "transketolase–transaldolase" pathway in the synthesis of ribose.

A further point of interest is the higher level of labeling in C-1 and C-2 relative to C-3 in ribose isolated from the liver and skin of the newborn animals as compared to ribose from young adults. There seems to be no obvious explanation for this difference.

The biosynthesis of deoxyribose from acetaldehyde and glyceraldehyde 3-phosphate catalyzed by deoxyribose phosphate aldolase should produce label only in C-3 of the deoxypentose. This presumes that the acetaldehyde would arise by decarboxylation of [1-^{14}C]pyruvic acid and would therefore be unlabeled (Fig. 3). Even if the acetaldehyde were provided from C-3 and C-4 of threonine, as suggested by BOXER AND SHONK[4], one would not expect it to be highly labeled since threonine is an essential amino acid in the rat. Since in all experiments cited in Table II C-1 or both C-1 and C-2 were significantly labeled, it would appear that a labeled source of acetaldehyde is available or that this mechanism is not important in the synthesis of deoxyribose in the rat although its occurrence is not entirely ruled out by these data.

If deoxyribose were synthesized from ribose by direct reduction or if both pentoses have a common precursor, then the patterns of ^{14}C in ribose and deoxyribose should be similar whatever the distribution of isotope in the former. The data of Expt. 5 are consistent with such a concept. The distributions of labeling in the two pentoses

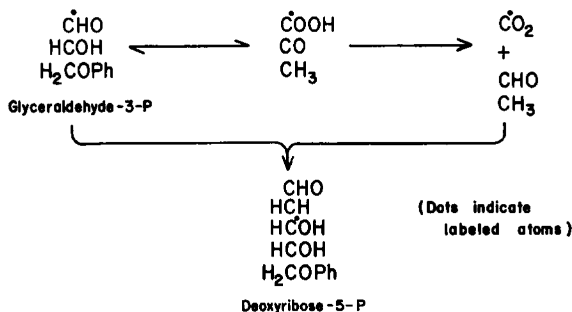


Fig. 3. "Deoxyribosephosphate aldolase" pathway for the synthesis of deoxyribose.

isolated from the skin and from the liver of newborn rats were quite similar. A comparison of the patterns of ^{14}C in the ribose and deoxyribose isolated from the livers of normal young adult animals, however, showed a marked difference. This difference in the distribution of tracer is even more obvious in the animals which were thiamine deficient. The data obtained with young adult rats seem inconsistent with the "direct reduction" or "common precursor" hypotheses for synthesis of deoxyribose.

It is, of course, possible that in the young adult, deoxyribose is made by direct reduction from a pool of ribose which is separated in some manner from the ribose which goes into RNA and whose pattern of ^{14}C is like that found in the deoxyribose rather than that of the ribose of RNA. This is a possibility since RNA and DNA are probably synthesized at different periods in the cell cycle and since, in the liver of the young adult rat, the number of cells synthesizing the latter nucleic acid at any unit time would be a very small portion of the total cell population. However, as shown in Expts. 1–4, the same general distribution of ^{14}C was found in the deoxyribose whether the experiment took 2.5 or 24 h, whether the time between doses was 0.5 or 4 h, whether the animals were starved or fed *ad libitum* before the experiment, or whether the animals were normal or thiamine deficient (having, in the last case, an abnormal pattern of ^{14}C in the ribose of RNA). Surprisingly enough, the presence of an ascitic tumor in the young adult (Expt. 6) does alter the isotopic pattern found in the deoxyribose of the adult liver although there is no gross evidence of abnormality in this organ. In this situation the pattern of ^{14}C in the deoxypentose is the same as that in the ribose of RNA and the distributions are similar to those found in the newborn. Further biological and biochemical study of this phenomenon is in progress.

Nevertheless, if there is a pool of ribose which is the precursor of deoxyribose by direct reduction and which is somehow separated (possibly by being in the nucleus) from that which contributes to RNA then it seems reasonable to consider that there is an, as yet, undefined pathway for the biosynthesis of ribose. Neither known pathway for synthesis of ribose would produce the considerably higher level of labeling in C-1 relative to C-2 as shown in deoxyribose in Expts. 1–4. Incidentally, as far as the present authors know, no data have been published which demonstrate that the cell contains separate pools of ribose which become labeled differently in isotopic experiments.

The data of Table II are not the only results which suggest that synthesis of deoxyribose may occur by a mechanism other than the direct reduction of ribose or from a precursor common to ribose. Even in *E. coli* where the distribution of ^{14}C in

the two pentoses is generally similar, significant differences have been reported. For example the two pentoses did not have similar patterns when isolated during the lag phase from the nucleic acids of "acetate-grown" cells put on [2-^{14}C]glucose[36]. Even cells grown on specifically labeled glucose and harvested at the end of the log phase of growth showed certain marked differences in the distributions of tracer in ribose and deoxyribose[12]. In *Pseudomonas saccharophila* grown on [6-^{14}C]glucose the primary labeling in the ribose was in C-1 and C-5 while in the deoxyribose considerable activity appeared in C-2 as well as in C-1 and C-5 (ref. 37). These cells were harvested at the end of the log phase of growth. Also SHREEVE[18], HORECKER *et al.*[19] and LALAND AND SMITH-KIELLAND[20] have previously published data showing differences in the labeling of ribose and deoxyribose in rat liver from ^{14}C-labeled precursors other than bicarbonate.

The reason for the quantitative difference in the patterns of ^{14}C in the deoxyribose of Expts. 1, 2 and 3 is not obvious. However, it is probably not a result of the analytical procedure since the results from duplicate degradations carried out on the same sample agreed to within 5 %. The differences may arise from biological variation. It does not seem sufficient, however, to alter the interpretation given the data.

It is interesting to note that the ribose from the skin had a specific activity higher than that of the liver in Expt. 5, indicating the high rate of metabolism of young rat skin.

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