## Reduction of 5'-cytidylic acid to deoxycytidylic acid by mammalian enzymes

This report describes the conversion of $^{14}C$-labeled CMP to deoxycytidine nucleotides by a cell-free extract of a rat tissue, the Novikoff ascites tumor. It has been demonstrated previously (see REICHARD[1]) that *in vivo* and in living-cell systems cytidine can be incorporated into DNA deoxycytidine with the glycosidic bond intact. Small conversions of ribonucleosides and ribonucleotides to the deoxyribose derivatives have been obtained by GROSSMAN[2,3] in bacterial extracts and by REICHARD[1] in chick-embryo extracts. The conversion has not been obtained previously in extracts of mammalian tissues.

The tumor cells were ruptured by homogenization in 1.5 vol. of distilled water and centrifuged at 100,000 $\times$ *g* to obtain the supernatant fraction. Dialysis of the extracts increased the enzyme activity. $HClO_4$ extracts were made of the incubation mixtures, carrier dCMP was added, and the extracts were heated to hydrolyze polyphosphates. The dCMP was re-isolated by chromatographic procedures, on Dowex-50 and paper, based on the methods of REICHARD[4]. The amount of [$^{14}C$]dCMP formed was calculated from the amount of carrier dCMP added and the specific radioactivity of the isolated dCMP.

Table I illustrates the requirements for ATP, TPN, and a reducing system in the reaction. In the complete system, [$^{14}C$]CMP (prepared from [6-$^{14}C$]orotic acid[5]) was phosphorylated and maintained as cytidine triphosphate; in the absence of ATP, the CMP was destroyed with consequent low conversion to dCMP. TPN was clearly required and the activity was augmented by a TPN-reducing system, although a DPN-lactate system was nearly as effective. The reaction rate was constant for 2 h of incubation but decreased subsequently.

Added [$^{14}C$]cytidine was rapidly phosphorylated by the enzyme extract, and was as effective a substrate as [$^{14}C$]CMP. When the substrate was "uniformly-labeled" [$^{14}C$]cytidine (Schwarz Laboratories, Inc.), with a ratio of specific activities (base/nu-

TABLE I

SUBSTRATE REQUIREMENTS FOR THE CONVERSION OF [$^{14}C$]CMP TO DEOXYCYTIDINE NUCLEOTIDES BY NOVIKOFF-TUMOR EXTRACT

The complete system contained, in a final volume of 1.5 ml: 25 $\mu$moles Tris buffer, pH 7.0; 11.2 $\mu$moles $MgCl_2$; 7.5 $\mu$moles ATP; 0.6 $\mu$mole TPN; 6.0 $\mu$moles glucose 6-phosphate; 0.39 $\mu$mole [$^{14}C$]CMP, 37,500 counts/min; 0.1 unit of commercial glucose-6-phosphate dehydrogenase; and 1.0 ml of enzyme extract (dialyzed for 12 h against 0.02 *M* Tris buffer, pH 6.5 at 4°). Sodium lactate replaced glucose 6-phosphate in No. 6.

| *Conditions* | *Counts/min in dCMP* |
|---|---|
| 1. Complete system | 2100 |
| 2. 2.5 $\mu$moles ATP instead of 7.5 $\mu$moles | 500 |
| 3. ATP omitted | 5 |
| 4. Glucose 6-phosphate omitted | 1600 |
| 5. TPN omitted | 20 |
| 6. DPN instead of TPN | 1700 |

Abbreviations: CMP, cytidine 5'-phosphate; dCMP, 2'-deoxycytidine 5'-phosphate; Tris, tris(hydroxymethyl)aminomethane hydrochloride; gluc-6-P, glucose 6-phosphate; ATP, adenosine triphosphate; TPN, triphosphopyridine nucleotide; DPN, diphosphopyridine nucleotide; DNA, deoxyribonucleic acid.

cleoside) of 0.68, it was found that the ratio of the product dCMP was 0.66. This indicated that the deoxyribose of the dCMP was derived from the ribose of the substrate. The radioactivity of deoxycytidine (isolated with carrier) was negligible with either [$^{14}$C]CMP or [$^{14}$C]cytidine as substrate.

Centrifugation of the enzyme extract, adjusted to pH 5 with acetic acid, yielded "pH-5 soluble" and "pH-5 precipitate" fractions, both of which were found to be necessary for the overall reaction. Both fractions were labile to heat and stable to dialysis. As shown in Table II, the sequence and substrate requirements of the two enzyme fractions were explored. The action of the pH-5 soluble enzyme with the TPN-reducing system and possibly with ATP was necessary before or simultaneously with the incubation of the pH-5 precipitate with [$^{14}$C]CMP. The pH-5 soluble fraction appears to produce an essential, unidentified component, while the pH-5 precipitate fraction appears to catalyze the reduction of CMP nucleotides.

TABLE II

SEQUENCE OF ACTION OF THE TWO FRACTIONS

After the first incubation for 1 h, the tubes were heated 2 min in a boiling-water bath, cooled and the additional substrates and enzyme were added for the second 1-h incubation. The final amounts of components were the same as in Table I.

| *Components in first incubation* | | *Additional components in second incubation* | | *Counts/min in dCMP* |
|---|---|---|---|---|
| *enzyme preparation* | *substrates* | *enzyme preparation* | *substrates* | |
| 1. pH-5 precipitate | ATP, $MgCl_2$, TPN, Gluc-6-P, [$^{14}$C]CMP | pH-5 soluble | — | 0 |
| 2. pH-5 soluble | — | pH-5 precipitate | ATP, $MgCl_2$, TPN, Gluc-6-P, [$^{14}$C]CMP | 20 |
| 3. pH-5 soluble | ATP, $MgCl_2$, [$^{14}$C]CMP | pH-5 precipitate | TPN, Gluc-6-P | 120 |
| 4. pH-5 soluble | ATP, $MgCl_2$, TPN, Gluc-6-P, [$^{14}$C]CMP | pH-5 precipitate | — | 270 |
| 5. pH-5 soluble | TPN, Gluc-6-P | pH-5 precipitate | ATP, $MgCl_2$, [$^{14}$C]CMP | 270 |
| 6. pH-5 soluble | ATP, $MgCl_2$, TPN Gluc-6-P | pH-5 precipitate | [$^{14}$C]CMP | 410 |
| 7. pH-5 precipitate + pH-5 soluble | ATP, $MgCl_2$, TPN Gluc-6-P, [$^{14}$C]CMP | (no second incubation) | | 710 |
| 8. whole extract | ATP, $MgCl_2$, TPN, Gluc-6-P, [$^{14}$C]CMP | (no second incubation) | | 790 |

These experiments support the concept that the deoxyribose of DNA deoxycytidine may be derived from the ribose of cytidine by a reductive step at the nucleotide level.

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*The University of Texas, M.D. Anderson Hospital and Tumor Institute, Houston 25, Tex. (U.S.A.)*

E. C. MOORE
R. B. HURLBERT

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