INTESTINAL ABSORPTION OF CYTIDINE DIPHOSPHATE CHOLINE AND ITS CHANGES IN THE DIGESTIVE TRACT

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Summary Intestinal absorption of cytidine diphosphate choline (CDP-choline), its structural changes in the digestive tract, and hepatic uptake have been investigated in rats using 14C-labeled (14CH3 attached to N of choline) and 3H-labeled (at C5 of pyrimidine) compounds. The results indicate that: 1) CDP-choline is relatively stable in the stomach, but is quickly degraded into cytidine and choline in the intestine; 2) The hepatic uptakes of 14C and 3H reach the maximum in two to three hours after oral administration; 3) Whereas the amount of 14C remaining in the gut is inversely related to the hepatic uptake, no similar correlation is seen with 3H-labeled CDP-choline, and 4) Extrahepatic uptake of 14C and 3H is very small. The possibility of phosphorylation in the mucosa of choline and cytidine has been discussed, based on the differences in relative amount of radioactivity in individual broken-down products in the intestinal lumen and mucosa.

It has been established that B vitamins exist in nature mostly in their coenzyme forms. Despite the general interest in the intestinal absorption of these vitamins and coenzyme forms subsequent to their recent clinical uses for therapeutic purposes, our knowledge is very limited. Even the long dispute of whether vitamin B1 is absorbed after phosphorylation (1, 2), or passively without chemical changes (3), has not as yet been settled, although more recent data suggest active transport across the intestinal mucosa involving phosphorylation (4, 5).

Coenzyme forms of B vitamins are relatively unstable in the gut. Flavine
adenine dinucleotide, for instance, is broken down into flavine mononucleotide and riboflavin before being absorbed (5, 6). Cytidine diphosphate choline (CDP-choline) (7) is an important cofactor in the biosynthesis of lecithin, and has come to be used clinically of late.

The present investigation has been undertaken to study the intestinal absorption of this nucleotide and chemical changes this compound may undergo in the digestive tract.

MATERIALS AND METHODS

Wistar rats weighing 120-200 g were used. They were fasted overnight and CDP-choline in solution was fed into the stomach by a stomach tube.

Two labeled compounds were used (Fig. 1). $^{14}$CH$_3$-labeled compound was diluted with nonradioactive CDP-choline to yield an aqueous solution of 0.84 $\mu$Ci/4mg/ml or 4.2 $\mu$Ci/5mg/ml; 1.0 ml was fed. $^3$H-labeled compound (at C$_5$ of pyrimidine) was also used in solutions of 3.22 $\mu$Ci/4mg/ml or 12.88 $\mu$Ci/4mg/ml.

![Fig. 1. Structure of CDP-choline and positions of the labels.](image)

Preparation of samples. For the time study, 5 rats each were killed at 0.5, 1, 2, and 3 hr after oral administration of labeled CDP-choline. For chromatographic studies, 3 rats were used for each experiment and typical data are shown in the figures. They were killed by a blow on the head 30 min after oral administration. The stomach and the upper half of the small intestine were removed after ligation. The stomach was opened and gastric juice was collected. Intestinal washing was collected after injecting a few milliliters of saline from one end of the excised intestine and draining it. Gastric juice and intestinal washing were centrifuged and the supernatant was used for study.

Intestine mucosa obtained by scraping and the liver were homogenized with saline to yield a 10% homogenate or extracted with perchloric acid.

Radioactivity counting. A Beckman LS-233 liquid scintillation counter was used. The scintillator consisted of 1,000 ml of dioxane, 200 ml of methylcellosolve,
12 g PPO, 600 mg POPOP, and 60 g of naphthalene. Gel powder (Cab-O-Sil) was used as suspender of homogenate (30 g/1,000 ml scintillator). Quenching was corrected by the internal standard method.

**Thin layer chromatography (TLC).** Eastman Chromatogram Sheet 6065 Cellulose with two kinds of developing solvent systems was used (8).

(A) 1 m NH₄-Ac (pH 7.5), 95% ethanol and concentrated NH₄OH (3: 7: 0.05 v/v), and (B) iso-propanol, water, trichloroacetic acid and concentrated NH₄OH (75: 25: 5: 0.3 v/v).

An aliquot of 0.05-1.0 ml of the sample was applied at each origin and developed together with the standards of non-radioactive CDP-choline, choline phosphate, and choline chloride for the study of ¹⁴C-CDP-choline. Cold CDP-choline, CDP, CMP, and cytidine were similarly developed as standards for ³H-CDP-choline.

Spots of CDP-choline, CDP, CMP, and cytidine were made visible with ultraviolet light (Toshiba SL-30) having a central frequency at 2,537 Å, and choline phosphate and choline by color reaction with HANES (9) and DRAGENDORFF (10) reagents, respectively. After development and air drying, the cellulose was divided into bands 5 mm in width, and each was scraped into 10 ml of dioxane scintillator to be counted. The cellulose mixed in the scintillator had no effect on the counting efficiency.

¹⁴CO₂ evolved from the rat was trapped in a closed apparatus using phenethylamine. The details have already been described (11).

**EXPERIMENTAL**

1) **Purity of ¹⁴C- and ³H-CDP-choline**

¹⁴C-CDP-choline, to which a small amount of non-radioactive CDP-choline had been added to give a sufficient absorption of ultraviolet rays, was analysed by TLC. At the same time, non-radioactive choline chloride was chromatographed, using both A and B solvent systems. After air drying, the cellulose was divided into 5 mm widths, scraped and counted for ¹⁴C. The chromatogram (Fig. 2) shows the radioactivity exclusively in the CDP-choline fraction and a trace of radioactivity in choline phosphate separated using B solvent.

³H-CDP-choline was developed by TLC together with cold CDP-choline, CDP, CMP, and cytidine and similarly analysed. Figure 3 shows that most of the radioactivity was in the CDP-choline fraction and a trace in CMP and cytidine.

2) **Distribution of radioactivities following ingestion of ¹⁴C- and ³H-compounds**

The rats were fed either ¹⁴C- or ³H-CDP-choline (0.8 μCi/4mg and 3.22 μCi/
Fig. 2. TLC of $^{14}$C-CDP-choline preparation.

Fig. 3. TLC of $^3$H-CDP-choline preparation.
INTESTINAL ABSORPTION OF CDP-CHOLINE

4mg, respectively). The distribution of radioactivities with time in the intestinal lumen and each organ was investigated. Practically no radioactivity of either $^{14}$C or $^3$H appeared in the heart, brain, spleen, and pancreas, nor in the urine, up to 3 hr after administration. Both labeled compounds were absorbed into the blood increasingly with time. The radioactivity remaining in the stomach and intestine was gradually decreased up to 3 hr, to 5% of the dose with $^{14}$C, and to 15% with $^3$H. The hepatic uptake of $^{14}$C reached a maximum in 2–3 hr in a reversed relation to the radioactivity remaining in the gut. The hepatic uptake of $^3$H was only 1/4–1/5 of $^{14}$C and the relation to the gut $^3$H was not as apparent as with $^{14}$C (Fig. 4). The radioactivities in the mucosa remained rather consistent regardless of the label.

3) Biochemical behavior of $^{14}$C-CDP-choline in the gut

The stomach and the intestine were flushed with a small amount of saline 30 min after oral administration of 4 mg (0.84 μCi) of $^{14}$C-CDP-choline. The washings were developed with solvents A and B. The bulk of radioactivity in the gastric juice was found in the CDP-choline fraction and a little in the choline phosphate fraction, although separation was not too clear with solvent B (Fig. 5). In the intestinal washing, there was practically no activity in the CDP-choline fraction, and the bulk of activity was with the choline position (Fig. 6).
Fig. 5. TLC of the stomach washing 30 min after ingestion of $^{14}$C-CDP-choline (0.84 μCi/4mg).

Fig. 6. TLC of the intestinal washing 30 min after ingestion of $^{14}$C-CDP-choline (0.84 μCi/4mg).
4) Biochemical behavior of $^3$H-CDP-choline in the gut

Similar experiments were carried out with 3.22 $\mu$Ci/4mg of $^3$H-CDP-choline. The bulk of radioactivity was found in the CDP-choline position, and a little activity in the position of cytidine (Fig. 7). With the intestinal washing, no activity was found in the CDP-choline and the bulk of activity in the area compatible with cytidine (Fig. 8).

\begin{align*}
\text{cpm} & \quad \text{A} & \quad \text{B} \\
750 & \quad 500 & \quad 250 & \quad 0 \\
0 & \quad 2 & \quad 4 & \quad 6 & \quad 8 \text{ cm} \\
\end{align*}

![Fig. 7. TLC of the stomach washing 30 min after ingestion of $^3$H-CDP-choline (3.22 $\mu$Ci/4mg).](image)

5) $^{14}$CO$_2$ expiration after ingestion of $^{14}$C-CDP-choline

$^{14}$CO$_2$ expired after ingestion of 0.84 $\mu$Ci/4ml of $^{14}$C-CDP-choline was absorbed with phenethylamine, and the radioactivity was measured. $^{14}$CO$_2$ expiration was 0.2% in the first hour, 1.0% in the second hour, and 1.55% in the third and fourth hours. It declined very slowly thereafter. The total expiration in 12 hr was 13% of the dose, and the radioactivity excreted into the urine during the 12 hr was about 0.4%.

6) Determination of $^{14}$C-labeled compounds in liver shortly after absorption

The rats were fed $^{14}$C-CDP-choline (4.2 $\mu$Ci/5mg) and after 2 hr, at the time of peak hepatic uptake of $^{14}$C, they were killed, intestinal mucosa and livers were
Fig. 8. TLC of the intestinal washing 30 min after ingestion of \(^3\)H-CDP-choline (3.22 μCi/4mg).

Table 1.

| TISSUE               |
|----------------------|
| homogenized with     |
| 0.6 N PCA 2 vol.     |
| 4,000 rpm 10'        |

Supernatant (Sup.)     Precipitate (Prec.)
neutralized with       dissolved in
5 N KOH pH 6.0–7.0     0.5 N NaOH
Sup.                  conc. HCOOH
TLC & counting         counting
Table 2. Acid extraction rates of $^{14}$C and $^3$H in the liver and intestinal mucosa.

Doses: $^{14}$C-CDP-choline 0.84 µCi/4mg/ml (intestine), 4.2 µCi/5mg/ml (liver)
$^3$H-CDP-choline 12.88 µCi/4mg/ml

| Labeled compound       | Tissue          | Homogenate (%) | Acid soluble fraction (%) | Precipitate (%) |
|------------------------|-----------------|----------------|--------------------------|-----------------|
| $^{14}$C-CDP-Choline    | Liver           | 45.2           | 22.1                     | 22.8            |
|                        | Small intestine | 9.8            | 6.0                      | 3.1             |
|                        | (Mucosa)        |                |                          |                 |
| $^3$H-CDP-Choline      | Liver           | 17.8           | 8.5                      | 6.4             |
|                        | Small intestine | 6.6            | 2.5                      | 2.6             |
|                        | (Mucosa)        |                |                          |                 |

Figures are in percent of the dose administered.

Fig. 9. TLC of acid extract of intestinal mucosa 2 hr after ingestion of $^{14}$C-CDP-choline (4.2 µCi/5mg).

homogenized with perchloric acid in ice, and acid extraction was carried out as shown schematically in Table 1. A comparison between the radioactivities of the homogenate of mucosa and liver and those of acid extracts is shown in Table 2, together with the results for $^3$H-CDP-choline.

It was found that about one-half of the radioactivity in the homogenate was extractable by the acid. Subsequently, acid extracts of the mucosa and liver were analyzed by TLC after neutralization. Radioactivities were found in the spots corresponding to CDP-choline, choline phosphate and choline both in the extracts
of intestinal mucosa and liver, with least activity in the CDP-choline fraction (Figs. 9 and 10).

7) Determination of $^3$H-labeled compounds in the liver shortly after absorption

Two hours after ingestion of 8 mg (25.7 $\mu$Ci) of $^3$H-labeled CDP-choline, the acid extracts of the liver and intestinal mucosa were analyzed, as in the preceding study, with $^{14}$C-CDP-choline. It was found that radioactivity had spread among the spots for CDP-choline, CDP, CMP, and cytidine, and that most of the activity was in the CMP and cytidine fractions (Figs. 11 and 12).

DISCUSSION

As the structure of CDP-choline in Fig. 1 indicates, $^{14}$CH$_3$ attached to the quaternary ammonium is stable and is metabolized in the liver mitochondria through transmethylation and other reactions. The $^3$H attached to C$_5$ of the pyrimidine is also stable and not exchanged by the environmental H$_2$O. These preparations were checked for purity by TLC and found to be adequate for the study.

The distribution of $^{14}$C and $^3$H in the rat after oral administration of $^{14}$C- and $^3$H-labeled compounds showed that disappearance of the former from the gastrointestinal canal was somewhat faster than the latter, and that absorption was maximum 2–3 hr after oral administration. Hepatic uptake was greater for
Fig. 11. TLC of acid extract of intestinal mucosa 2 hr after ingestion of ^3^H-CDP-choline (25.7 μCi/8mg).

Fig. 12. TLC of acid extract of liver 2 hr after ingestion of ^3^H-CDP-choline (25.7 μCi/8mg).
$^{14}\text{C}$, about three times that of $^{3}\text{H}$, suggesting cleavage of the moieties containing $^{14}\text{C}$ and pyrimidine.

When the gastric content was analyzed at 30 min after oral administration of the labeled compounds, the bulk of CDP-choline in the gastric juice was found to be intact. The analysis of intestinal washing obtained at the same time disclosed that most of the $^{14}\text{C}$ was in the spot compatible with choline, suggesting that CDP-choline is stable in the stomach but is quickly split to yield choline in the small intestine. Similarly, $^{3}\text{H}$ label was found mainly in the fraction compatible with cytidine in the intestinal washing. Evidently, CDP-choline is broken down in the intestine releasing choline and cytidine. Such changes may be effected by the action of intestinal esterases and pyrophosphatases. These split compounds as well as the original CDP-choline may be absorbed from the intestinal mucosa as such.

The analysis of an acid extract of liver 2 hr after oral administration of $^{14}\text{C}$-CDP-choline disclosed that the soluble label was in the fractions compatible with CDP-choline, choline phosphate and choline. The relative activity in choline was greater in the intestinal mucosa during the absorptive phase as compared with that in the liver. It may be that choline phosphate and choline produced in the intestine are further broken down, and that some are reutilized for synthesis of CDP-choline in the liver. That the relative amount of choline phosphate in the intestinal mucosa was greater than that in the lumen may suggest the possibility of phosphorylation of choline in the mucosa. The observation that $^{3}\text{H}$ was mostly with cytidine in the intestinal lumen, but more $^{3}\text{H}$ was found with CMP and CDP in the mucosa, may also suggest the possible phosphorylation of the base in the mucosa.

The measurement of $^{14}\text{CO}_2$ following oral administration of $^{14}\text{C}$-CDP-choline demonstrated that about 14% of the radioactivity was lost from the lung and into the urine as CO$_2$, indicating a relatively small rate of complete breakdown of the choline moiety.

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