Absorption and Metabolism of Pyridoxamine in Mice
I. Pyridoxal as the Only Form of Transport in Blood

Tae Sakurai, Tadashi Asakura, Aritake Mizuno, and Makoto Matsuda

Department of Biochemistry, The Jikei University School of Medicine, Nishi-Shinbashi, Minato-ku, Tokyo 105, Japan
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Summary [3H] Pyridoxamine was orally administered to mice in physiological amounts, and the distribution of isotope between the six recognized forms of vitamin B₆ and pyridoxic acid was determined at different times in the intestine, liver, blood, and brain. After 7 min about 50% of the radioactivity in pyridoxamine had been absorbed by the intestine and transported to the blood and other organs. Labeled pyridoxal phosphate was found in the intestine and liver. Labeled pyridoxamine could not be detected in the peripheral blood, but substantial amounts of labeled pyridoxal and pyridoxal phosphate were found in the blood. However, when a large amount (40–140 nmol) was given, a significant amount of labeled pyridoxamine was found in the blood, together with labeled pyridoxal and pyridoxal phosphate. These results suggest that the intestine and/or liver play a major role in completely converting physiological amounts of pyridoxamine to circulating pyridoxal, which is then taken up and phosphorylated by other organs.

Key Words pyridoxamine, pyridoxal phosphate, pyridoxal kinase, pyridoxine phosphate oxidase, intestine, liver

The general pathway for the metabolic interconversion of B₆ vitamers is well known. For example, pyridoxine (PN) and pyridoxamine (PM) are phosphorylated to pyridoxine phosphate (PNP) and pyridoxamine phosphate (PMP), respectively, by pyridoxal (PL) kinase, which has been detected in all mammalian tissues investigated (1). PNP and PMP are commonly converted to pyridoxal phosphate (PLP) by pyridoxine phosphate (PNP) oxidase (2), which, in contrast to the wide distribution of PL kinase, is confined to a few tissues (3–8). The great activity of PNP oxidase is found in the liver, with only limited activity in the intestine, brain, and kidney, and almost no activity in the lung, heart, muscles, and pancreas. PLP can be converted to PL by hydrolysis with phosphatase (9). Part of
the PL is then converted again to PLP by PL kinase and the remaining part is oxidized to pyridoxic acid by PL oxidase (4).

Our previous work (10, 11) has shown that, when a physiological dose of pyridoxine (PN) is orally administered to mice, PN is completely converted to circulating PL by sequential participation of PL kinase, PNP oxidase and phosphatase of the intestine and/or liver, and thus PL may be used as the only source of PLP for most tissues. This result suggests that when PM is given as a dietary B₆ vitamer it may be completely converted to circulating PL by the same enzyme system described above.

In this study, various amounts of [¹³H]PM were orally administered to mice, and the distribution of isotope among B₆ vitamers in various organs, including blood, was determined to investigate the absorption and metabolism of the vitamers in live animals.

MATERIALS AND METHODS

Chemicals. [¹³H]PM with a specific activity of about 3.87 Ci/mm mol was obtained from the Radiochemical Centre (Amersham, England). Purification was performed with an Amberlite column (12). Acid phosphatase was purchased from Sigma Chemical Co.

Animals and tissue preparation. Male DDY mice (4 weeks old) served as experimental animals. The animals were fasted for 6 h before the experiments, then 100 μl of saline solution containing [¹³H]PM (1.4-140 nmol, 54 μCi) was injected through a cannula into the gastric cavity. The mice were decapitated at different times after the administration. The organs were quickly removed, weighed and then homogenized in 9 volumes of 1 N perchloric acid, followed by centrifugation at 20,000 × g for 15 min. Blood removed from the heart was also homogenized in 9 volumes of 1 N perchloric acid and centrifuged. The precipitate was washed once with 0.2 N perchloric acid and all supernatants were combined. The combined supernatant was neutralized with 5 N KOH to pH 3-4 and centrifuged at 20,000 × g for 15 min. In some cases, blood was mixed with EDTA (a few mg) and centrifuged at 3,000 × g for 5 min to separate plasma and erythrocytes, which were extracted with perchloric acid as described here.

Chromatographic procedures. B₆ vitamers were separated into fractions by the chromatographic procedures described by Loo and Badger with some modification (12). The same quantity of each extract was transferred to an Amberlite CG-120 column (0.4 × 1.75 cm) equilibrated in 0.1 M acetate buffer (pH 4) and washed through the column with 1.5 ml of the same buffer. The first effluent, containing PLP, PNP, PMP, and pyridoxic acid, was collected in one fraction and kept for later separation into individual components. The column was sequentially washed to elute the nonphosphorylated B₆ vitamers as follows: PL, 1.2 ml of 0.1 M Na phosphate buffer (pH 6.0) after 1.0 ml acetate buffer (pH 4.0); PN, 2.0 ml of 0.1 M Na phosphate buffer (pH 6.5); and PM, 1.5 ml of 0.1 M Na phosphate buffer (pH

8.5). The first effluent was adjusted to pH 5 with 5 N KOH, and treated with acid phosphatase (0.5 mg/ml, incubation for 60 min at 37°C) to hydrolyze PLP, PNP, and PMP to PL, PN, and PM, respectively. The treated solution was adjusted to pH 3-4 with acetic acid and again applied to the same Amberlite column. Pyridoxic acid was eluted with 1.5 ml of 0.1 M acetate buffer (pH 4), and the PL, PN, and PM derived, respectively, from PLP, PNP, and PMP, were separately eluted in the same manner as described above.

In some cases, the first effluent containing PLP, PNP, PMP, and pyridoxic acid was applied to a 0.4 × 3.5 cm Dowex 1 × 8 (formate-type) column equilibrated in 0.05 M K formate buffer (pH 4.25). Elution was performed with a continuous gradient from 0.05 M potassium formate (pH 4.25) to 0.05 M potassium citrate plus 0.2 M potassium chloride (pH 4.25), as described by Bain and Williams (13). B6 vitamers were well resolved and came off the column in the order of PMP, PNP, PLP, and pyridoxic acid.

Isotope determination. Radioactive assays were carried out on the column fractions using a liquid scintillation spectrometer. Aliquots of 2 ml were added to 4.5 ml of the scintillator ACS II.

RESULTS

The absorption of [3H]PM by the intestine and the distribution of 3H among the B6 vitamers in the organ are presented as a function of time in Fig. 1a. At 7 min after the oral administration of [3H]PM (1.4 nmol, 5.4 μCi), about half of the isotope in the intestine was absorbed, and labeled PLP and PL started to appear in the organ. The amounts of labeled PMP were very small as compared with labeled PLP and PL (data not shown).

The distribution of 3H among the B6 vitamers in the liver is illustrated as a function of time after [3H]PM administration in Fig. 1b. The greater part of the isotope in the liver was found in PLP, with a small amount in PL. A considerable amount of [3H]pyridoxic acid was found in the liver in the 15 min following administration, whereas [3H]PM was not found in the organ during that period, in spite of continuous absorption of [3H]PM by the intestine.

Figure 1c shows the distribution of 3H in B6 vitamers in the blood according to the length of time after [3H]PM administration. In contrast to its distribution in the liver, in blood the isotope was first distributed in PL and secondly in PLP, but not in pyridoxic acid. Labeled PL rapidly reached a maximum level after 15 min and then diminished somewhat gradually. At 15 min most labeled PL in the blood was located in the plasma, while most of the labeled PLP was located in the blood cells (Fig. 2a). Labeled PMP and PM were scarcely found in either the plasma or blood cells.

Figure 1d shows the distribution of 3H in B6 vitamers in the brain as a function of time after [3H]PM administration. In the brain, the isotope was distributed first in PLP and secondly in PL, but was scarcely found in PMP and PM.
Fig. 1. Distribution of $^3$H in B$_6$ vitamers in mouse tissues after oral administration of $[{}^3$H]pyridoxamine (5.4 $\mu$Ci). ■, $[{}^3$H]pyridoxamine; ○, pyridoxal; ◦, pyridoxal phosphate; △, pyridoxic acid.

The mechanism of transport of $[{}^3$H]PM was investigated with saturation experiments involving the addition of various amounts of cold PM to 5.4 $\mu$Ci of $[{}^3$H]PM. When $[{}^3$H]B$_6$ vitamers in blood were examined 7 min after the administration of a 10-fold amount of $[{}^3$H]PM (14 nmol, 5.4 $\mu$Ci), labeled PL and PLP predominated, with labeled PM found only in trace amounts.

However, when the amount of $[{}^3$H]PM administered was increased to 33-fold (46 nmol, 5.4 $\mu$Ci), a considerable amount of labeled PM was found, together with $J. Nutr. Sci. Vitaminol.$
large amounts of labeled PL and PLP. Moreover, \( ^3H \) PM was detected as a larger peak than those of \( ^3H \) PL and \( ^3H \) PLP in the chromatograms when a 100-fold amount of \( ^3H \) PM (140 nmol, 5.4 \( \mu \)Ci) was administered. Figure 3 shows the relationship between ratios of \( ^3H \) PL, PLP, and PM to all \( ^3H \)B\(_6\) vitamers in blood and amounts of \( ^3H \) PM administered.

When a 100-fold amount of \( ^3H \) PM was administered, most labeled PM and PL were located in the plasma, while a large amount of labeled PLP was located in the blood cells (Fig. 2b).

**DISCUSSION**

The present experiment shows that, although \( ^3H \) PM in physiological amounts is rapidly absorbed across the intestinal wall, it is not found in the circulating blood. Instead of \( ^3H \) PM, a noticeable amount of \( ^3H \) PL appeared rapidly in the blood (Fig. 1c). These results suggest that PM is taken up by the intestinal wall and liver and converted there to PL, with only the product PL released into the circulating blood. In fact the isotope of \( ^3H \) PM appeared in PLP and PL in the intestinal wall and liver following \( ^3H \) PM administration, suggesting
Fig. 3. Relationship between ratios of $[^3H]B_6$ vitamers in blood and amounts of administered $[^3H]$pyridoxamine. Values are means ± SD of three experiments.

- $[^3H]$pyridoxal
- $[^3H]$pyridoxal phosphate
- $[^3H]$pyridoxamine

that $[^3H]$PL was synthesized from $[^3H]$PM, probably through the PM-PMP-PLP-PL pathway (14). It has been reported that intestinal wall and liver cells exhibit adequate levels of activity of PL kinase, PNP oxidase and phosphatase (6, 8, 15–17).

The intestine and liver may provide a means of completely converting dietary PM to circulatory PL, which can then serve as the only source of the coenzyme PLP in all tissues that contain PL kinase, whether they contain PNP oxidase or not. The results shown in the present report suggest that in a physiological state PL may play a very important role in transport between organs and may be the only source of PLP for these cells. $[^3H]$PLP in the brain (Fig. 1d) therefore is considered to have come from $[^3H]$PL in the blood.

The observation that most $[^3H]$PL in the blood is located in the plasma (Fig. 2a) suggests that plasma PL may be the source of PLP for most tissues and that the loose binding of PL to plasma albumin (18) and the resulting accumulation of this vitamer in the plasma may provide a means for PLP to function as a principal form of transport in the blood (Fig. 4).

In any case, it is important to note that when a physiological level of PM is orally administered to mice, no PM reaches the circulating blood; rather, PL appears as the only form in the circulating blood. In this condition, therefore, all organs other than the intestine and liver are thought to obtain not PM but PL from the plasma and then convert it to PLP via PL kinase. Whether the $[^3H]$PL found in the blood originated in the intestine wall or liver or both remains to be clarified in future experiments.

When amounts of $[^3H]$PM larger than 46 nmol were administered, a considerable amount of $[^3H]$PM appeared in the blood without being completely trans-
Fig. 4. Metabolic transformation of pyridoxamine by the intestine, liver, and brain, and suggested role of the intestine or/and liver as a source of pyridoxal for the brain and other organs.

formed to $[^{3}H]$PL (Fig. 3). This result suggests that the ability of the intestinal wall and/or liver to completely convert dietary PM to circulating PL has a limit and that this limit is between 14 and 46 nmol of PM per mouse. The PM that leaked into the circulation was distributed to the plasma. This leaked PM in plasma may serve as a source of the coenzyme PLP in some tissues that contain both PL kinase and PNP oxidase.

Our previous reports have already shown that when less than 46 nmol of PN is given orally to mice, PN is entirely transformed to PL, which can serve as the only source of vitamin B$_6$ for most tissues (10, 11). Thus, it is important to note that when a physiological level of B$_6$ vitamers (PN and PM) is administered orally to mice, each vitamer is entirely transformed to PL in the intestinal wall and/or liver, and only this PL is used as a principal source of vitamin B$_6$ by tissues and organs.

REFERENCES


