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Research Article

The plasma content of B6 vitamers is governed by, among other factors, dietary supply and metabolic interconversion. This study examines the effect of pyridoxine supplementation on the plasma content of B6 vitamers and pyridoxic acid in man, and the metabolic conversion and release of B6 compounds in isolated rat hepatocytes. Six healthy human subjects were given 100 mg pyridoxine-HCl/d orally for 1--3 wk. Before pyridoxine supplementation, the mean total plasma level of B6 vitamers was 114 +/- 9 nM; and pyridoxal-P, pyridoxamine-P, pyridoxal, pyridoxine, and pyridoxamine accounted for 54, 3, 11, 27, and 5%, respectively. Plasma level of pyridoxic acid was 40 +/- 7 nM. Thus, pyridoxal-P is the principal B6 vitamer in plasma. During pyridoxine supplementation, mean plasma levels of the B6 vitamers and pyridoxic acid increased to 655 +/- 122 and 222 +/- 55 nM, respectively. The plasma content of pyridoxal-P and pyridoxic acid increased 6--7-fold and that of pyridoxal, 12-fold, but the pyridoxine level did not increase. Isolated hepatocytes, 1 g/15 ml, were incubated for 2 h with 3.33 microM [¹⁴C]pyridoxine (6 microCi/mumol). At zero time, the cells contained about 35 nmol pyridoxal-P and 25 nmol pyridoxamine-P. After 2 h incubation, the cellular content of pyridoxal-P and pyridoxamine-P did not change significantly, but the medium contained 5.9 nmol pyridoxal-P, 0.3 nmol pyridoxamine-P, 7.2 nmol pyridoxal, 26.6 nmol [...]

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Plasma Content of B₆ Vitamers and Its Relationship to Hepatic Vitamin B₆ Metabolism

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ABSTRACT The plasma content of B₆ vitamers is governed by, among other factors, dietary supply and metabolic interconversion. This study examines the effect of pyridoxine supplementation on the plasma content of B₆ vitamers and pyridoxic acid in man, and the metabolic conversion and release of B₆ compounds in isolated rat hepatocytes. Six healthy human subjects were given 100 mg pyridoxine-HCl/d orally for 1–3 wk. Before pyridoxine supplementation, the mean total plasma level of B₆ vitamers was 114±9 nM; and pyridoxal-P, pyridoxamine-P, pyridoxal, pyridoxine, and pyridoxamine accounted for 54, 3, 11, 27, and 5%, respectively. Plasma level of pyridoxic acid was 40±7 nM. Thus, pyridoxal-P is the principal B₆ vitamer in plasma. During pyridoxine supplementation, mean plasma levels of the B₆ vitamers and pyridoxic acid increased to 655±122 and 222±55 nM, respectively. The plasma content of pyridoxal-P and pyridoxic acid increased 6–7-fold and that of pyridoxal, 12-fold, but the pyridoxine level did not increase. Isolated hepatocytes, 1 g/15 ml, were incubated for 2 h with 3.33 μM [¹⁴C]pyridoxine (6 μCi/μmol). At zero time, the cells contained about 35 nmol pyridoxal-P and 25 nmol pyridoxamine-P. After 2 h incubation, the cellular content of pyridoxal-P and pyridoxamine-P did not change significantly, but the medium contained 5.9 nmol pyridoxal-P, 0.3 nmol pyridoxamine-P, 7.2 nmol pyridoxal, 26.6 nmol pyridoxine, 0.3 nmol pyridoxamine, and 7.5 nmol pyridoxic acid. Whereas the specific radioactivity of pyridoxal-P, pyridoxal, and pyridoxic acid in the medium approached that of [¹⁴C]pyridoxine, the specific radioactivity of cellular pyridoxal-P and pyridoxamine-P was only 20% of that of pyridoxine. Thus, newly synthesized pyridoxal-P is not freely exchangeable with endogenous pyridoxal-P, but is preferentially released or degraded to pyridoxal and pyridoxic acid. The latter B₆ compounds are also released. These results suggest that orally ingested pyridoxine is rapidly

metabolized in liver and its products are released into the circulation in the form of pyridoxal-P, pyridoxal, and pyridoxic acid.

INTRODUCTION

Vitamin B₆ is metabolized in vivo to multiple vitameric forms and is ultimately excreted as pyridoxic acid in the urine. Pyridoxal-P and pyridoxamine-P, the coenzyme forms, are the major B₆ compounds in tissues (1), but pyridoxal-P (2) and pyridoxal (3) appear to be the major forms in blood plasma. The quantitative relationship between these B₆ vitamers and other B₆ compounds in plasma has not been defined. Such knowledge is important to the understanding of the process of transport of vitamin B₆ among the different organs and tissues of the body.

Recently, we have modified an existing method (4) for the separation and analysis of the B₆ compounds. By using this method, we have measured the plasma content of B₆ vitamers and pyridoxic acid in man before and during pyridoxine supplementation. Because the liver is the most active organ in the metabolism of pyridoxine (5–8) and the sole source of pyridoxal-P in blood plasma (9), we have also studied the metabolic conversion and release of B₆ compounds in isolated rat hepatocytes. The data indicate a close relationship between hepatic metabolism and the plasma level of the various B₆ compounds.

METHODS

Studies on humans. Six healthy human subjects were studied, 5 males and 1 female, 23–38 yr of age. Venous blood samples were collected when they were on a regular diet before and during oral supplementation of pyridoxine-HCl, 100 mg/d. Blood samples were taken in all subjects after 1 wk of pyridoxine supplementation, and in three subjects, samples were collected at weekly intervals for 3 wk. The samples were drawn after an overnight fast and immediately before the next daily dose of pyridoxine was administered. Blood samples were collected with 2 mM EDTA, pH 7.4, as anti-coagulant, and plasma was separated by centrifugation within 30 min.

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Studies on isolated rat hepatocytes. Male Sprague-Dawley rats weighing 200–300 g were purchased from Harlan Industries, Inc., Indianapolis, Ind. They were maintained for at least 1 wk on a standard laboratory chow (Wayne Lab-Blox, Allied Mills, Inc., Chicago, Ill.) before use. This diet contains 8.13 ppm pyridoxine-HCl, 1.13 ppm in excess of the amount recommended by the National Research Council, National Academy of Sciences (10). Isolated rat liver cells were prepared as described previously (11). The cell preparations consistently contained >95% parenchymal cells. Immediately following isolation, $95 \pm 1\%$ (\pm SE, $n = 8$) of the cells excluded trypan blue, and $84 \pm 2\%$ of the cells continued to exclude the dye following 2 h of incubation. Cells prepared in this manner exhibited high rates of gluconeogenesis (12), maintained intracellular K^+ concentrations at 130–150 meq/liter of cell water (13), and were active in protein synthesis (14) and amino acid transport (11).

Freshly isolated hepatocytes, 1 g wet wt, were incubated at 37°C in 15 ml Krebs-Henseleit medium (pH 7.4) containing 10 mM glucose and 2.5 g/100 ml fatty acid-free bovine serum albumin. Where indicated, [^{14}C]pyridoxine (6 μ Ci/ μ mol) was also added to the medium to bring its concentration to 3.33 μ M. All incubations were performed in Erlenmeyer flasks in a shaking water bath and in the presence of 95% O_2 and 5% CO_2 . At the end of 2 h incubation, the cell suspensions were centrifuged to separate the cells from the medium as described previously (11).

Analyses. B_6 compounds were extracted from samples of plasma and incubation medium by treating 14 ml of the sample with 1.56 ml of 75% trichloroacetic acid. Trichloroacetic acid was added dropwise at 4°C with constant stirring. The treated samples were then heated to 37°C for 10 min and centrifuged for 30 min at 32,800 g and 4°C. The clear supernatant fractions were collected and divided into 2-ml aliquots. The trichloroacetic acid was extracted by water-saturated and peroxide-free diethyl ether until the pH was at least 4.5. The samples were frozen at $-20^\circ C$ overnight and used for assay the next day.

B_6 compounds in cell pellets were extracted by adding 6 ml of 7.5% trichloroacetic acid and homogenizing with a Polytron PT20 homogenizer (Polytron Corp., Elkhart, Ind.) at a power setting of 6 for 1 min. The volume of the suspension of precipitated protein was brought up to 10 ml by adding 7.5% trichloroacetic acid. The suspension was then heated to 50°C for 20 min and centrifuged. The resultant pellets of precipitated protein were re-extracted by adding 8 ml of 7.5% trichloroacetic acid and centrifuging. The clear supernatant fractions from the two extraction steps were combined and then divided into 2-ml aliquots for ether extraction. The use of 7.5% trichloroacetic acid did not chemically alter added B_6 vitamins in the extraction procedure. The recovery of pyridoxal-P, the B_6 compound most tightly bound to proteins, was $104 \pm 6\%$ (mean \pm SD, $n = 8$).

Fractionation of the B_6 compounds was performed by a modification of the method originally described by Loo and Cort (4). The strongly acidic cation exchange resin (Bio-Rad AG 50 W-X8, 200–400 mesh; Bio-Rad Laboratories, Richmond, Calif.) was washed three times in 1 N HCl to remove fluorescent contaminants. Excess acid was then removed by washing with deionized, distilled water. After washing, the resin was washed again with 1 N NaOH and rinsed with water until the pH was 7.4. The washed resin, free of fluorescent contaminants, was next rinsed with 100 mM formic acid, then 10 mM formic acid, until the pH was 3.2 or lower. The resin was finally rinsed with 10 mM ammonium formate buffer (pH 3.2) to bring the pH and conductivity of the effluent to those of the formate buffer. The resin was placed in chromatographic columns 0.7 \times 30 cm (Bio-Rad Econo-Columns, No. 737-1252; Bio-Rad Laboratories) and packed to a bed height of 10 cm.

The columns were connected by plastic tubings to a Technicon Auto-Analyzer proportioning pump (Technicon Instruments Corp., Tarrytown, N. Y.) to maintain the flow rate at 1 ml/min.

Pyridoxal-P in 10 mM ammonium formate (pH 3.2) did not adsorb to the cationic resin and it could be eluted with 20 ml of this buffer. By contrast, all the other B_6 compounds adsorbed to the resin in 10 mM ammonium formate (pH 3.2) and could be displaced sequentially by increasing the pH and ionic strength (Fig. 1): pyridoxic acid with 60 ml of 100 mM ammonium formate (pH 3.2); pyridoxamine-P with 75 ml of 95 mM sodium acetate (pH 4.2) and 30 ml of 100 mM sodium acetate (pH 5.2); pyridoxal with 60 ml of 120 mM sodium acetate (pH 5.6); pyridoxine with 60 ml of 100 mM sodium phosphate (pH 6.5); and pyridoxamine with 60 ml of 120 mM sodium phosphate (pH 7.5).

All of the B_6 vitamins were assayed by fluorometric measurements essentially as described by Contractor and Shane (15). Pyridoxic acid was measured by the method of Reddy et al. (16). The recoveries of pyridoxal-P, pyridoxic acid, pyridoxamine-P, pyridoxal, pyridoxine, and pyridoxamine were 98 ± 3 , 102 ± 7 , 86 ± 5 , 109 ± 7 , 111 ± 7 , and $107 \pm 12\%$ ($n = 9$), respectively. These recovery data were obtained by adding authentic vitamins in amounts equivalent to those present in the samples.

The chromatographic procedure described above did not separate pyridoxine-P from pyridoxal-P. By fluorometric assay using the cyanide reaction (15), however, only pyridoxal-P was measured, and pyridoxine-P did not interfere. Indeed, results based on fluorometric assay of tissue and plasma pyridoxal-P after chromatographic separation agreed well with those assayed by tyrosine apodecarboxylase. It is known that pyridoxine-P occurs only in trace amounts in tissues (17).

Deproteinized samples of plasma and medium, 7 ml, were diluted with 0.77 ml 100 mM ammonium formate (pH 3.2) and 17.23 ml 10 mM ammonium formate (pH 3.2) to bring the final pH to 3.2 and the conductivity to about 3 mmho. 20 ml deproteinized cell extracts were diluted with 2.25 ml 100 mM ammonium formate (pH 3.2). These samples were then used for chromatographic separation of the B_6 compounds.

For each run in which the unknown samples were separated, a sample blank and a standard solution containing 100–1,000 ng of each B_6 compound were also analyzed. Aliquots

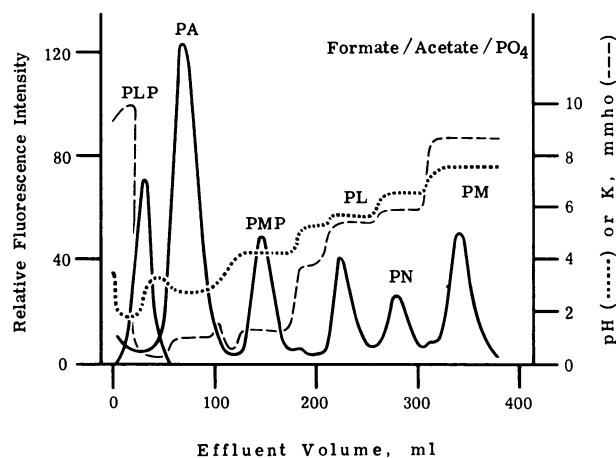


FIGURE 1 Chromatographic separation of the B_6 compounds by a strongly acidic cationic exchange resin, AG 50 W-X8. PA, pyridoxic acid; PL, pyridoxal; PLP, pyridoxal-P; PM, pyridoxamine; PMP, pyridoxamine-P; PN, pyridoxine.

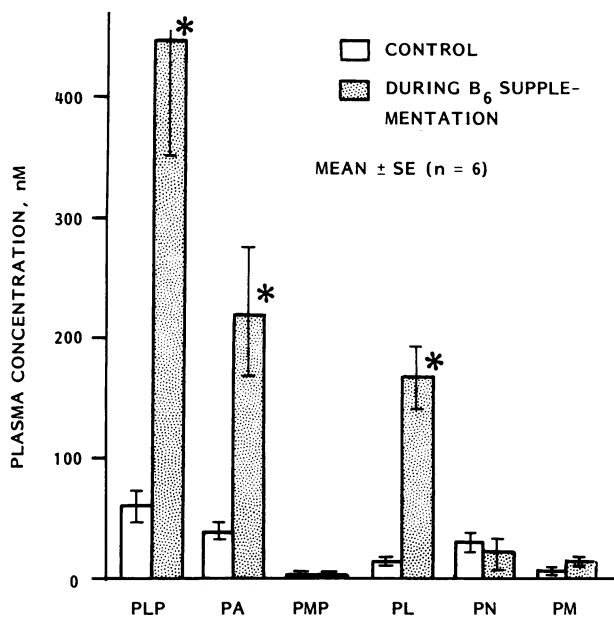


FIGURE 2 Plasma content of B₆ compounds before and during pyridoxine supplementation (100 mg pyridoxine-HCl/d). Abbreviations are the same as in Fig. 1. *, $P < 0.01$.

of the effluents from these columns were used for fluorometric and/or isotopic measurements.

All steps and specimens were protected from ordinary room light by the use of darkened rooms and yellow fluorescent lights to avoid photolysis of the B₆ compounds.

Materials. All of the B₆ compounds and other reagents were purchased from Sigma Chemical Co., St. Louis, Mo. [4,5-¹⁴C]pyridoxine-HCl was a gift of Dr. Myron Brin (Hoffmann-LaRoche, Inc., Nutley, N. J.). The purity of [¹⁴C]pyridoxine-HCl was verified by chromatography.

Statistical analysis. Student's *t* test was used to determine the significance of the difference between mean values. The results are reported as mean ± SE.

RESULTS

In individuals consuming a regular diet, pyridoxal-P and pyridoxic acid were the major B₆ compounds found in blood plasma (Fig. 2). Pyridoxal-P constituted >53% of the active vitamers. After 1 wk of pyridoxine supplementation, the plasma concentration of pyridoxal-P and pyridoxic acid increased 6–7-fold, whereas that of pyridoxal increased ~12-fold. In three subjects, the plasma levels of the B₆ compounds were determined weekly during supplementation for 3 wk. They did not fluctuate significantly after the 1st wk, indicating that they had attained new steady-state levels within this period. Since pyridoxal-P and pyridoxal are biologically active, and are known to turn over rapidly in vivo (18, 19), the results indicate that these compounds are the major transport forms in the circulation.

To define the relationship of the plasma content of B₆ compounds to hepatic vitamin B₆ metabolism, studies were performed with intact isolated rat hepatocytes. Hepatocytes were incubated in the presence of [¹⁴C]-

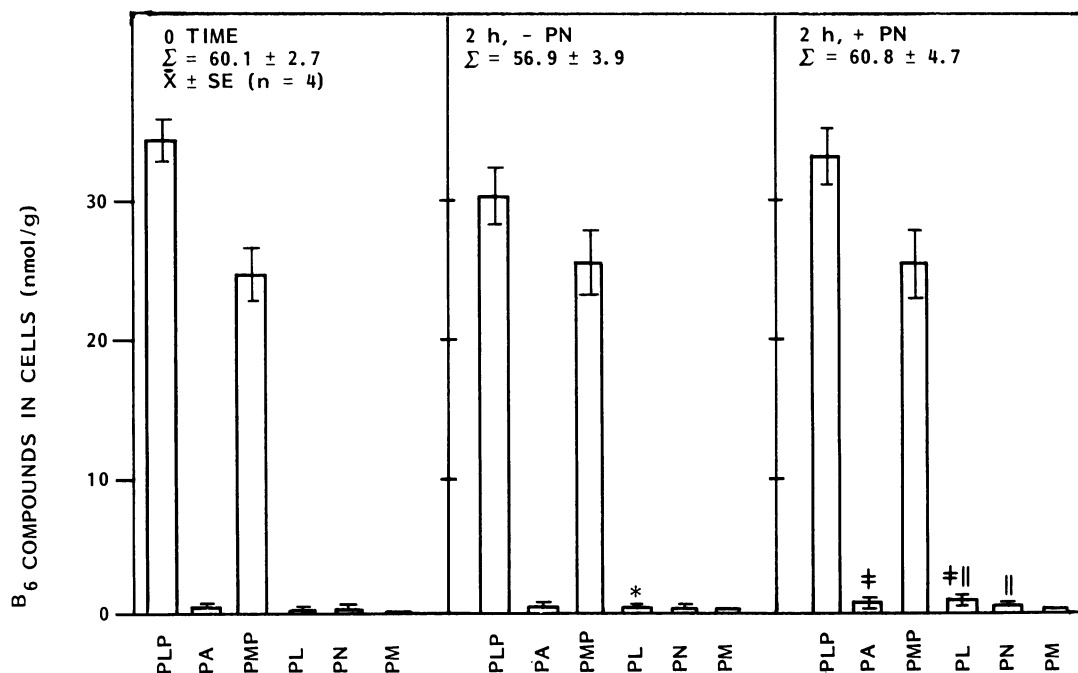


FIGURE 3 Cellular level of B₆ compounds in isolated hepatocytes before and after incubation with (+PN) and without (-PN) pyridoxine. *, $P < 0.05$, 0 time vs. 2 h -PN; ‡, $P < 0.05$, 0 time vs. 2 h +PN; †, $P < 0.02$, 2 h -PN vs. 2 h +PN. Abbreviations are the same as in Fig. 1.

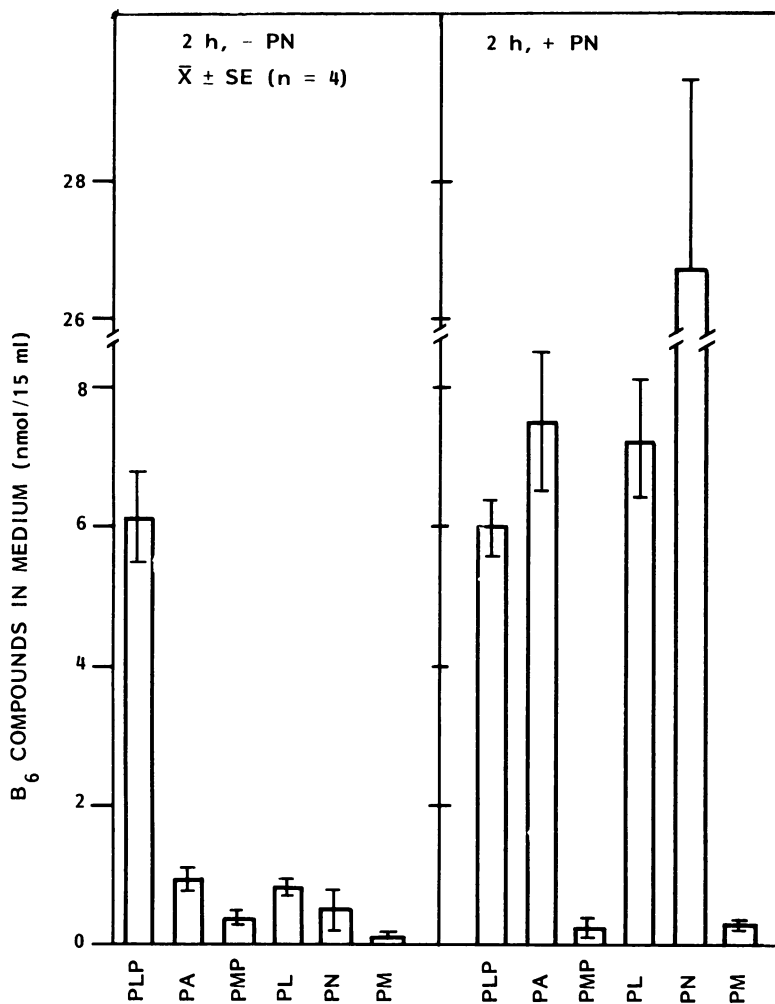


FIGURE 4 Level of B₆ compounds released into the medium by isolated hepatocytes after incubation in the absence (-PN) and presence of (+PN) of pyridoxine. Abbreviations are the same as in Fig. 1.

pyridoxine, and the hepatic metabolism and release of different B₆ compounds into the incubation medium were examined by both fluorometric and isotopic measurements.

Fig. 3 shows the cellular level of B₆ compounds in isolated hepatocytes and the effect of incubation in the absence and presence of [¹⁴C]pyridoxine. At zero time, the hepatocytes contained ~35 nmol of pyridoxal-P and 25 nmol of pyridoxamine-P per gram wet weight. The contents of these coenzymes are similar to those found in the freeze-clamped liver of B₆-sufficient rats *in situ* (20). The content of the other B₆ compounds was extremely low. After 2 h incubation in the absence of radiolabeled pyridoxine, the intracellular levels of the B₆ compounds did not change except for that of pyridoxal, which increased ($P < 0.05$) from 0.14 to 0.29 nmol/g. The level of pyridoxal-P tended to decrease, but the change was not statistically significant. Hepato-

cytes incubated in the presence of radiolabeled pyridoxine also did not exhibit significant change in the cellular levels of pyridoxal-P and pyridoxamine-P. However, the cellular content of pyridoxic acid increased from 0.36 (at zero time) to 0.73 nmol/g, and that of pyridoxal, from 0.14 to 0.95 nmol/g.

Fig. 4 depicts the levels of the B₆ compounds released into the medium by isolated hepatocytes after incubation in the absence and presence of [¹⁴C]pyridoxine. In the absence of added radiolabeled pyridoxine, pyridoxal-P was the major B₆ compound released. However, after 2 h incubation in the presence of added radiolabeled pyridoxine, large amounts of pyridoxal-P, pyridoxic acid, and pyridoxal were found in the medium. Of the 50 nmol of labeled pyridoxine added to the incubation mixture, ~21 nmol was converted to pyridoxal-P, pyridoxic acid, and pyridoxal in the medium and ~26 nmol remained unmetabolized. Recovery

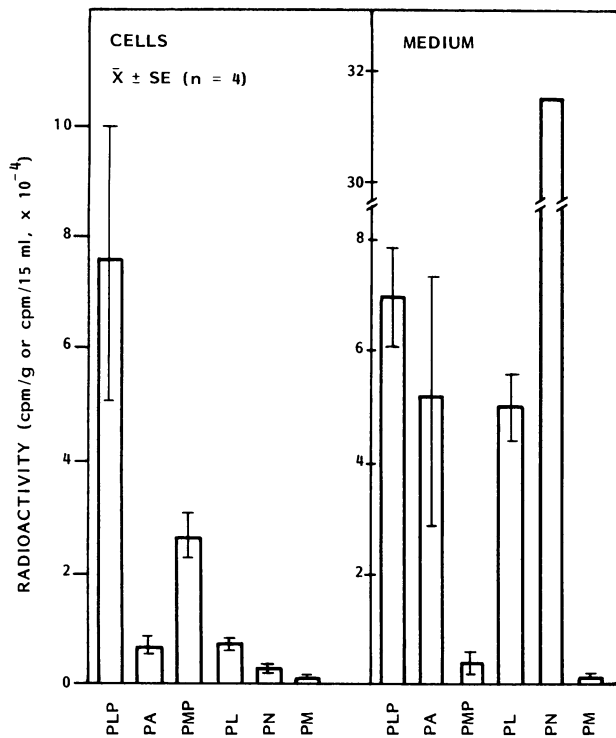


FIGURE 5 Metabolic conversion of [¹⁴C]pyridoxine by isolated hepatocytes. Abbreviations are the same as in Fig. 1.

was 94%. These findings *in vitro* with isolated hepatocytes mimic the situation seen in the human subjects during pyridoxine supplementation, i.e., pyridoxal-P, pyridoxic acid, and pyridoxal exhibit the largest increments in plasma (Fig. 2).

Fig. 5 summarizes the metabolic conversion of [¹⁴C]pyridoxine by isolated liver cells based on isotopic measurement. The recovery of radioactivity counts in these experiments was 102% of that of [¹⁴C]pyridoxine added. About 20% of the recovered counts were intracellular, and the remainder was in the medium (51% as unmetabolized [¹⁴C]pyridoxine and 29% as radiolabeled B₆ compounds other than [¹⁴C]pyridoxine). Labeled pyridoxal-P and pyridoxamine-P accounted for 85% of the total counts in the cell pellet. By contrast, ¹⁴C-labeled pyridoxal-P, pyridoxic acid, and pyridoxal accounted for >96% of the products of [¹⁴C]pyridoxine that appeared in the medium. The data thus agree well with those shown in Figs. 3 and 4, which assayed the total contents of the B₆ vitamers by means of fluorometric analysis.

Fig. 6 shows the metabolic conversions of [¹⁴C]pyridoxine by isolated hepatocytes in the cell pellet and the medium, expressed as specific radioactivities. The specific radioactivity of pyridoxine in the cell and medium was ~12,000 cpm/nmol, similar to that of [¹⁴C]pyridoxine added to the incubations. The specific ra-

dioactivities of pyridoxal-P and pyridoxamine-P in the cell pellet were <20% of that of pyridoxine, however. This enormous dilution of radioactive counts indicates that the intracellular endogenous pools of pyridoxal-P and pyridoxamine-P are not readily miscible with the newly synthesized compounds. By contrast, the specific radioactivity of pyridoxal-P in the medium approached that of pyridoxine, suggesting that there is a small but rapidly mobilizable pool of newly synthesized pyridoxal-P in the cell associated with secretion. In the incubation medium, the specific radioactivities of pyridoxic acid and pyridoxal were higher than those of intracellular pyridoxal-P and pyridoxamine-P, suggesting that the newly synthesized pyridoxal-P also tends to be degraded rapidly to pyridoxal and pyridoxic acid and released into the circulation.

Because pyridoxine-P could not be separated from pyridoxal-P by chromatography, and because the fluorometric assay was specific for the latter, its presence can potentially elevate falsely the calculated specific radioactivities of pyridoxal-P shown in Fig. 6. The values for pyridoxal-P in the medium were found to be no higher than that of pyridoxine, however, indicating that little or no radiolabeled pyridoxine-P was in the medium. Within the cell, the specific radioactivity of pyridoxal-P was somewhat higher than that of pyridoxamine-P. Conceivably, there could be a small amount of radiolabeled pyridoxine-P present within the hepatocytes under the conditions of the experiment.

DISCUSSION

The quantitative determination of the plasma content of vitamin B₆ has been problematic because of the existence of multiple forms of this vitamin *in vivo*. Although plasma pyridoxal-P can be assayed enzymatically (2) and both plasma pyridoxal and pyridoxal-P fluorometrically after conversion to their methyl anthranilate derivatives (3), these methods are not directly suitable for the measurement of other B₆ compounds in plasma. As reported in this communication, we have accomplished successfully the analysis in a single plasma sample of six of the seven B₆ compounds known to be present *in vivo*. The method of Loo and Cort (4) was employed to separate the B₆ compounds, but major modifications were introduced in the elution buffers to improve chromatographic separation. Additionally, the specificity of the fluorometric assays of the B₆ compounds was enhanced by prior chemical conversion (15).

In human subjects on regular diets, we found that pyridoxal-P accounted for >50% of the active B₆ vitamers in plasma (Fig. 2). Upon supplementation with oral pyridoxine, the levels of both pyridoxal-P and pyridoxal increased 6–12-fold, but pyridoxal-P remained the preponderant B₆ vitamer. Importantly,

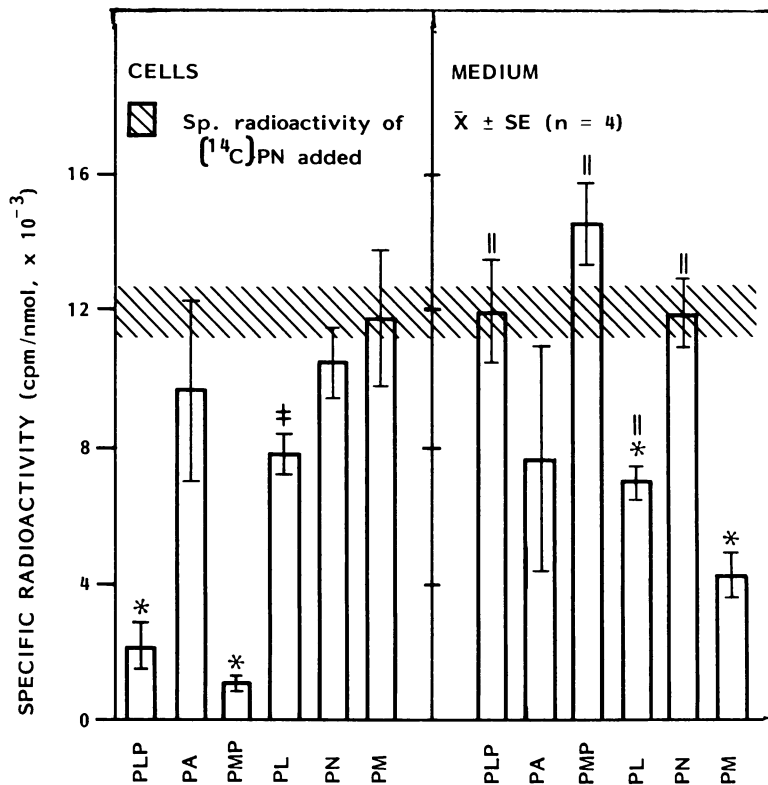


FIGURE 6 Metabolic conversion of [¹⁴C]pyridoxine by isolated hepatocytes, expressed as specific (Sp.) radioactivity. *, $P < 0.001$ and †, $P < 0.005$, compared with the specific radioactivity of pyridoxine. ‡, $P < 0.005$, compared with the specific radioactivities of cellular PLP and PMP. Abbreviations are the same as in Fig. 1.

plasma pyridoxamine-P occurred only in trace amounts (<3 nM) both before and during pyridoxine supplementation. Therefore, the content of B₆ compounds in blood plasma differs significantly from that of whole blood which is known to contain about equimolar amounts (~60–70 nM) of pyridoxal-P and pyridoxamine-P (15). This major difference is consistent with the general observation that pyridoxamine-P is mainly an intracellular B₆ vitamers (1, 20). Accordingly, the high level of pyridoxamine-P in whole blood must be due to the occurrence of this coenzyme in erythrocytes and other cellular elements. The above findings also confirm the earlier notion (21) that pyridoxal-P is the major B₆ vitamers in blood plasma, but implicate both pyridoxal-P and pyridoxal as the transport forms of vitamin B₆. The propensity of these B₆ vitamers to circulate in plasma is most likely due to protein binding: pyridoxal-P is known to bind very avidly to albumin, and pyridoxal, less so (9, 22). On the other hand, both pyridoxal-P and pyridoxal are also cleared rapidly from plasma in vivo (18, 19). Since pyridoxal-P is not excreted at all and pyridoxal only minimally in the urine (15), physiological mechanisms must exist for their transfer into tissues. Because albumin-bound pyri-

doxal-P can be dephosphorylated by phosphatases (9) and pyridoxal can readily permeate biological membranes (20), the findings suggest that pyridoxal is the transport form that ultimately enters various tissues in the body.

Since pyridoxal-P, pyridoxal, and pyridoxic acid exhibited the largest increments in plasma in vivo during supplementation with oral pyridoxine (Fig. 2), it is pertinent to consider the organ sources of these plasma B₆ compounds. Previous studies on the effect of organ ablation in dogs (9) have shown that the liver is the sole organ responsible for the formation of plasma pyridoxal-P from either pyridoxine or pyridoxal. On the other hand, pyridoxal in plasma may derive from multiple organ sources. Anderson et al. (18) reported that human erythrocytes can convert pyridoxine into pyridoxal and release the latter compound into the medium. The results reported here with isolated hepatocytes indicate that the liver can also release pyridoxal into the circulation, as well as pyridoxal-P and pyridoxic acid (Figs. 4 and 5). Although the relative and quantitative importance of erythrocytes and liver in converting ingested pyridoxine to plasma pyridoxal is uncertain at this time, it is known that the liver is the most

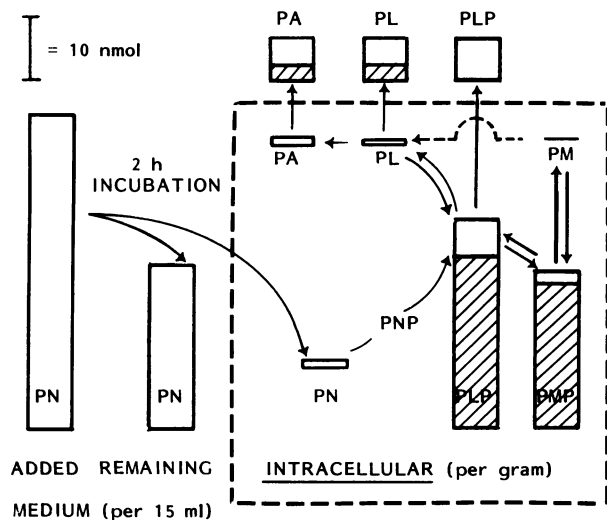


FIGURE 7 Schematic representation of the metabolic conversion of pyridoxine by isolated hepatocytes. The scheme depicts the amount of pyridoxine added at zero time and the content of pyridoxine and other B₆ compounds in the cells and the medium after 2 h incubation. The height of each bar represents the content of each B₆ compound calibrated according to the scale shown. The clear areas represent [¹⁴C]-pyridoxine and its radiolabeled metabolic products, with a specific radioactivity of 12,053±635 cpm/nmol. The shaded areas represent the endogenous nonradiolabeled pyridoxal-P and pyridoxamine-P in isolated cells and their metabolic products released into the medium.

active organ in the metabolism of pyridoxine in vivo (5–8). The results reported here with isolated hepatocytes also indicate that the liver cannot export pyridoxamine-P into the circulation (Fig. 4). This finding in vitro simulates the situation in vivo, wherein the plasma level of pyridoxamine-P failed to rise upon supplementation with oral pyridoxine.

Colombini and McCoy (6) have reported previously that, within 1 min after the intravenous injection of [¹⁴C]pyridoxine in a mouse, a large amount of radiolabeled pyridoxal appeared in the carcass. Similarly, Contractor and Shane (7) observed that the amount of radiolabeled pyridoxal exceeded the level of [¹⁴C]-pyridoxine in the brain and the fetus of pregnant rats shortly after the injection of [¹⁴C]pyridoxine. These observations suggested that there is either a high rate of direct conversion of pyridoxine to pyridoxal in these tissues or there is a rapid uptake of pyridoxal from the circulation into these tissues. If indeed the liver functions in vivo in a manner to convert rapidly ingested pyridoxine to plasma pyridoxal, this process favors the latter explanation for the aforementioned observations.

The pathways for the metabolic interconversion of the B₆ vitamers have been summarized by Snell and Haskell ([23], cf. Fig. 7). The studies with isolated

hepatocytes reported here provide further insights on the relationship of intracellular pool sizes and the metabolic flux of B₆ vitamers in the liver of B₆-sufficient animals. The major findings, summarized in Fig. 7, permit the following conclusions: (a) Pyridoxal-P and pyridoxamine-P are the major endogenous B₆ compounds in liver cells, and their intracellular content is tightly regulated so that excess amounts of these coenzymes do not accumulate in the presence of non-phosphorylated B₆ precursors (24). (b) Newly synthesized pyridoxal-P and pyridoxamine-P do not exchange freely with endogenous intracellular pools of the coenzymes and, to a large extent, constitute a rapidly mobilizable pool destined for degradation and/or secretion into the circulation. (c) Pyridoxal-P, pyridoxal, and pyridoxic acid, but not pyridoxamine-P, are the principal B₆ compounds released by the liver.

Although it has been suggested that pyridoxal kinase (25) and pyridoxine-P oxidase (26) may serve as control points in the regulation of pyridoxal-P metabolism, studies reported from our laboratory have emphasized the role of protein binding and the hydrolysis of unbound or loosely bound pyridoxal-P in governing the cellular level of this coenzyme (20). The results reported here suggest that hepatic pyridoxamine-P, similar to pyridoxal-P, may also be regulated in this manner. Accordingly, newly synthesized pyridoxal-P and pyridoxamine-P, being unbound or loosely bound to B₆ enzymes, represent a rapidly mobilizable B₆ pool in liver. Pyridoxal-P, synthesized in excess, is either released into the medium or degraded to pyridoxal and pyridoxic acid. Whereas a transport mechanism does exist in liver for the secretion of pyridoxal-P, a similar mechanism is apparently absent for pyridoxamine-P. Thus, excess pyridoxamine-P is either transformed to pyridoxal-P or degraded to pyridoxamine and further oxidized to pyridoxal and pyridoxic acid. In addition to this functional compartmentalization, presumably due to coenzyme-protein interaction, structural compartmentation may also exist (27, 28).

ACKNOWLEDGMENTS

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