Vitamin B₆ Metabolism in McA-RH7777 Cells

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ABSTRACT

The metabolism of vitamin B₆ in McA-RH7777 cells has been characterized with respect to pyridoxal 5'-phosphate (PLP) levels, and the activities of pyridoxine (PN) kinase (EC 2.1.7.35) and pyridoxamine 5'-phosphate (PNP) oxidase (EC 1.4.3.5). PLP levels (12.4 ± 4.4 ng/mg protein) were at the lower end of the range found for Morris hepatomas, carcinogen-induced rat hepatomas, and liver from rats fed a PN-deficient diet. PN kinase activity was about one-third of that found in normal rat liver. PNP oxidase appeared to be absent in high-speed supernatants of homogenates prepared from McA-RH7777 cells. The absence of PNP oxidase was supported by enzymatic and immunological data. These findings resemble those found previously for Morris hepatoma 7777. In contrast to rat liver, such preparations caused little or no release of volatile counts upon incubation with either [3H]-C4'IPN or [3H]-C4'IPNP. High-speed supernatants of homogenates prepared from both McA-RH7777 cells and Morris hepatoma 7777 were very much less capable than similar preparations from rat liver in converting [G-3H]PNP to PLP and pyridoxamine 5'-phosphate. Despite the apparent absence of PNP oxidase, intact confluent or log-phase McA-RH7777 cells were capable of converting [G-3H]PNP to PLP and pyridoxamine 5'-phosphate. These findings are discussed in terms of tumor nutrition and vitamin B₆ metabolism in a rat hepatoma cell line.

INTRODUCTION

Vitamin B₆ metabolism has been examined in a variety of species and tissues (1). In mammals, the liver is the organ of particular interest owing to its central role in the conversion of the various B₆ vitamins to coenzymatically active PLP and its export of PLP to extracellular tissues as a PLP-albumin complex (2). Studies on vitamin B₆ uptake and metabolism in isolated rat hepatocytes have provided information on these processes (3, 4). The uptake and metabolism of vitamin B₆ by subcellular organelles, i.e., rat liver mitochondria, also have been studied (5, 6).

There have been relatively few studies on vitamin B₆ metabolism in mammalian cells capable of dividing in vitro. Eagle first established in 1956 that vitamin B₆ was necessary for the survival and growth under tissue culture conditions of HeLa human carcinoma cells and a mouse fibroblast cell line designated L (7). Subsequently, the uptake of PL and PLP (8) and the enzymes involved in vitamin B₆ metabolism were studied in cells grown as ascites tumors (9, 10). The effects of vitamin B₆ deprivation on the activities of PLP-dependent enzymes in cultured human fibroblasts have been examined (11). In 1982, DiSorbo and Litwack reported that PN at millimolar concentrations was toxic to a rat hepatoma cell line grown in vitro (12). A cell line selected for its resistance to the toxic effects of PN was examined with respect to its uptake of PN and its PLP content (13).

Previous reports from our laboratory have dealt with vitamin B₆ metabolism in normal rat liver (14), regenerating rat liver (15), transplanted Morris rat hepatomas (16-18), and carcinogen-induced rat hepatomas (19). The present study is concerned with vitamin B₆ metabolism in cells grown in tissue culture, specifically, the McA-RH7777 rat hepatoma cell line derived from the corresponding transplanted Morris hepatoma (20).

MATERIALS AND METHODS

Materials. B₆ vitamins and PA were purchased from Sigma Chemical Co. (St. Louis, MO). PLP concentrations of stock solutions were determined spectrophotometrically after suitable dilution into 0.1 N KOH using an extinction coefficient of 6600 at 388 nm (21). [3H]PN hydrochloride (1.4 Ci/mmol), [3H]tryptamine hydrochloride (3.5 Ci/mmol), and PCS liquid scintillation counting fluid were from the Amersham Corp. (Arlington Heights, IL). N-[5'-phosphoryridoxyl]-[3H]tryptamine was synthesized by the method of Langham et al. (22). [3H]-C4'IPN and [3H]-C4'IPNP were from a previous study (14). L-[1-14C]Tyrosine (53 mCi/mmol) was obtained from New England Nuclear (Boston, MA). Ion exchange resins (AG 1-X8, 200—400 mesh, and AG 50W-X8, 200—400 mesh) were purchased from Bio-Rad (Rockville Centre, NY). Tyrosine decarboxylase apoenzyme used in the quantification of PLP was partially purified from dried Streptococcus faecalis cells (Sigma) by the procedure of Lumeng and Li (23). The sources of antibodies, immunochemical reagents, and supplies used in an ELISA for rat PNP oxidase (17) have been published previously (19) McA-RH7777 cells (ATCC No. CRL 1601) were obtained from the American Type Culture Collection (Rockville, MD). Components used in the medium for the growth of McA-RH7777 cells in tissue culture were purchased from Gibco Laboratories (Grand Island, NY). Falcon plasticware used in tissue culture work was from Becton Dickinson Labware (Oxnard, CA). House-distilled water was further purified with a Milli-Q water purification system (Milliapore Corp., Bedford, MA). All other chemicals and reagents used in these experiments were of the highest quality commercial grade available.

Cells. McA-RH7777 cells were grown in antibiotic-supplemented (alternating penicillin/streptomycin and gentamicin) Ham's F-12 medium with glutamine, made 14 mM in sodium bicarbonate, plus 10% fetal bovine serum in a humidified 95% air/5% CO₂ atmosphere (37°C); the doubling time was approximately 24 h.

Cell Extracts. Extracts of McA-RH7777 cells for PLP analyses were prepared as follows. Confluent cells were washed with PBS, scraped from the flasks, combined, and washed twice by suspension and centrifugation (10 min, 1000 g) from PBS. The cell pellet obtained from one T75 flask was suspended in 1.0 ml of 80 mM sodium phosphate buffer (pH 7.4). The suspension was homogenized with ice/water cooling (45 sec followed by 15 sec) using a Polytron PT-10 homogenizer (Brinkmann Instruments, Westbury, NY) at a setting of 10. Trichloroacetic acid (75% w/v) was added to the cell homogenate to a final concentration of 7.5%. PLP was then quantified as described elsewhere (14, 16, 18).

Extracts of McA-RH7777 cells used for assays involving PNP oxidase were prepared as follows. Confluent or log-phase cells were scraped from T75 flasks, collected, and washed with PBS as described above. Cell pellets were suspended in 0.25 M sucrose and homogenized as described above. The numbers of cells per ml of 0.25 M sucrose varied, depending on the particular experimental requirements. Homogenates...
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were centrifuged for 1 h at 100,000 g. Supernatants obtained in this fashion were used in analyses for PNP oxidase activity, immunochemically reactive PNP oxidase protein, PN kinase activity, and PLP-derived tumor. PNP oxidase activity was determined by a sensitive radiochemical method developed by Langham et al. (22). The ELISA method for the detection of rat PNP oxidase using an antibody fraction from immune rabbit serum has been described previously (17, 19). PN kinase activity was determined by a modification (14) of the procedure of Karawya and Fonda (24). High-speed supernatants of extracts from McA-RH7777 cells also were used in experiments, described below, on the conversion of [G-3H]PNP to other B6 vitamer forms. Protein was determined by the method of Lowry et al. (25) using bovine serum albumin as the reference standard.

Metabolism of [G-3H]PN. The metabolism of [G-3H]PN was studied using high-speed supernatants (100,000 g; 1 h; 4°C) of homogenates from McA-RH7777 cells grown in culture, Morris hepatoma 7777 grown in the hindlegs of Buffalo rats, liver of Morris 7777 hepatoma-bearing Buffalo rats, and homogenates of liver obtained from 75- to 100-g male Sprague-Dawley Crl:CD(SD)BR rats (Canadian Breeding Laboratories, St. Constant, Quebec, Canada). Morris 7777 hepatoma-bearing Buffalo rats were provided by Dr. Jen-Fu Chiu to whom the authors are indebted. Methods for the preparation of hepatoma extracts have been published previously (16). Rats were housed in the Animal Care Facility of the University of Vermont College of Medicine where they were allowed free access to water and a nonpurified Purina Laboratory Chow 5001 (Ralston Purina Co., St. Louis, MO). Animals were killed by a crushing blow to the skull. Livers were excised, minced, and homogenized, as described above, in 9 volumes of 0.25 M sucrose. Incubation mixtures (2.0 ml) contained 0.125 M sucrose, 0.08 μM zinc sulfate, 1 mM ATP, 3 μM FMN, and 3.33 μM [G-3H]PN (61.4 μCi/μmol) in 80 mM potassium phosphate buffer (pH 7.2; 37°C). At the desired times, 0.1 ml of 100% trichloroacetic acid (w/v) was added to stop the reactions. The acidified incubation mixtures were processed for the identification and quantification of [G-3H]PN-derived radioactive B6 vitamers. These analyses were done, with minor modifications, by the open-column chromatography method described by Lumeng and Li (3). Authentic B6 vitamers and PA (1 mg of each) were used as markers in every experiment. They were added to the trichloroacetic acid-treated incubation mixtures. The elution of the various B6 vitamers and PA were followed by measuring the absorption at 290 nm of acidified eluent fractions.

In experiments dealing with the metabolism of [G-3H]PN by intact McA-RH7777 cells, the growth medium contained 0.3 μM [G-3H]PN (247 μCi/μmol) for confluent cultures; 491 μCi/μmol for log-phase growth cells. Confluent cells were incubated with the radioactive vitamin for 2 h at 37°C; cells in log-phase growth were incubated with the radioactive vitamin for 24 h. Cells were collected and washed essentially as described above. Cell pellets obtained by centrifugation were homogenized with 7.5% trichloroacetic acid and processed for the analysis of [G-3H]PN-derived B6 vitamers by the open-column chromatography method. Controls comprised of radioactive vitamin in medium alone were carried through the same procedures.

Determination of Volatile Counts Released from [H-4′]PN and [H-4′]PNP. Volatile counts were determined by vacuum distillation of incubation mixtures and collection of the distillate in a cold finger trap. In experiments with [H-4′]PNP, the substrate was 3.33 μM (205 μCi/μmol) in a reaction mixture (2.0 ml) containing liver or McA-RH7777 extracts, 3 μM FMN, 0.18 M Tris, and 80 mM potassium phosphate (pH 7.2). The reactions were stopped by the addition of 0.1 ml of 100% trichloroacetic acid (w/v) was added to stop the reactions. The acidified incubation mixtures were processed for the identification and quantification of [G-3H]PN-derived radioactive B6 vitamers. These analyses were done, with minor modifications, by the open-column chromatography method described by Lumeng and Li (3). Authentic B6 vitamers and PA (1 mg of each) were used as markers in every experiment. They were added to the trichloroacetic acid-treated incubation mixtures. The elution of the various B6 vitamers and PA were followed by measuring the absorption at 290 nm of acidified eluent fractions.

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RESULTS

PLP Content. The PLP content of confluent McA-RH7777 cells was determined in this study to be 12.4 ± 4.4 ng/mg protein (n = 9). Using data obtained in previous studies (14, 16, 18, 19), we calculate that the PLP content of Morris hepatoma 7777 grown in the hindlegs of Buffalo rats is in the range of 14 to 19 ng/mg protein. Similarly, the PLP content of carcinogen-induced rat hepatomas is in the range of 14 to 22 ng/mg protein. These values compare with 37.0 ± 2.5 and 19.8 ± 2.7 ng PLP/mg protein for liver tissue obtained from rats fed PN-sufficient and -deficient diets, respectively.

Activities of Enzymes Involved in the Metabolism of Vitamin B6. The conversion of PN to coenzymatically active PLP conventionally requires the sequential actions of PN kinase (EC 2.7.1.35) and PNP oxidase (EC 1.4.3.5) (1). We have measured the activities of these enzymes in McA-RH7777 cells to obtain information on the capability of these cells to use these two enzymes to form PLP from PN, and to compare the data with corresponding data obtained previously for liver and liver-derived tumors (14–19). Both PN kinase and PNP oxidase activities were determined by radiochemical methods.

In the case of PN kinase, the activity found in supernatants of homogenates obtained from McA-RH7777 cells was 8.1 ± 1.1 nmol/h per mg protein (n = 7); this is approximately 36% of the activity found in supernatants of homogenates prepared from normal rat liver. In comparison, Morris hepatoma 7777 grown in vivo has 28% of the PN kinase activity found in normal rat liver (18, 19). Carcinogen-induced hepatomas and fetal rat liver also exhibit low PN kinase activities relative to normal rat liver (18, 19).

We could find no PNP oxidase activity in extracts of either log-phase or confluent McA-RH7777 cells using N-[(S)-3-phosphopyridinolyl]-[G-3H]tryptamine as the substrate in a sensitive radiochemical assay (22). Nor was there any immunologically detectable protein in extracts from either log-phase or confluent McA-RH7777 cells that corresponded to rat liver PNP oxidase; the detection method involved a microtiter plate ELISA technique that used rabbit antiserum to rat liver PNP oxidase (Fig. 1).

Release of Volatile Counts from [H-4′]PN and [H-4′]PNP by Extracts of McA-RH7777 Cells. Any conversion of [H-4′]PN or [H-4′]PNP to PLP necessarily must involve the release of volatile counts, either as H2O or its decomposition product, H2O2 (26). Accordingly, release of volatile counts over and above that of the background provides a measure of the enzymatic
oxidation of these substrates. In the case of [3H-C4']PNP, volatile count release provides a measure of the activity of PNP oxidase (EC 1.4.3.5) and any other enzyme(s) capable of effecting the oxidation of the C4'-hydroxymethyl group of PNP to higher oxidation states, i.e., carboxaldehyde and carboxylic. The results of these experiments are provided in Figs. 2 and 3. When [3H-C4']PNP was used as the substrate and the enzyme source was an extract of normal rat liver, the release of volatile counts was linear with respect to both time and protein concentration (Fig. 2). In contrast, extracts of Mca-RH7777 cells had little or no such activity. In the case of [3H-C4']PNP and normal rat liver extracts, there was also an increase in volatile counts with time (Fig. 3). Under the same conditions, there was only a slight release of volatile counts when extracts of Mca-RH7777 cells were used in the incubation mixture.

Metabolism of [G-3H]PN by Extracts of Rat Liver, Host Liver of Morris Hepatoma 7777-bearing Buffalo Rats, Morris Hepatoma 7777 Grown in the Hindlegs of Buffalo Rats, and Mca-RH7777 Cells Grown in Tissue Culture. Fig. 4 provides the results of experiments dealing with the metabolism of [G-3H]PN by extracts of rat liver as a function of time. At the lower protein concentration (2 mg/ml), one readily can infer precursor-product relationships from the data which indicate that the sequence of conversions is in the following order: PN → PNP → PLP. The rate-limiting enzyme in this sequence must be PNP oxidase. Were that not the case, one would anticipate that PNP at all times would be either at very low concentrations or not detectable. The experimental results at the higher protein concentration (9.3 mg/ml) reveal that PLP constitutes more than 80% of the B6 vitamer forms present in the incubation mixture after 1 h. After 2 h, equilibrating transamination reactions convert PLP to PMP, the only other coenzymatically active B6 vitamer form.

In contrast, incubation mixtures containing extracts (7.5 mg protein/ml) of Morris hepatoma 7777 grown in the hindlegs of Buffalo rats appear to be compromised in their ability to convert [G-3H]PN to coenzymatically active PLP and PMP (Fig. 5). For example, after a 1-h incubation, the labeled (PN + PNP):(PLP + PMP) ratio is approximately 2.9 for extracts of Morris hepatoma 7777, compared to the corresponding ratios, using extracts of normal rat liver, of approximately 1.1 (2 mg protein/ml) and 0.08 (9.3 mg protein/ml) (Fig. 4). Likewise, extracts of confluent Mca-RH7777 cells poorly convert [G-3H]PN to PLP and PMP (Fig. 6). Thus, incubation mixtures

![Graph](image-url)
In the latter study, PLP concentrations were reported to be vitamins by extracts of confluent McA-RH7777 cells. The amount of protein in the incubation mixtures was 1.9 mg/ml.

After a 1-h incubation with radioactive substrate, approximately 24 h of growth in medium containing radioactive PN, log-phase McA-RH7777 cells, intact cells clearly were capable of producing PLP constituted almost one-half of the B6 vitamer forms. After 12.4 ± 4.4 ng PLP/mg protein, have very low levels of PLP, falling at the lower limits of PLP concentrations determined by us for Morris hepatomas grown in vivo, carcinoogen-induced rat hepatomas, and liver from rats fed a vitamin B6-deficient diet (14, 16, 18, 19).

In addition to the low PLP content of McA-RH7777 cells, there is a much lower level of activity of the two enzymes responsible for the conventional conversion of PN to PLP, i.e., PN kinase (EC 2.1.7.35) and PNP oxidase (EC 1.4.3.5). Both of these enzyme activities were determined by sensitive and specific radiochemical methods. In the case of PN kinase, we have determined that the activity of this enzyme in supernatants of homogenates obtained from McA-RH7777 cells is only about one-third that measured in normal rat liver. More striking is the apparent absence of PNP oxidase activity which can be attributed to the absence or near absence of the enzyme as determined by an immunochromatographic ELISA detection method which uses antibodies to authentic rat liver PNP oxidase (Fig. 1). Similar observations have been made for Morris hepatoma 7777 and carcinoogen-induced rat hepatomas (17, 19).

In related experiments, we measured the appearance of volatile counts in incubation mixtures containing high-speed supernatants of homogenates obtained from McA-RH7777 cells or normal rat liver, using as substrates PNP or PN specifically labeled with tritium at C4′ (Figs. 2 and 3). If the conventional biosynthetic pathway of PLP from PN, i.e., PN to PNP to PLP, were operative, oxidation of a C4′-hydroxymethyl to a carboxaldehyde group would necessarily lead to the appearance of volatile counts in the medium. This is because PNP oxidase is a flavin-dependent enzyme which produces volatile hydrogen peroxide, a species which would be expected to undergo decomposition in the incubation mixture to oxygen and another volatile product, water. The results provided in Fig. 2 clearly indicate that normal liver extracts, upon incubation with [3H- C4′]PNP, cause the release of volatile counts in a time- and protein-dependent fashion. In contrast, under otherwise identical conditions, high-speed supernatants of homogenates obtained from McA-RH7777 cells cause the release of very few volatile counts. While such counts are for the most part statistically significant relative to the controls, there is no apparent dependency on time or protein concentration for their release. When [3H-C4′]PN is used as the substrate and high-speed supernatants of homogenates of McA-RH7777 cells as the source of enzyme, there appears to be a time dependency associated with the small release of volatile counts but it is difficult to assess the meaningfulness of these data (Fig. 3).

Extracts of normal rat liver efficiently converted [3H]PN to PLP via PNP; PLP was then equilibrated with PMP by transamination reactions (Fig. 4). At longer incubation times and higher protein concentrations, PLP and PMP constituted the majority (approximately 90%) of the six B6 vitamer forms.

To the best of our knowledge, these studies represent the first detailed report on vitamin B6 metabolism in mammalian cells grown in tissue culture. The PLP concentration in McA-RH7777 cells was determined by us to be 12.4 ± 4.4 ng/mg protein compared to 37.0 ± 2.5 ng/mg protein for normal young adult rat liver. For isolated rat hepatocytes, the PLP content has been determined to be approximately 80 ng/mg protein (27). The only mammalian cell lines other than McA-RH7777 studied in this regard are a human fibroblast line (11) and two rat hepatoma cell lines selected for their resistance to pyridoxine toxicity (13). In the former study, the PLP content was determined from 27 ng/mg protein, for cells grown in PL-supplemented medium, to approximately 1-2 ng/mg protein for cells that were passed 4 times in PL-depleted media. In the latter study, PLP concentrations were reported to be high, approximately 81 and 137 ng/mg protein for the two cell lines studied. Thus, McA-RH7777 cells, with 12.4 ± 4.4 ng PLP/mg protein, have very low levels of PLP, falling at the lower limits of PLP concentrations determined by us for Morris hepatomas grown in vivo, carcinoogen-induced rat hepatomas, and liver from rats fed a vitamin B6-deficient diet (14, 16, 18, 19).
These results are in general accord with previous studies on the metabolism of radioactive pyridoxine in mice (28, 29), rats (30–33), perfused rat liver (34), and isolated rat hepatocytes (35; see also Ref. 36).

The data obtained from similar studies using high-speed supernatants of homogenates of Morris hepatoma 7777 and confluent McA-RH7777 cells are plotted in Figs. 5 and 6, respectively. It is readily apparent that extracts of both Morris hepatoma 7777 grown in vivo and the derivative McA-RH7777 cell line grown in vitro differed markedly from liver extracts with respect to their metabolism of \([\text{G}^3\text{H}]\text{PN}\). In the case of Morris hepatoma 7777, PPN constituted more than 80% of the six B<sub>6</sub> vitamin forms after 15 min and declined only to about 70% after 60 min even when a relatively high protein concentration of 7.5 mg/ml was used in the incubation mixture; PLP and PMP levels rose only very slowly during the 60-min incubation (Fig. 5). In the case of extracts from McA-RH7777 cells (1.9 mg protein/ml), PN and PNP constituted 84% of the B<sub>6</sub> vitamin forms after a 1-h incubation, with PLP and PMP accounting for a total of only 8% (Fig. 6). Under similar conditions, incubation mixtures containing liver extracts (2 mg protein/ml) had 52% of the B<sub>6</sub> vitamins in the forms of PN and PNP and 45% in the forms of PLP and PMP (Fig. 4).

Thus, extracts of both Morris hepatoma and McA-RH7777 cells are able to convert \([\text{G}^3\text{H}]\text{PN}\) to radioactive PLP and PMP in small but measurable amounts by mechanisms that appear to differ significantly from those that operate in the parent tissue from which they were derived.

The metabolism of \([\text{G}^3\text{H}]\text{PN}\) by both confluent and log-phase McA-RH7777 cells was also studied. The results of these experiments are provided in Fig. 7 which reveals that intact McA-RH7777 cells indeed were able to effect the conversion of PN to PLP and PMP. PLP accounted for almost one-half of the intracellular B<sub>6</sub> vitamin pool in confluent cells incubated for 2 h with radioactive PN. In the case of log-phase cells incubated for 24 h with radioactive PN, PLP and PMP comprised 87% of the radioactive intracellular B<sub>6</sub> vitamin pool, a distribution resembling that found in normal rat liver.

If EC 1.4.3.5 PNP oxidase is missing, it follows that there must be an alternate pathway for PLP synthesis from PN in intact McA-RH7777 cells since both intact confluent and log-phase McA-RH7777 cells are capable of converting PN to PLP (Fig. 7). It is likely that selection pressures attendant to the growth of these cells in culture necessitate the phenotypic property of independent production of the coenzymatically active form of vitamin B<sub>6</sub> from its coenzymatically inactive precursors.

All of the studies to date involving vitamin B<sub>6</sub> metabolism in Morris hepatomas (16–18) and carcinogen-induced rat hepatomas (19) indicate that rat hepatomas have very low PLP levels, low levels of PN kinase, and very low to absent levels of PNP oxidase. The present studies on vitamin B<sub>6</sub> metabolism in McA-RH7777 cells grown in tissue culture are in accord with these findings but indicate as well that intact rat hepatoma cells growing in tissue culture can make PLP from PN. Therefore, hepatomas arising in vivo may, in principle, obtain their PLP either by acquisition, as demonstrated for at least one subcellular organelle, i.e., mitochondria (5, 6), or by limited synthesis which is quite sufficient for its needs, as demonstrated in the present study for McA-RH7777 cells.<sup>4</sup>

That cells growing in culture may require only very low intracellular levels of PLP is demonstrated dramatically by the experiments of Lipson et al. (11) who showed that reduction of the intracellular PLP content of cultured human fibroblasts to approximately 1 ng/mg protein (3% of control levels) resulted in no evident changes in the morphology or growth rate of these cells even after 20 days (four passages) in culture. Conservation of the residual PLP no doubt involves its stabilization as PLP-protein complexes (2).

Given these observations, it seems unlikely that a general physiological deprivation of vitamin B<sub>6</sub> or administration of agents which lead to a generalized vitamin B<sub>6</sub> deficiency will be effective as nutrition-based interventions in the treatment of cancer. Such possibilities were explored originally almost 25 yr ago and the potential difficulties were appreciated at that time (37, 38). Also conceptually unattractive are approaches that involve long-term oral administration of B<sub>6</sub> vitamins in very high doses in the hope that there will be selective toxicity on tumors in the host organism. In the first place, high concentrations (mm) of PN are required to affect cell growth in vitro (12).

It is now clearly established that high dietary intake of PN (>500 mg/day; approximately 2 mmol as PN hydrochloride) is hazardous, particularly affecting sensory nerves (for a review, see Ref. 39). Secondly, all B<sub>6</sub> vitamins are pyridine derivatives. Pyridine itself has been recognized by toxicologists for decades as an industrial hazard that can cause central nervous system depression (40); its maximal acceptable concentration is 5 parts per million (41). Thirdly, owing to their free carboxaldehyde groups, PL and PLP, in particular, have special nondiscriminating chemical reactivity toward lysine side chains in proteins. This property indeed has led to the use of PLP as a protein-labeling reagent and reporter group (42). Finally, it has been shown that PN-resistant cell lines will grow under the selective pressure of high concentrations of PN (13), an adaptation that is unlikely to be available to normal cells. On the other hand, targeted approaches aimed at specific inhibition of PLP-dependent enzymes that are critical to cell viability or cell division, such as ornithine decarboxylase, have considerable attraction (43, 44). In addition, vitamin B<sub>6</sub> status may exert effects on tumor growth in an indirect fashion, such as by enhancement of immune system function (45). Finally, there is the possibility that there may be specific instances in which there is reason to believe that vitamin B<sub>6</sub> status may exert effects on carcinogenesis and tumor growth (46).

REFERENCES


4 Growth of rat hepatoma-derived HTC cells in suspension culture has enabled us recently to obtain relatively large amounts of cells. We have shown, using the sensitive radiochemical PNP oxidase assay of Langham et al. (22) with high concentrations of protein in the assay mixture, that there is a low but measurable amount of activity in high-speed supernatants of HTC cell extracts. In addition, there is a similarly small but measurable activity in cell pellets. When supernatants and pellets are combined, the activity appears to be more than additive. One possibility is that a microsomal cytochrome P450 system may be at least in part responsible for this apparent PNP oxidase activity.
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