Pyridoxine-derived B₆ Vitamers and Pyridoxal 5'-Phosphate-binding Proteins in Cytosolic and Nuclear Fractions of HTC Cells*

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The nuclear fraction of rat hepatoma-derived HTC cells contained approximately 8% of the total cellular pyridoxal 5'-phosphate. HTC cells were able to metabolize [3H]pyridoxine to coenzymatically active pyridoxal 5'-phosphate and pyridoxamine 5'-phosphate. As HTC cells did not have any demonstrable pyridoxal 5'-phosphate oxidase activity, the conversion of pyridoxine to pyridoxal 5'-phosphate must have taken place by a nonconventional route. The ratio of pyridoxal 5'-phosphate to pyridoxamine 5'-phosphate in the nunnuclear fraction of HTC cells was approximately 1:1, whereas in the nuclear fraction it was approximately 17:1, indicating that there was selective acquisition of pyridoxal 5'-phosphate by the nucleus. With the aid of a monoclonal antibody specific for the 5'-phosphopyridoxyl group, it was shown that there was one major pyridoxal 5'-phosphate-binding protein in a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)-resolved nucleoplasmic extract of HTC cells. This finding was confirmed by radioautography of an SDS-PAGE-resolved nucleoplasmic extract obtained from cells grown in a medium containing [3H]pyridoxine. Isoelectric focusing followed by SDS-PAGE also indicated the presence of one major pyridoxal 5'-phosphate-binding protein in the nucleoplasmic extract of HTC cells having a relatively high isoelectric point (approximately 7. Data were obtained indicating that the protein might exist in a higher molecular weight form, probably a dimer. Currently, these findings constitute virtually all of the available information on vitamin B₆ and the cell nucleus.

The subcellular distribution of pyridoxal 5'-phosphate (PLP)¹ and the major cytosolic PLP-binding proteins in rat liver have been examined by Boerum et al. (1). The fraction of the total PLP found in the nuclei of liver cells was reported to be approximately 21% in the case of rats fed a diet adequate in vitamin B₆ and 39% in the case of rats fed a diet deficient in vitamin B₆; the mitochondrial plus lysosomal fraction contained about 10% of the total cellular PLP. In studies on the effect of different dietary levels of vitamin B₆ on the content of PLP in rat liver, van den Berg et al. (2) found that 65-85% of total liver PLP was in the supernatant fraction after high speed centrifugation of rat liver homogenates, leaving 15-35% in the pellet. Accordingly, a significant amount of PLP is associated with the noncytosolic fraction of the cell, most of it being in the nucleus. It is safe to say that virtually nothing is known about how the nucleus acquires its PLP or what PLP is doing there. The experiments reported herein are a first step in the direction of addressing these questions. They involve studies on the metabolism of pyridoxine by a rat hepatoma cell line, the identification of pyridoxine-derived PLP in the nucleus, and the demonstration of a readily extractable nuclear PLP-binding protein.

EXPERIMENTAL PROCEDURES

Cultivation of Cells and Preparation of Nuclei—Rat hepatoma-derived HTC cells were provided by Dr. Jen-Fu Chiu of our department. They were grown in antibiotic-supplemented (alternating penicillin/streptomycin and gentamycin) Ham's F-12 medium with glutamine, made 26.2 mM in sodium bicarbonate and 5% in fetal bovine serum, in a humidified 95% air, 5% CO₂ atmosphere (37 °C); the doubling time was approximately 18 h. For growth of HTC cells in suspension culture, the medium used was Eagle's minimal essential medium for suspension culture with glutamine, made 26.2 mM in sodium bicarbonate and 5% in fetal bovine serum. Nuclei from HTC cells were isolated by a sucrose centrifugation method essentially as described by Chiu et al. (3) with minor modifications such as the substitution of Hepes buffer for Tris buffer. Rat liver nuclei were prepared as described previously (4). Nuclear preparations were routinely checked by light microscopy; examination of a typical preparation by electron microscopy revealed that the nuclei were essentially free of cytoplasmic tags. The material remaining on the top of the sucrose cushion used in the purification of nuclei was designated the nonnuclear fraction. Chromatin was prepared by hypotonic lysis of nuclei in 10 mM Hepes, 1 mM EDTA, and 0.1 mM PMSF (pH 7.9) (4). The protein/DNA ratio in chromatin preparations from HTC cells was approximately 2. The initial nucleoplasmic supernatant obtained after centrifugation of the chromatin was designated nucleoplasmic extract. The cytosolic fraction was the supernatant obtained after high speed centrifugation of cell homogenates prepared as described previously (5).

Reductions with Sodium Cyanoborohydride—Endogenous PLP was reductively and covalently linked to cellular PLP-binding proteins as P-Pxy residues by treatment with sodium cyanoborohydride. Sodium cyanoborohydride has a significant advantage over sodium borohydride for this purpose in that there is less foaming of the samples owing to the greater stability of sodium cyanoborohydride in aqueous solutions. For cytosolic proteins, 0.2 volume of sodium cyanoborohydride (10 mg/ml in phosphate-buffered saline (137 mM NaCl, 2 mM KCl, 1.5 mM KH₂PO₄, 8.1 mM Na₂HPO₄, pH 7.2-7.4), 0.1 mM PMSF) was added, and the resulting solution was stirred on ice for 30 min. Samples were then dialyzed against phosphate-buffered saline, 0.1 mM PMSF, with a final dialysis against 10-fold diluted phosphate-buffered saline, 0.1 mM PMSF followed by frozen storage or lyophilization. Chromatin and nucleoplasmic extract were prepared by hypotonic lysis of nuclei with sodium cyanoborohydride (1 mg/ml) present in the lysis buffer. Excess sodium cyanoborohydride was

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² The abbreviations used are: PLP, pyridoxal 5'-phosphate; PMP, pyridoxamine 5'-phosphate; PNP, pyridoxine 5'-phosphate; P-Pxy, 5'-phosphopyridoxyl-; PMSF, phenylmethanesulfonyl fluoride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
removed from the nucleoplasmic extract by dialysis as described. The chromatin pellet was rehydrated and washed by suspension and centrifugation from 1.5 mM NaCl, 0.15 mM sodium citrate, 0.5 mM PMSE (pH 7.0). Exhaustive digestion (1 h, 4 °C) of nuclei (1 mg of DNA/ml with DNase I (50 μg/ml) in 10 mM Tris, 1 mM MgCl₂, 0.5 mM PMSE prior to reduction with sodium cyanoborohydride or sodium borohydride (0.1 volume; 10 mg/ml in the same buffer) had no effect on the nuclear P-Pxy protein pattern found on Western blots.

Electrophoresis and Immunoblot Detection of P-Pxy Proteins—SDS-PAGE (5% stacking gel, 7.5% running gel) and Western blotting of SDS-PAGE-resolved proteins were performed essentially as described previously (6). Nuclear and chromatin preparations were subjected to one-dimensional electrophoresis. Two-dimensional gel electrophoresis was carried out by the O'Farrell method (7) essentially as described by Adams (8); the second dimension had a 5% stacking gel and a 10% running gel. Monoclonal antibody E6(22) is specific for the P-Pxy group, has been described previously (6), as has its application for the identification of P-Pxy proteins on Western blots by a horseradish peroxidase-dependent immunoblot procedure (6, 9, 10).

In the present study, PLP-depleted human serum (6,11) was replaced by PLP-depleted goat serum in the initial blocking solution. In addition, both E6(22) and goat anti-mouse IgG antibody-horseradish peroxidase conjugate were dissolved in 2.5% PLP-depleted goat serum, 1% bovine serum albumin in PBS.

RESULTS

PLP Content of HTC Cells—The PLP content of confluent HTC cells grown in F-12 medium was determined to be 10.7 ± 0.9 ng/mg of protein (n = 5). Of the total cellular PLP, approximately 8% was associated with the nuclear fraction obtained from HTC cells. We have reported previously that the PLP content of McA-RH7777 cells, another rat hepatoma cell line, was 12.4 ± 4.4 ng/mg of protein (5). For hepatomas grown in vivo, PLP has been found to be in the range of 14.22 ng/mg of protein; for liver tissue obtained from rats fed pyridoxine-sufficient and pyridoxine-deficient diets, PLP contents were 37.0 ± 2.5 and 19.8 ± 2.7 ng/mg of protein, respectively (12, 17-19). When HTC cells were grown in pyridoxine-free medium for six passages, their PLP concentration fell to 4.9 ± 0.8 ng/mg of protein (n = 5). These cells apparently grew perfectly well under these conditions and must have met their vitamin B⁶ requirements from the vitamer forms available in the 5% fetal bovine serum in the medium. Ljason et al. (11) have reported that the PLP content of human fibroblasts was maintained at approximately 4 ng/mg of protein during four passages in vitamin B⁶ (pyridoxal)-free Eagle's minimal essential medium containing 10% fetal bovine serum—an observation similar to our own.

Activities of Pyridoxine-Metabolizing Enzymes in HTC Cells—PNP oxidase activity in the cytosolic fraction from HTC cells grown in F-12 medium was measured as described previously (5) by a sensitive radiochemical method that uses N-5'-P-Pxy-[3H]tryptamine as substrate (13). As was the case with McA-RH7777 cells (5), there was no demonstrable PNP oxidase activity in HTC cytosols. Pyridoxine kinase activity in HTC cytosolic fractions was determined by a radiochemical method (12) to be 39% of that found in rat liver; the comparable figure for McA-RH7777 cells was 36% (5). Metabolism of [3H]Pyridoxine by HTC Cells—HTC cells were passed four times in vitamin B⁶ (pyridoxine)-free F-12 medium so as to deplete endogenous stores of pyridoxine. Cells were then grown in medium containing [3H]pyridoxine (0.126 μM; 0.176 μCi/ml). Nuclear and nonnuclear fractions were analyzed for [3H]pyridoxine-derived B⁶ vitamer forms by ion-exchange chromatography. Of the total radioactivity applied to ion-exchange columns in three replicate experiments, the average recovery in the form of B⁶ vitamers was 92% in the case of the nuclear fraction and 94% in the case of the nonnuclear fraction. The results are provided in Table I. Of particular interest is the finding that the ratio of [3H]PLP to [3H]PMP in the nucleus is approximately 17 compared with a ratio of approximately 1 in the rest of the cell. When isolated HTC cell or rat liver nuclei were incubated with [3H]pyridoxine, there was no conversion to any of the other B⁶ vitamer forms.

For experiments involving studies on the conversion of [3H]pyridoxine to [3H]pyridoxine-derived B⁶ vitamer forms, cells were first passed on plates in pyridoxine-free F-12 medium followed by incubation with [3H]pyridoxine-containing medium. We infer from this that HTC cells growing on plates can meet, their vitamin B⁶ requirements from the serum in the medium and by conversion of whatever pyridoxine is added to the medium to other B⁶ vitamer forms in sufficient amounts for growth. Presumably, the conversion of pyridoxine to pyridoxine-deprived B⁶ vitamer forms in HTC cells

| Table I
| Subcellular distribution of [3H]pyridoxine-derived B⁶ vitamer forms in HTC cells

| Vitamin | Nuclear fraction | Nucleoplasmic fraction |
|---------|-----------------|-----------------------|
| Pyridoxine | 0.4 ± 0.1 | 0.7 ± 0.6 |
| Pyridoxamine | 0 | 0 |
| Pyridoxal | 0 | 2.8 ± 0.5 |
| PNP | 0.9 ± 0.4 | 1.4 ± 0.3 |
| PMP | 0.1 ± 0.1 | 4.6 ± 0.5 |
| PLP | 0.8 ± 0.2 | 4.2 ± 0.7 |
ine to other B₆ vitamer forms is by way of a nonconventional pathway that does not require the action of pyridoxine phosphate oxidase, as demonstrated previously for McA-RH7777 cells (5). On the other hand, HTC cells grown in suspension culture with Eagle's minimal essential medium for suspension culture, which contains pyridoxal, did not grow well when pyridoxal was replaced by pyridoxine; in addition, [³H]pyridoxine was very poorly converted to coenzymatically active B₆ vitamer forms. Thus, HTC cells grown in suspension appear to require pyridoxal, a vitamer form that can readily be converted to PLP by the action of a kinase found in these hepatoma cells in an amount equal to 39% of that found in normal rat liver, as noted above.

**PLP-binding Proteins in HTC Cells**—A monoclonal antibody, designated E6(2)2, directed against the P-Pxy group enables the detection on Western blots of P-Pxy proteins formed by the action of reducing agents such as sodium borohydride or sodium cyanoborohydride on P-pyridoxylidene proteins (see “Experimental Procedures” and references therein). The discriminating power of E6(2)2, as used in the present modification of the Western immunoblot detection method for P-Pxy proteins, is shown in Fig. 1. *Lane 1* shows endogenous PLP-binding proteins in rat liver. *Lane 2* demonstrates the reagent properties of PLP, i.e. its ability to derivatize proteins nonspecifically when it is present in excess (4). *Lane 3* reveals that failure to link PLP to PLP-binding proteins reductively and covalently will result in loss of PLP during SDS-PAGE. It is apparent from Fig. 1 that a positive reaction is dependent on the presence of PLP, the addition of a reducing agent such as sodium borohydride, and monoclonal antibody E6(2)2.

The patterns of P-Pxy proteins obtained after reduction with sodium cyanoborohydride of nuclear and nonnuclear fractions obtained from HTC cells are shown in Fig. 2. The major finding from the patterns provided in Fig. 2 is that there is one very prominent P-Pxy protein in nuclei obtained from HTC cells, having an apparent molecular mass after SDS-PAGE in the range of 50–55 kDa. This protein is in the nucleoplasmic extract obtained after hypotonic lysis of nuclei and not in the chromatin fraction, as shown in *lanes B* and *C*. This band has been found in the nuclei of HTC cells grown on plates in F-12 medium (*n* = 4), in the nuclei of HTC cells grown in suspension culture in Eagle’s minimal essential medium for suspension culture (*n* = 4), and in rat liver nuclei (*n* = 1). Comparison of Figs. 1 and 2 reveals that there are many fewer bands in the nonnuclear fraction obtained from HTC cells compared with the nonnuclear fraction obtained from normal rat liver. It has been demonstrated previously that hepatomas have far fewer detectable P-Pxy proteins than normal rat liver and that the pattern of P-Pxy proteins in hepatomas resembles that found in fetal rat liver (19–21). A rationalization for the differences between normal liver and hepatomas with respect to vitamin B₆ metabolism and requirements has been proposed (22).

HTC cells were grown in medium containing [³H]pyridoxine. Fig. 3 provides radioautographs of a sodium cyanoborohydride-reduced SDS-PAGE-resolved cytosolic and nucleoplasmic extracts from HTC cells grown in medium containing [³H]pyridoxine were resolved by SDS-PAGE and horizontally electroeluted to nitrocellulose. *Panel A*, radioautograph of gel; *lane 1*, cytosolic extract (approximately 400 µg of protein); *lane 2*, nucleoplasmic extract (approximately 370 µg of protein). *Panel B*, Western blot corresponding to panel A. The numbers on the left represent molecular masses in kDa.
Detection of PLP-binding Proteins in the Nucleoplasmic Extract of HTC Cells—Fig. 4 is a composite of two isoelectric focusing experiments (panels A and C) and the corresponding Western blots (panels B and D). The Western blots demonstrate the presence of one major PLP-binding protein in the nucleoplasmic extract of HTC cells. The protein of interest focused at the extreme basic end of the tube gel when the pH gradient was in the range of 5.0-6.5 (Fig. 4, panel B). Accordingly, ampholytes having a higher pH range were used in the focusing step (panels C and D). Panel D reveals that the major P-Pxy protein in the nucleoplasmic extract had a relatively high PI, which placed it at a considerable distance from the majority of the proteins in the nucleoplasmic extract, most of which focused at the acidic end of the tube gel when the pH range in the tube gel was from 6.0 to 7.4. This property should be of considerable use in the purification of this protein.

Evidence for the Existence of Higher Molecular Mass Forms of the Major PLP-binding Protein in the Nucleoplasmic Extract of HTC Cells—When SDS-PAGE gels were run in the absence of mercaptoethanol, we observed the presence of bands whose mobility corresponded approximately to what one would expect for dimeric species. Attempts to convert completely the 50-55 kDa form to the higher molecular mass form by incubation overnight in the absence of mercaptoethanol and in the presence of oxidized glutathione (5 mM in phosphate-buffered saline, pH 7.4) were not completely successful (Fig. 5). Nevertheless, it is apparent from Fig. 5 that under these conditions, there were bands that approximately corresponded to dimers of the 50-55 kDa form. One potential candidate protein is ornithine decarboxylase, a dimeric PLP-dependent enzyme that has been found in nucleoplasmic extracts (24). Subunit molecular masses reported for ornithine decarboxylase are 50 kDa (rat liver) (25), 55 kDa (mouse

**Fig. 4.** Two-dimensional gel patterns of nucleoplasmic extracts obtained from HTC cells. Panels A and C, Coomassie Blue-stained polyacrylamide gels; panels B and D, P-Pxy proteins in Western blots from polyacrylamide gels A and C, respectively, detected by antibody E6(2). Approximately 350 µg of protein was applied to the 2-mm x 10-cm tube gels. Molecular mass markers are shown on the right and left edges in panels A and C. Molecular mass markers in strips cut from both edges of Western blot D were detected by staining with Amido Black. The numbers at the tops of the panels are the average pH values of two 1-cm sections cut from control tube gels; the sections were vortexted in 0.5 ml of 0.01 M KCl to extract the ampholytes. The Coomassie Blue-stained protein(s) identified by the arrows in panels A and C most closely correspond to the P-Pxy proteins identified by the arrows in panels B and D. For further details, see "Experimental Procedures."
lymphoma cells) (26), and 53 kDa (mouse kidney) (27). However, the isoelectric points reported for these enzymes are 4.1, less than 5.6, and 4.8, respectively. Thus, ornithine decarboxylase from a variety of sources have pi values that are markedly lower than that found for the P-Pxy protein in the nucleoplasmic extracts of HTC cells (pi approximately 7). Nevertheless, with the information in hand, one cannot definitively rule out at this time an ornithine decarboxylase variant in the nucleoplasmic extract of HTC cells having an unusually high isoelectric point.

**DISCUSSION**

A fraction of the PLP found in rat liver and rat hepatoma cells resides in the nucleus. There is nothing known about its function. The results reported in this study establish that HTC cells contain pyridoxine-derived PLP in the nucleus, that PLP comprises the only significant B6 vitamer form in the nucleus, and that there is one particularly prominent PLP-binding protein in the nucleoplasmic fraction of nuclear preparations obtained from HTC cells. In addition, we have shown the presence of a corresponding protein in rat liver nuclei.

One possibility is that PLP happens to leak into the nucleus somehow, and its presence there is of no physiological significance. If this were the case, one would expect PLP to be primarily and nonspecifically associated with lysine-containing histones in the nucleus. Along these lines, Pal and Chris- tensen (28) have reported that the pellet obtained from broken Ehrlich ascites tumor cells became intensely yellow upon incubation with PLP, no doubt a consequence of Schiff base formation between PLP and lysine side chains. However, there does not appear to be any significant association of PLP with the histones found in the nucleus of HTC cells grown in media containing pyridoxine. Rather, the major PLP-binding protein is found in the nucleoplasmic extract and has an apparent molecular mass of 50–55 kDa after SDS-PAGE (Fig. 2). In addition, one would expect that PLP would be the least likely B6 vitamer form to diffuse across the nuclear membrane, as it is generally accepted that free phosphorylated forms of B6 vitamers do not readily cross membranes of mammalian cells (29). Indeed, PLP has been used as a labeling reagent for the external surface of cell membranes owing to the fact that it will spontaneously react with the side chains of lysine residues that are accessible to it on the surface of cell membranes (30). Accordingly, it is reasonable to infer that the presence of PLP in the nucleus is of physiological significance and that it gets there by a specific mechanism(s).

What possible role(s) might PLP have in the nucleus of a cell? There is considerable evidence that PLP might affect steroid hormone activity by altering the interaction of steroid receptor complexes with DNA, chromatin, and nuclei (31–38). As an example, DiSorbo and Litwack have shown that rat hepatoma cells, when grown in the presence of 5 mM pyridoxine, have a significantly decreased glucocorticoid-dependent induction of tyrosine aminotransferase; the reverse was the case when the medium was depleted of pyridoxine (35). Similar findings have been reported for the glucocorticoid-dependent induction of HeLa cell alkaline phosphatase (36). Majumdar et al. (39) have shown that the addition of PLP to an incubation medium containing mouse mammary gland explants resulted in a significant inhibition of both the binding of dexamethasone to nuclear steroid receptor as well as of dexamethasone-stimulated casein mRNA synthesis. In addition to its effects on steroid receptors, PLP has been very effectively used as a site-specific affinity-labeling reagent for other DNA-binding proteins, e.g. the gene 5 DNA unwinding binding protein from bacteriophage fd (40) and for nucleotide and polynucleotide-binding sites on enzymes such as Escherichia coli DNA polymerase I (41) and the recBC enzyme of E. coli (42). It is assumed that the pyridine ring of PLP mimics the base portion of a nucleotide, with a phosphate group being common to both PLP and a nucleotide. Thus, there is an excellent chemical rationale for the possibility that PLP might act as a small molecular modulator of protein-DNA interactions. It must be emphasized that the physiological significance of observations that are dependent on nonphysiological concentrations of B6 vitamers remain problematical because of the reagent properties of B6 vitamer forms, particularly PLP (4). It is important to note that the findings reported in the present study were made under experimental conditions employing no excess amount of vitamin.

We have no information at this time as to how PLP gets into nuclei of HTC cells or rat liver. One possibility is that it gains entry by a conventional route involving transport in an unphosphorylated form across the nuclear membrane followed by phosphorylation and binding to nuclear protein(s) (43). A second is that PLP is transported intact across the nuclear membrane, as demonstrated for PLP acquisition by mitochondria (44). A third is that it enters the nucleus only in association with a specific protein(s).

It is intriguing that the form and substance of the present report resemble in many ways those published recently in this journal for a nuclear retinoic acid-binding protein (mass, 55–60 kDa) found in the human leukemia cell line HL60 (45). Since retinoic acid, a vitamin A derivative, is a potent inducer of terminal differentiation of HL60 cells, it was postulated that the retinoylated nuclear protein may be involved in regulation of retinoic acid effects at the gene level. The possibility that PLP may exert similar effects in the nucleus via the protein we have identified in the nucleoplasmic extract of HTC cells is of considerable interest.

In conclusion, this is the first substantive report concerned with PLP in the nucleus of cells. Of particular interest is the finding that there is one major PLP-binding protein in the nucleoplasmic fraction of nuclei obtained from HTC cells and rat liver. The purification and characterization of this protein are in progress.

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REFERENCES

1. Bosron, W. F., Veitch, R. L., Lumeng, L., and Li, T-K. (1978) J. Biol. Chem. 253, 1488-1492
2. van den Berg, H., Boghadee, J. J. P., Sinkeldam, E. J., and Schreurs, W. H. P. (1982) Int. J. Vitam. Nutr. Res. 52, 407-416
3. Cito, J-F., Fujita, H., and Hulilac, L. S. (1977) in Methods in Cell Biology (Stein, G., Stein, J., and Kleinsmith, J., eds) Vol. 16, pp. 283-296, Academic Press, New York
4. Kittler, J. M., Meisler, N. T., Viceps-Madore, D., Cidlowski, J. A., and Thanassi, J. W. (1984) Anal. Biochem. 137, 210-216
5. Meisler, N. T., and Thanassi, J. W. (1983) Cancer Res. 43, 1080-1085
6. Viceps-Madore, D., Cidlowski, J. A., Kittler, J. M., and Thanassi, J. W. (1985) J. Biol. Chem. 258, 2689-2696
7. O’Farrell, P. H. (1975) J. Biol. Chem. 250, 4007-4021
8. Adams, L. D. (1987) in Current Protocols in Molecular Biology (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Strobel, K., eds) Vol. 1, Part A, pp. 577-594, John Wiley & Sons, New York
9. Kittler, J. M., Viceps Madore, D., Cidlowski, J. A., Meisler, N. T., and Thanassi, J. W. (1986) Methods Enzymol. 122, 120-127
10. Kittler, J. M., and Thanassi, J. W. (1986) in Coenzymes and Cofactors: Vitamin B6, Pyridoxal Phosphate. Chemical, Biochemical, and Medical Aspects (Dolphin, D., Pouleau, R., and Avramovic, G., eds) Vol. 1, Part A, pp. 871-944, John Wiley & Sons, New York
11. Lipson, M. H., Kraus, J. P., Solomon, L. R., and Rosenberg, L. E. (1980) Arch. Biochem. Biophys. 204, 496-493
12. Meisler, N. T., and Thanassi, J. W. (1980) J. Nutr. 110, 1965-1975
13. Langham, L., Garber, B. M., Roe, D. A., and Kazarinoff, M. N. (1982) Anal. Biochem. 129, 329-334
14. Kawanie, E., and Fonda, M. (1978) Anal. Biochem. 90, 525-528
15. Lumeng, L., and Li, T-K. (1980) J. Biol. Chem. 255, 1488-1492
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
17. Thanassi, J. W., Nutter, L. M., Meisler, N. T., Coners, P., and Cito, J-F. (1981) J. Biol. Chem. 256, 3370-3376
18. Meisler, N. T., Nutter, L. M., and Thanassi, J. W. (1982) Cancer Res. 42, 5338-5343
19. Kittler, J. M., Meisler, N. T., and Thanassi, J. W. (1986) J. Nutr. 116, 588-598
20. Kittler, J. M., Viceps-Madore, D., Cidlowski, J. A., and Thanassi, J W (1980) Biochem. Biophys. Res. Commun. 112, 61-65
21. Thanassi, J. W., Meisler, N. T., and Kittler, J. M. (1985) in Vitamin B6: Its Role in Health and Disease (Reynolds, R. D., and Lekien, J. E., eds) pp. 319-336, Alan R. Liss, Inc., New York
22. Thanassi, J. W. (1984) in Vitamins, Nutrition, and Cancer (Pra- tice, K. N., ed) pp. 251-265, Karger, Basel
23. Straubhaus, P. H., Kent, A. B., Hedrick, J. H., and Fischer, K. H. (1967) Methods Enzymol. 11, 671-675
24. Emaneusson, H., and Heby, O. (1982) Cell Biol. Int. Rep. 6, 951-954
25. Kameji, T., Murakami, Y., Fujita, K., and Hayashi, S-I. (1982) Biochim. Biophys. Acta 717, 111-117
26. McConlogue, L., and Coffino, P. (1983) J. Biol. Chem. 258, 8384-8388
27. Seely, J. R., Piso, H., and Pegg, A. E. Biochemistry 21, 3394-3399
28. Fal, P. R., and Christensen, H. N. (1961) J. Biol. Chem. 236, 894-897
29. Ink, S. L., and Henderson, L. M. (1964) Annu. Rev. Nutr. 4, 455-470
30. Rifkin, D. B., Compasno, R. W., and Reich, E. (1972) J. Biol. Chem. 247, 6432-6437
31. Cidlowski, J. A., and Thanassi, J. W. (1981) J. Steroid Biochem. 15, 11-16
32. DiSorbo, D. M., and Litwack, G. (1982) in Biochemical Actions of Hormones (Litwack, G., ed) Vol. 9, pp. 205-219, Academic Press, New York
33. Schmidt, T. J., and Litwack, G. (1982) Physiol. Rev. 62, 1131-1192
34. Groddy, W. W., Schrader, W. T., and O’Malley, B. W. (1982) Endocrinol. Rev. 3, 141-163
35. DiSorbo, D. M., and Litwack, G. (1981) Biochem. Biophys. Res. Commun. 99, 1205-1208
36. Compton, M. M., and Cidlowski, J. A. (1986) Enocrinol. Rev. 7, 140-148
37. Bender, D. A. (1987) World Rev. Nutr. Diet. 51, 140-188
38. Litwack, G. (1988) Cancer Res. 48, 2636-2640
39. Majumdar, P. K., Joshi, J. B., and Banerjee, M. R. (1983) J. Biol. Chem. 258, 6795-6798
40. Axelrod, H., Greene, S. M., and McPherson, A. (1981) Biochim. Biophys. Acta 670, 443-453
41. Hazra, H. K., Detera-Wadleigh, S., and Wilson, S. H. (1984) Biochemistry 23, 2073-2078
42. Anni, M., Fujioaki, T., Nakayama, J., and Takagi, Y. (1979) J. Biol. Chem. 254, 10853-10856
43. Lumeng, L., and Li, T-K. (1980) in Vitamin B6 Metabolism and Role in Growth (Trytates, G., ed) pp. 27-51, Food and Nutrition Press, Westport, CT
44. Lui, A., Lumeng, L., and Li, T-K. (1981) J. Biol. Chem. 256, 6041-6046
45. Takahashi, N., and Breitman, T. R. (1989) J. Biol. Chem. 264, 5169-5163
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N T Meisler and J W Thanassi

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