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# Enzyme Activities in Vitamin $B_6$-Deficient, Normal, and Tumor-Bearing Animals: Effect of Hydrocortisone (38014)

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It is well-known that vitamin $B_6$, i.e., pyridoxine, pyridoxal, pyridoxamine, and their phosphorylated derivatives, is required for the normal growth of young rats and that pyridoxal phosphate is the coenzyme of transaminase and decarboxylase enzymes. It has been reported that the absence of this growth factor from the diet affects the activity of several enzymes in animal tissues (1–6) as well as steroid induction of enzyme activity (2, 5, 6). Whether lack of vitamin $B_6$ also affects the development of enzymatic activity and steroid enzyme induction in tumor-bearing animals has not been investigated. This study reports the effect of vitamin $B_6$ deficiency on the activity of tyrosine transaminase and serine dehydratase (two vitamin $B_6$-requiring enzymes) of normal, host liver, and hepatoma #7794A and the induction of these enzymes by hydrocortisone.

*Materials and Methods. Animals.* Buffalo male rats weighing approximately 60–70 g were inoculated intramuscularly in the hind leg muscles with hepatoma 7794A cells, generation 39 (4), at Howard University, Washington, DC. The animals were immediately shipped to Morgantown by air express where they were housed individually in a windowless, air-conditioned room with an automatic on-off light switch. The lights were off from 8:00 PM to 8:00 AM and on from 8:00 AM to 8:00 PM.

*Determination of enzyme activity.* All animals were sacrificed between 8:30 and 11:30 AM. The average weight of the groups of animals fed the vitamin $B_6$-deficient diet was 130 ± 15 (range) g while that of those fed the vitamin-supplemented diet was 260 ± 20 g. Livers and tumors were quickly removed and washed with cold 0.14 *M* KCl. Excess connective tissue was removed from each tumor and the weight recorded. Homogenization of tissues was carried out with a Blessing glass homogenizer (L-pestle clearance, 0.006 in.) in 7 vol of cold 0.14 *M* KCl, as previously described (6–9). Homogenates were centrifuged for 30 min at 4° at 105,000*g* in the Spinco Model L ultracentrifuge. The clear supernatant was used to measure enzymatic activity.

*Tyrosine transaminase.* L-Tyrosine:2-oxoglutarate aminotransferase (EC 2.6.1.5) activity was measured as previously described (10, 6) using the method of Diamondstone (11), and all results presented here (Tables I, II) were obtained by this method (11). During the course of the study, however, some samples were also assayed (spot check) for transaminase activity by the method of Canellakis and Cohen (12) as previously described (13) for further verification of our observations. The enzyme unit is defined as that amount catalyzing the formation of 1 μmole of *p*-hydroxybenzaldehyde in 10 min at 37° (11, 6).

*Serine dehydratase.* L-Serine hydrolyase, deaminating, EC 4.2.1.13, activity was determined as described by Suda and Nakagawa (14). The enzyme unit is defined as the amount catalyzing the formation of 1 μmole of pyruvate per minute at 37° (14).

*Protein.* Protein was measured by the





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TABLE I. Tyrosine Transaminase Activity of Normal, Host Liver, and Hepatoma #7794A of Buffalo Rats Fed Vitamin $B_6$-Deficient and Supplemented Diets.[a]

| Regimen | Treatment | Enzyme units per mg protein tyrosine transaminase | | |
|---|---|---|---|---|
| | | Normal liver | Host liver | Hepatoma |
| Basal diet | None | 129 ± 41 (3)[b] | 181 ± 23 (7) | 64 ± 9 (14) |
| | HC | — | 300 ± 15 (6) | 84 ± 12 (12) |
| | HC + CX | — | 187 ± 31 (3) | 58 ± 8 (6) |
| Basal diet plus pyridoxine[c] | None | 170 ± 30 (8) | 176 ± 20 (11) | 95 ± 10 (11) |
| | HC | 370 ± 35 (3) | 420 ± 25 (5) | 140 ± 20 (8) |

[a] Buffalo strain male rats were inoculated with hepatoma #7794A cells at weanling and were fed *ad lib.* either a vitamin $B_6$-deficient or a vitamin $B_6$-supplemented commercial diet for six weeks. Enzyme activity was determined at the time of sacrifice. Additional details in *Materials and Methods.* HC = hydrocortisone; CX = cycloheximide.

[b] Number in parenthesis is the number of animals or the number of tumors. The number following ± sign is the standard deviation.

[c] See *Materials and Methods.*

method of Lowry *et al.* (15). Hydrocortisone hemisuccinate (Mann) and cycloheximide (Actidione, Upjohn) were dissolved in 0.9% NaCl and, where indicated, were administered intraperitoneally 4 and 2 hr before sacrifice, respectively. The dosage of the hormone and antibiotic was 6 mg and 1.250 mg per 100 g body wt, respectively.

*Vitamin $B_6$ deficiency.* Vitamin $B_6$ deficiency was induced by feeding *ad lib.* weanling rats a basal diet lacking vitamin $B_6$, as described previously (6). Control animals received the basal diet supplemented with 70 mg of pyridoxine HCl/kg diet. Diets were prepared by Nutritional Biochemicals Corporation, Cleveland, OH, as described by French (20). The protein content was 30% (20).

*Results.* In attempts to relate the effect of vitamin $B_6$ deficiency on metabolic activity, the specific activity of two pyridoxal phosphate requiring enzymes, i.e., serine dehydratase and tyrosine transaminase, were measured in normal, host liver, and hepatoma 7794A, in both vitamin $B_6$-deficient and nondeficient Buffalo strain rats. The effect(s) of hydrocortisone and hydrocortisone plus cycloheximide on the activity of these two enzymes was also examined. The results are presented in Tables I and II. The levels of the two enzymes were, in general, lower in the groups fed the basal diet lacking the vita-

TABLE II. Serine Dehydratase Activity of Normal, Host Liver, and Hepatoma #7794A of Buffalo Rats Fed Vitamin $B_6$-Deficient and Supplemented Diets.[a]

| Regimen | Treatment | Enzyme units per mg protein serine dehydratase | | |
|---|---|---|---|---|
| | | Normal liver | Host liver | Hepatoma |
| Basal diet | None | 0.49 ± 0.15 (3) | 0.43 ± 0.19 (4) | 0 |
| | HC | — | 0.30 ± 0.10 (5) | 0 |
| | HC + CX | — | 0.17 ± 0.04 (3) | 0 |
| Basal diet plus pyridoxine | None | 0.81 ± 0.1 (3) | 1.43 ± 0.2 (4) | 0 |
| | HC | — | 1.90 ± 0.5 (4) | 0 |

[a] For experimental details see Table I.

min. Hydrocortisone administration resulted in increased tyrosine transaminase activity. The rise in activity above the noninduced level was 31% in hepatomas from vitamin $B_6$-deficient animals and did not occur if cycloheximide was given in addition to the hormone. A 47% increase in transaminase activity was observed in hepatomas from animals fed the vitamin-supplemented basal diet. Data regarding serine dehydratase are presented in Table II. Enzymatic activity could not be demonstrated in this hepatoma using the assay method of Suda and Nakagawa (14). In some instances, however, low values were observed which were not significant. Further, the data of Table II demonstrate that depletion of coenzyme (vitamin $B_6$ deficiency) reduced the level of the enzyme in both the liver of normal animals and the host liver of hepatoma-bearing animals. Although a slight increase in dehydratase activity was observed in the host liver of nondeficient animals after hydrocortisone injection, induction of the enzyme did not occur in either the hepatoma or the host liver of animals fed the basal diet lacking the vitamin.

*Discussion.* The results presented in Table I show that the activity level of normal liver and hepatoma transaminase was greater in animals fed the vitamin-supplemented basal diet. Further, induction of the enzyme by hydrocortisone was affected by lack of dietary vitamin $B_6$. A 65% increase over the basal activity level was observed in host liver transaminase of deficient animals and a 140% increase in the host liver transaminase of nondeficient controls (Table I). It is interesting to note that vitamin $B_6$ supplementation resulted in greater hormonal induction in hepatoma transaminase. Increases of 31 and 47% over the basal enzyme level were observed, respectively, in hepatomas from animals fed the basal diet and the basal diet supplemented with pyridoxine. This finding further demonstrates the involvement of vitamin $B_6$ in the induction process of this enzyme, as reported earlier (6). In the previous study (6), the rise of tyrosine transaminase activity following the administration of hydrocortisone was further stimulated in the presence of puromycin. The results presented here, however, show that cycloheximide inhibited the rise in enzymatic activity, due to steroid administration. In both cases, the animals were fed the same basal diet and were deficient in vitamin $B_6$. In the previous study (6), however, Wistar strain animals without tumors were used while in this study tumor-bearing Buffalo strain animals were employed. These observations suggest that the animal strain and/or the presence of tumor affect hepatic transaminase activity and its inhibition by protein synthetic inhibitors in the presence of the steroid. Additional tests are planned in this regard.

Potter and coworkers (16) have examined the activity of this enzyme in various Morris hepatoma lines. The reported activity values varied greatly from almost zero to even greater than that of normal liver. The response of the enzyme to hydrocortisone in different hepatoma lines also varied from complete unresponsiveness to significant increases (17). Our results show a direct involvement of vitamin $B_6$ in the hormonal induction of this enzyme since minimal induction was seen in host livers and hepatomas of animals fed the basal diet lacking the vitamin.

Absence of vitamin $B_6$ from the diet resulted in reduced serine dehydratase levels in both normal and host liver (Table II). The specific activity of this enzyme was reduced by 32% and by as much as 3 times in normal and host liver, respectively. Further, while only a minimal increase in enzyme activity due to steroid administration could be demonstrated in the host livers of animals fed the basal diet supplemented with the vitamin, none occurred in the hepatoma. Vitamin $B_6$ supplementation facilitated the expression of this enzyme in normal and host liver and also its induction in host liver by hydrocortisone. Although hormonal induction of hydratase activity was not observed in animals fed the basal diet, administration of cycloheximide in addition to hydrocortisone resulted in further reduction (47%) in

enzymatic activity. Administration of hydrocortisone alone reduced the basal hydratase level by 30%. Since a higher enzyme basal level and hormonal induction of enzyme activity occurred in animals fed the basal diet supplemented with the vitamin, it appears that pyridoxine affords a "protective or stabilizing effect" of hydratase. The observations with cycloheximide are not understood and further tests are planned in this regard. The observations reported here are in agreement with the findings by other workers (1–6) of reduced levels of protein catabolic enzymes in vitamin $B_6$ deficiency.

Potter *et al.* (16) reported a very low serine dehydratase activity level (10–15 $\mu$moles/g tumor) in hepatoma #7794A, generation 18, which was not influenced by dietary protein intake. These workers used an automated assay method to determine enzymatic activity (19) which for technical difficulties we could not adopt in our laboratory. Instead, the enzyme assay method recommended by Suda and Nakagawa (14) was employed. The presence of serine dehydratase activity in hepatoma 7704A, generation 39(4), was not detected by this method. It is also of interest to note that Davis *et al.* (18) reported that in some hepatomas, serine dehydratase levels were not measurable, while in others they were high and the higher the chromosome number of the hepatoma the higher the activity of this enzyme. The correlation of activity with chromosome number corresponds with the findings of Potter *et al.* (16). Further, Watanabe *et al.* (17) reported an increase of this enzyme in hepatoma 7800 following repeated injections of hydrocortisone for seven days. This, however, was not the case with other hepatoma lines examined by these workers.

*Summary.* The effect of vitamin $B_6$ deficiency on the activity of two vitamin $B_6$-requiring enzymes and the induction of these enzymes by hydrocortisone was studied in normal and hepatoma #7794A-bearing Buffalo rats. Enzyme activities were generally lower in vitamin $B_6$-deficient animals. Serine dehydratase was absent in this hepatoma. Administration of hydrocortisone resulted in increased transaminase activity of normal, host liver, and hepatoma. The increase in activity was greater in animals fed the pyridoxine-supplemented basal diet. Cycloheximide blocked the hormonally induced rise in enzyme activity. Hormonal induction of serine dehydratase was not seen in the host liver or tumor of animals fed the basal diet. However, a small increase in activity did occur in the host liver of animals fed the pyridoxine-supplemented basal diet. Administration of hydrocortisone with or without cycloheximide resulted in lower activity values of this enzyme in the host liver of vitamin $B_6$-deficient animals.

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