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STUDIES WITH TRYPTOPHAN METABOLITES *IN VITRO*—II

EFFECT OF TARTAR EMETIC ON KYNURENINE METABOLISM BY NORMAL MOUSE LIVER

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Abstract—Potassium antimonyl tartrate (tartar emetic) inhibited the conversion of kynurenine to both kynurenic and anthranilic acids in normal mouse liver homogenates. The inhibition is mainly due to a reduction in the level of active pyridoxal. Since the inhibition is easily reversed by the supplementation of exogenous pyridoxal phosphate but not with pyridoxal and ATP, it would seem that tartar emetic inhibited the phosphorylation of pyridoxal. Similar inhibitory effects on the metabolism of kynurenine were observed with deoxypyridoxine phosphate. The latter inhibition is removed in presence of a specific concentration of tartar emetic. The antimony moiety of tartar emetic appears to form an inactive chelate with both pyridoxal phosphate and deoxypyridoxine phosphate which replaces the active counterpart at the active sites of both kynureninase and kynurenine transaminase enzyme systems. Evidence for the formation of these chelates was obtained spectrophotometrically.

THE ASSOCIATION between vesicle schistosomiasis and bladder cancer is generally accepted, but the exact nature of this association is still not clear.[1]

In a previous work from this laboratory,[2] it was observed that infestation with *Schistosoma mansoni* created a deficiency in the phosphorylated pyridoxal (Plp) of the infested liver. This was accomplished by concentrating the cofactor and simultaneously inhibiting the phosphorylation of pyridoxal. Since active pyridoxal is essential for the two main reactions utilizing kynurenine, kynureninase, and kynurenine transaminase, the observed lack of Plp in the infested liver resulted in modified levels of the two metabolites of kynurenine. The observation that several kynurenine metabolites are known bladder carcinogens[3–5] raises the possibility that the increased concentration of these metabolites may be implicated in the high incidence of bladder tumors observed in bilharzial patients.

Since potassium antimonyl tartrate (tartar emetic) has long been used in the treatment of bilharziasis both in the United Arab Republic and abroad, with obvious toxic effects, it was of interest to study its effects on kynurenine metabolism, particularly since the high incidence of bladder tumors in bilharzial patients seems to be associated with a disordered tryptophan metabolism.[6–8]

The present study was initiated to investigate the effects of tartar emetic on the metabolism of kynurenine in the normal mouse liver.

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EXPERIMENTAL

Animals

The animals used in the present experiments were adult albino mice weighing from 15 to 20 g, fed *ad libitum* on a specially prepared diet containing all the necessary factors.

Materials

The tryptophan metabolites; DL-kynurenine sulfate and kynurenic acid together with pyridoxal hydrochloride and deoxypyridoxine hydrochloride were obtained from Sigma Chemical Co. (St. Louis, Mo.). Anthranilic acid was chemically prepared and tested for purity. The adenosine triphosphate used was that marketed by Ormonoterapia Richter Ltd. (Milan, Italy) under the proprietary name ATIPI. α-Ketoglutarate was obtained from L. Light and Co. Ltd. (Colnbrook, England). Pyridoxal phosphate and deoxypyridoxine phosphate were prepared according to the method described by Beiler and Martin.[9] In some experiments pyridoxal phosphate from commercial sources was used (Grade A, Calbiochem, Los Angeles, Calif.). The results obtained with either sample of pyridoxal phosphate were indistinguishable. Tartar emetic was obtained from the Chemical Industries Development (CID, Cairo, U.A.R.). Only redistilled water from an all-glass still was used to make solutions.

Preparation of the homogenates

The mice were killed by exsanguination after being stunned by a blow on the head. The fresh livers were quickly removed and placed in ice-cold 0·25 M sucrose solution. Tissue homogenates were prepared in the isotonic sucrose in a Potter–Elvehjem homogenizer to make a 10% suspension based on the wet weight of the tissue.

Incubations

Incubations were carried out in 25-ml Erlenmeyer flasks shaken in a water bath at 37° with air as the gas phase. All flasks were brought to a final volume of 4 ml with distilled water. Immediately after incubation, 1 ml of 16% trichloroacetic acid was added to each flask and the mixture transferred to centrifuge tubes with 1 ml of distilled water. The precipitate was removed by centrifugation, and the supernatants were frozen until analyzed. Flasks were run in duplicates and a zero-time flask was included in each set of experiments.

The concentrations of the different materials, when present in the incubation medium, unless otherwise stated were as follows: DL-kynurenine sulfate, 5·0 μM; pyridoxal hydrochloride, 40·0 μg; deoxypyridoxine hydrochloride, 40·0 μg; adenosine triphosphate, 0·005 M; calcium chloride, 0·005 M; magnesium sulfate, 0·001 M; potassium phosphate buffer (pH 7·4), 0·05 M; α-ketoglutarate, 30·0 μM; pyridoxal phosphate, 40·0 μg; deoxypridoxine phosphate, 40·0 μg, 10% whole liver homogenate, 2·0 ml.

Quantitative estimation of metabolites

Kynurenine, anthranilic acid, kynurenic acid were determined by the method of Miller *et al.*[10] Kynurenic acid and anthranilic acid were also determined by the method of Mason and Berg.[11]

The concentration of kynurenine in the present study was not rate limiting for the enzymatic reactions studied.[2] An incubation period of 3 hr was found adequate for the optimal utilization of kynurenine and the formation of both metabolites.

RESULTS

The effect of increasing the concentration of the tartar emetic on kynurenine transaminase and kynureninase, as indicated by the amounts of kynurenic acid and anthranilic acid (in μmole/g liver) produced, respectively, are shown in Table 1. At a concentration of 3×10^{-5} M, tartar emetic caused a marked inhibition of

TABLE 1. EFFECT OF INCREASING THE CONCENTRATION OF TARTAR EMETIC ON THE METABOLISM OF KYNURENINE BY NORMAL MOUSE LIVER HOMOGENATES

Experiment No.	Concentration of tartar emetic (M)	Metabolites determined (μmole/g liver)*		
		Kynurenine utilized†	Kynurenic acid	Anthranilic acid
1	0	5·32 ± 0·23	1·46 ± 0·11	2·82 ± 0·14
2	3×10^{-5}	4·93 ± 0·19	0·93 ± 0·10	3·13 ± 0·21
3	3×10^{-4}	5·01 ± 0·27	0·10 ± 0·02	3·70 ± 0·19
4	3×10^{-3}	1·03 ± 0·13	0·00	0·10 ± 0·01
5	$7{\cdot}8 \times 10^{-3}$	0·69 ± 0·09	0·00	0·00

The incubation medium (4 ml) contained 5 μM DL-kynurenine sulfate, 30 μM α-ketoglutarate, and 10% whole liver homogenate (2 ml) in 0·05 M potassium phosphate buffer, pH 7.4. Incubations were carried out for 3 hr at 37°

* Average values of 4–12 experiments ± S.E.

† These values represent the difference between the kynurenine recovered and that originally present in the medium.

kynurenene transaminase as evidenced by the reduced levels of kynurenic acid. However, at this concentration no apparent effect was encountered on kynureninase (experiment 2). Increasing the concentration of tartar emetic produced a more pronounced inhibition of kynurenine transaminase (experiment 3). At concentrations above 3×10^{-4} M, the kynureninase enzyme became progressively inhibited. At a concentration of $7{\cdot}8 \times 10^{-3}$ M, both enzyme systems were completely inhibited, and neither of the metabolites was formed. The amounts of kynurenine utilized in this and other experiments reflected closely the amounts used in the synthesis of the two metabolites. However, slightly more kynurenine was utilized than that converted to both metabolites. The difference, though small and more or less constant, might reflect hydroxylation to 3-hydroxykynurenine or the further metabolism of either of the products. Several of the suspected metabolites were assayed by conventional methods, but extremely small amounts were obtained. It should be noted that no nicotinic acid was synthesized in any of the experiments reported here. The concentrations of tartar emetic used are very similar to the concentrations found in the liver of intact mice given a daily dose of 3 mg tartar emetic/kg for 5 days.[12] It is interesting to note the similarity between the effects of tartar emetic at the lower concentrations (experiment 2 and 3, Table 1) and those of *S. mansoni* infestation (cf. Ref. 2). The possible implications of this similarity will be discussed later.

The inhibition encountered with tartar emetic on either kynurenine transaminase or kynureninase could be reversed only in the presence of active pyridoxal (experiment

3–5 and 9–11, Table 2). Ca^{2+}, Mg^{2+}, ATP, and pyridoxal hydrochloride, individually or together in the concentrations used in the present study, were not able to counteract completely the inhibitory effects of tartar emetic on both enzymes (experiments 1, 2 and 6–8, Table 2).

The effect of increasing the concentration of pyridoxal phosphate on the inhibitory action of tartar emetic on both enzyme systems was also investigated, and the results are shown in Table 3. The results indicate that in the absence of tartar emetic, pyridoxal phosphate stimulated the synthesis of both kynurenic and anthranilic acids. In the presence of tartar emetic, however, pyridoxal phosphate reversed the inhibition to an extent dependent upon the amount of tartar emetic present in the medium. Furthermore, the addition of pyridoxal phosphate in the presence of varying concentrations of tartar emetic failed to result in the production of equivalent amounts of kynurenic and anthranilic acids to those obtained in the absence of tartar emetic; the difference became more pronounced when higher concentrations of tartar emetic were present in the incubation medium.

The effects of deoxypyridoxine phosphate, a known antagonist for pyridoxal phosphate, were also studied on this system (Table 4). The inhibitory effects of deoxypyridoxine phosphate were added to the inhibitory effects of tartar emetic in the concentrations studied (experiments 1 and 7, Table 4). However, pyridoxal phosphate was still able to counteract the combined inhibitory effects of both tartar emetic and deoxypyridoxine phosphate (experiments 4–6, Table 4). The effects of Ca^{2+} and Mg^{2+} were minimal at the concentrations employed (experiments 2, 3, 5, 6, 8–10, Table 4).

The added inhibition of deoxypyridoxine phosphate on the inhibitory effects of tartar emetic was not observed when lower concentrations of tartar emetic were used (Table 5). Thus it is clear that at concentrations of tartar emetic of 3×10^{-5} or $1{\cdot}5 \times 10^{-4}$ M (experiments 2 and 3, Table 5), the amounts of kynurenic and anthranilic acids produced were higher than those formed in the presence of either inhibitor alone (experiments 2–5, Table 1, and experiment 1, Table 5). The level of these metabolites was nearly of the same order as those formed in the presence of the endogenous pyridoxal phosphate without the addition of the cofactor (experimental, Table 1). It is conceivable that this "relief of inhibition" or even the slight stimulation observed when both inhibitors were present together is due to a competition of both endogenous pyridoxal phosphate and deoxypyridoxine phosphate for the antimony of tartar emetic. Since pyridoxal phosphate required the participation of a polyvalent cation for its action,[13] antimony may inhibit both reactions catalyzed by pyridoxal phosphate, i.e. kynurenine transaminase and kynureninase, by forming an inactive chelate with it. However, the presence of deoxypyridoxine phosphate, an antimetabolite of pyridoxal phosphate, might chelate all the antimony present. This would allow the endogenous pyridoxal phosphate to catalyze both reactions by chelating with the activating polyvalent cation. This is potentiated by the obvious dependency of this effect on the concentration of tartar emetic (Table 5). This hypothesis was tested spectrophotometrically. However, no evidence of chelation could be detected with the concentrations used in the incubation with liver homogenate. This might be due to the higher sensitivity of the biological system (mouse liver homogenates) which would allow for the detection of minute amounts of the chelation complex, in contrast to the low sensitivity of the physical method. Spectrophotometric

TABLE 2. EFFECT OF DIFFERENT ADDITIONS ON THE ACTION OF TARTAR EMETIC ON KYNURENINE METABOLISM *in vitro*

Experiment No.	Concentration of tartar emetic (M)	Pyridoxal	ATP	Ca^{2+}	Mg^{2+}	Pyridoxal phosphate	Metabolites determined* (μmole) Kynurenine utilized†	Kynurenic acid	Anthranilic acid
1	3×10^{-4}			+	+		5·62 ± 0·53	0·24 ± 0·02	3·30 ± 0·19
2	3×10^{-4}		+				6·32 ± 0·48	0·54 ± 0·02	3·50 ± 0·21
3	3×10^{-4}					+	22·10 ± 2·73	6·55 ± 0·37	9·40 ± 0·42
4				+		+	19·41 ± 2·36	6·30 ± 0·33	8·50 ± 0·73
5	3×10^{-4}				+	+	20·93 ± 3·02	6·55 ± 0·42	9·40 ± 0·91
6	3×10^{-3}		+				4·18 ± 0·90	0·39 ± 0·03	1·00 ± 0·07
7	3×10^{-3}		+	+	+		5·32 ± 0·43	0·66 ± 0·07	2·65 ± 0·31
8	3×10^{-3}	+	+	+	+		5·38 ± 0·55	0·48 ± 0·03	2·65 ± 0·27
9	3×10^{-3}					+	17·39 ± 3·78	4·63 ± 0·19	7·90 ± 0·41
10	3×10^{-3}	+	+	+	+	+	23·1 ± 4·93	4·41 ± 0·21	13·15 ± 1·30
11	$7{\cdot}8 \times 10^{-3}$					+	14·76 ± 2·11	2·50 ± 0·13	6·25 ± 0·41

Conditions as in Table 1.
* Average of 4–6 experiments ± S.E.
† These values represent the difference between the kynurenine recovered and that originally present in the medium.

TABLE 3. EFFECT OF PYRIDOXAL PHOSPHATE ON THE ACTION OF TARTAR EMETIC ON THE METABOLISM OF KYNURENINE BY NORMAL MOUSE LIVER HOMOGENATE

Experiment No.	Pyridoxal phosphate (μg/g liver)	Molar concentration of tartar emetic* 0 Kynurenic acid (μmole)	0 Anthranilic acid (μmole)	3×10^{-4} Kynurenic acid (μmole)	3×10^{-4} Anthranilic acid (μmole)	3×10^{-3} Kynurenic acid (μmole)	3×10^{-3} Anthranilic acid (μmole)	$7{\cdot}8 \times 10^{-3}$ Kynurenic acid (μmole)	$7{\cdot}8 \times 10^{-3}$ Anthranilic acid (μmole)
1	00	1·46	2·82	0·10	3·70	0·00	0·10	0·00	0·00
2	20	2·20	3·20	1·89	2·95	0·63	2·10	0·20	1·30
3	40	3·90	5·20	3·39	4·60	2·91	5·10	2·60	5·55
4	200	7·20	13·30	6·55	9·40	4·63	7·90	2·50	6·25

Conditions as in Table 1.
* Average of 4–6 experiments.

TABLE 4. EFFECT OF DIFFERENT CONCENTRATIONS OF ADDITIONS ON THE ACTION OF DEOXYPYRIDOXINE PHOSPHATE ON THE INHIBITORY EFFECT OF TARTAR EMETIC ON KYNURENINE METABOLISM BY NORMAL MOUSE LIVER

Experiment No.	Concentration of tartic emetic (M)	Ca^{2+} (M)	Mg^{2+} (M)	Pyridoxal phosphate (μg/g liver)	Deoxypyridoxine phosphate (μg/g liver)	Metabolites determined (μmole/g liver)*		
						Kynurenine utilized†	Kynurenic acid	Anthranilic acid
1	3×10^{-4}				200	2·56 ± 0·19	0·35 ± 0·02	1·60 ± 0·12
2	3×10^{-4}	0·055			80	3·12 ± 0·23	0·15 ± 0·02	1·40 ± 0·19
3	3×10^{-4}		0·011		80	3·12 ± 0·25	0·35 ± 0·02	1·40 ± 0·16
4	3×10^{-4}			200	200	23·75 ± 4·17	7·25 ± 0·32	15·05 ± 2·37
5	3×10^{-4}	0·015		200	200	19·43 ± 3·94	5·85 ± 0·41	9·20 ± 1·97
6	3×10^{-4}		0·003	200	200	17·61 ± 4·15	5·00 ± 0·29	9·10 ± 1·83
7	3×10^{-3}				200	3·73 ± 0·36	0·00	1·50 ± 0·12
8	3×10^{-3}	0·040			200	3·91 ± 0·19	0·065 ± 0·01	1·15 ± 0·16
9	3×10^{-3}		0·008		200	2·76 ± 0·21	0·095 ± 0·01	1·15 ± 0·12
10	3×10^{-3}	0·020	0·004		200	2·83 ± 0·30	0·085 ± 0·01	1·10 ± 0·12
11	$7·8 \times 10^{-3}$				200	1·92 ± 0·12	0·00	0·00

* Average of 4–6 experiments ± S.E.
† These values represent the difference between the kynurenine recovered and that originally present in the medium.

TABLE 5. EFFECT OF DEOXYPYRIDOXINE PHOSPHATE ON THE ACTION OF TARTAR EMETIC ON THE METABOLISM OF KYNURENINE BY NORMAL MOUSE LIVER HOMOGENATES

Experiment No.	Concentration of tartar emetic added (M)	Metabolites determined (μg/g liver)*		
		Kynurenine utilized†	Kynurenic acid	Anthranilic acid
1	0	5·1 ± 0·43	0·36 ± 0·02‡	2·30 ± 0·19‡
2	3×10^{-5}	7·9 ± 0·52	1·65 ± 0·21‡	3·80 ± 0·17‡
3	$1{\cdot}5 \times 10^{-4}$	6·3 ± 0·41	1·20 ± 0·07‡	3·50 ± 0·21‡
4	3×10^{-4}	2·56 ± 0·19	0·35 ± 0·02	1·60 ± 0·12
5	$1{\cdot}5 \times 10^{-3}$	3·80 ± 0·29	0·40 ± 0·02	1·65 ± 0·10
6	3×10^{-3}	3·73 ± 0·36	0·00	1·50 ± 0·12
7	$7{\cdot}8 \times 10^{-3}$	1·92 ± 0·12	0·00	0·00

The incubation medium (4 ml) contained 5 μM DL-kynurenine sulfate, 30 μM α-ketoglutarate, 40 μg deoxypyridoxine phosphate, and 10% whole liver homogenate (2 ml) in 0·05 M potassium phosphate buffer, pH 7·4. Incubations were carried out for 3 hr at 37°.

* Average of 4–6 experiments ± S.E.

† These values represent the difference between the kynurenine recovered and that originally present in the medium.

‡ The difference between the amount of kynurenic acid or anthranilic acid produced in experiment 1 and that produced in either experiment 2 or 3 is statistically significant.

evidence for this chelation phenomenon was obtained when slightly exaggerated conditions were used.

With pyridoxal phosphate solution (10 μg/ml), at pH 9·0 (Fig. 1), the increase in the absorbance at 195 mμ to overshadow the band at 227 mμ in the presence of tartar emetic solution (10 mg/ml), is evidence for the formation of a tartar emetic–pyridoxal

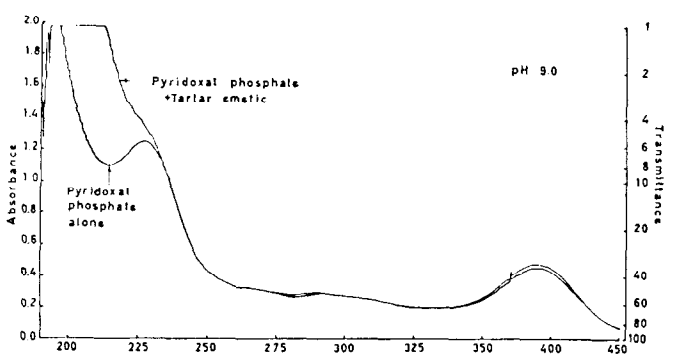

FIG. 1. Effect of tartar emetic on the absorption spectrum of pyridoxal phosphate at pH 9·0. Note the great increase in absorbance at 195 mμ. The band at 227 mμ is overshadowed.

phosphate chelate. At pH 3·0, the appearance of two energy-transfer bands at 220 mμ and 250 mμ is further evidence for the formation of this chelate (Fig. 2).

With deoxypyridoxine phosphate, at pH 9·0 (Fig. 3), no evidence of chelation could be detected. However, at pH 3·0 (Fig. 4), the high increase in the absorbance at 205 mμ to overshadow the band at 227 mμ is evidence for the formation of tartar emetic–deoxypyridoxine phosphate chelate.

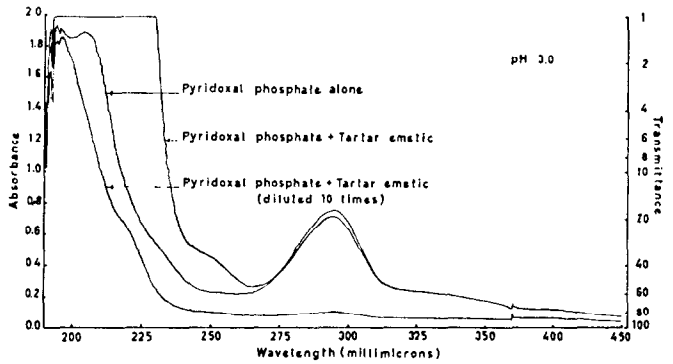

FIG. 2. Effect of tartar emetic on the absorption spectrum of pyridoxal phosphate at pH 3·0. Note the appearance of two energy-transfer bands at 220 mμ and 250 mμ.

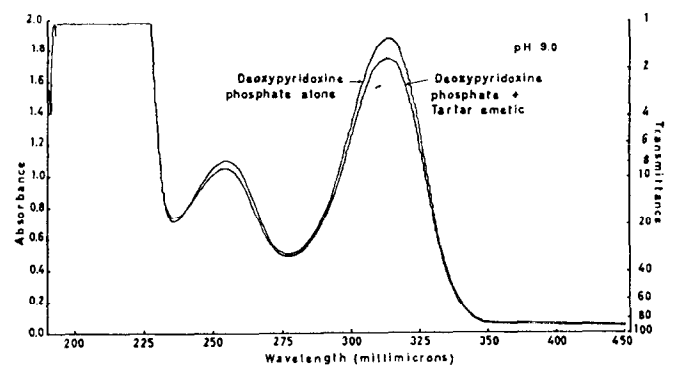

FIG. 3. Effect of tartar emetic on the absorption spectrum of deoxypyridoxine phosphate at pH 9·0. The difference observed is due to dilution.

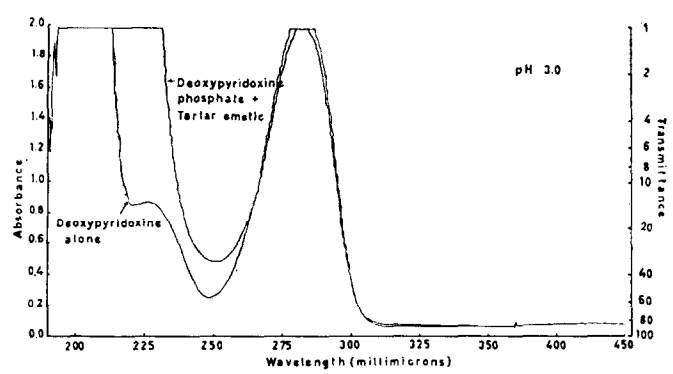

FIG. 4. Effect of tartar emetic on the absorption spectrum of deoxypyridoxine phosphate at pH 3·0. Note the increase in the band at 205 mμ to overshadow the band at 227 mμ.

DISCUSSION

It is evident from the present study that tartar emetic produces functional pyridoxine deficiency in the mouse liver. The deficiency encountered in the presence of low concentrations of the drug is mainly on the kynurenine transaminse system. This is manifested by the low production of kynurenic acid with no detectable effect on the kynureninase enzyme catalyzing the synthesis of anthranilic acid. However, marked inhibition of kynurenine transaminse is produced at higher concentrations of tartar emetic. At the same time, decreased accumulation of anthranilic acid indicates an inhibition of the kynureninase enzyme system also under these conditions. In the absence of pyridoxal phosphate, the higher sensitivity of kynurenine transaminase relative to kynureninase (observed in this study in the presence of tartar emetic) has been noted previously with deoxypyridoxine phosphate, an antimetabolite of pyridoxal phosphate.[2] Thus, it is now certain that kynurenine transaminase is more sensitive and probably has a higher requirement for pyridoxal phosphate than the kynureninase enzyme in the mouse liver. Different sensitivities of pyridoxal phosphate-requiring enzymes, along the formylkynurenine pathway of tryptophan metabolism, have also been reported in various species (review).[13]

The gradual counteraction of the effects of tartar emetic incident to increasing concentration of pyridoxal phosphate indicates that pyridoxal phosphate is the factor directly responsible for the observed inhibition (Table 3). Even in the presence of high concentrations of both tartar emetic and deoxypyridoxine phosphate, pyridoxal phosphate was still able to counteract their combined inhibitory effects (Table 4), thus indicating that pyridoxal phosphate is quite possibly the target of both inhibitors. Furthermore, the inability of pyridoxal hydrochloride, ATP, Ca^{2+}, and Mg^{2+} to counteract the effects of tartar emetic, though present in the concentrations needed to form pyridoxal phosphate,[14] indicates that the phosphorylation of pyridoxal with ATP is impaired in the presence of tartar emetic. As a matter of fact, tartar emetic has been shown to inhibit the phosphorylation of fructose 6-phosphate.[15, 16]

In investigating the site of action of tartar emetic, it has been now suggested that the antimony moiety forms an inactive chelate, substituting the polyvalent cation[13, 17, 18] necessary for the reactions catalyzed by the endogenous pyridoxal phosphate. The amount of pyridoxal phosphate that is not chelated with antimony would form an active chelate with the proper polyvalent cation, thereby being responsible for the varying activities of both enzymes at low tartar emetic concentrations (experimental, Table 1). Spectrophotometric analyses revealed that tartar emetic formed a chelate with pyridoxal phosphate at pH 3 and pH 9 and with deoxypyridoxine phosphate at pH 3, but not at pH 9. Therefore, it is suggested that the formation of an inactive chelate with pyridoxal phosphate and the inhibition of phosphorylation of pyridoxal are at least two main mechanisms by which tartar emetic produces its observed effects on kynurenine transaminase and kynureninase enzymes. The significance of these findings on the observed toxicity of tartar emetic in intact animals and in man remains to be established. Studies in the intact animals are now in progress.

The present study indicates that tartar emetic, which has long been used for the treatment of bilharziasis in man, produces similar effects on kynurenine metabolism as the infestation itself.[2] This is striking, since the effects produced by the infesting worms may result in a higher incidence of bladder tumors in the host.[1] The inhibitory effects of the worms on the reactions utilizing kynurenine[2] may be increased by this

drug. This could, at least in part, account for the sometimes observed high incidence of bladder tumors in the bilharzial patients repeatedly treated with tartar emetic.

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REFERENCES

1. J. CLEMMESEN (Ed.), *Symposium on Cancer of the Urinary Bladder*, p. 7. Karger, Basel (1963).
2. M. SAMIR AMER, M. H. ABDEL-DAIM and G. A. ABDEL-TAWAB, *Biochem. J.*, in press.
3. E. BOYLAND, *The Biochemistry of Bladder Cancer*. Thomas, Springfield, Illinois (1963).
4. G. T. BRYAN, R. R. BROWN and J. M. PRICE, *Cancer Res.* **24**, 596 (1964).
5. G. T. BRYAN, R. R. BROWN and J. M. PRICE, *Cancer Res.* **24**, 582 (1964).
6. M. A. M. ABUL-FADL and A. S. KHALAFALLACH, *Br. J. Cancer* **15**, 479 (1961).
7. A. S. KHALAFALLACH and M. A. M. ABUL-FADL, *Br. J. Cancer* **18**, 592 (1964).
8. G. A. ABDEL-TAWAB, F. S. KELADA, N. L. KELADA, M. H. ABDEL-DAIM and N. MAKHYOUN, *Int. J. Cancer* **1**, 377 (1966).
9. J. M. BEILER and G. J. MARTIN, *J. biol. Chem.* **169**, 345 (1947).
10. I. L. MILLER, M. TAUCHIDA and E. A. ADELBERG, *J. biol. Chem.* **203**, 205 (1953).
11. M. MASON and C. P. BERG, *J. biol. Chem.* **195**, 515 (1952).
12. G. F. OTTO and T. H. MAREN, *Am. J. Hyg.* **51**, 370 (1950).
13. P. HALTZ and D. PALM, *Pharmac. Rev.* **16**, 113 (1964).
14. I. C. GUNSALUS and W. E. RAZZELL, *Methods in Enzymology*, Vol. 2, p. 646. Academic Press, New York (1955).
15. E. BUEDING, *Br. J. Pharmac.* **12**, 159 (1957).
16. E. BUEDING, *Biochem. Pharmac.* **15**, 1107 (1966).
17. D. E. METZLER, M. IKAWA and E. E. SNELL, *J. Am. chem. Soc.* **76**, 648 (1954).
18. J. B. LONGENECKER and E. E. SNELL, *J. biol. Chem.* **213**, 229 (1955).