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STUDIES WITH TRYPTOPHAN METABOLITES *IN VITRO*—III

THE EFFECT OF SCHISTOSOMICIDAL DRUGS ON KYNURENINASE AND KYNURENINE TRANSAMINASE OF NORMAL MOUSE LIVER

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Abstract—The effects of four antischistosomal drugs on the metabolism of kynurenine were investigated. The antimony-containing drugs produced inhibition of both kynureninase and kynurenine transaminase. The inhibition was directly related to the antimonial contents of the drugs. 5-(Nitro-thiazolyl)-2-oxo-tetrahydroimidazole (NTOTI), which does not contain antimony, failed to inhibit either enzyme. The possibility of the formation of an inactive chelate between the antimony and pyridoxal phosphate, which is a needed cofactor in both enzyme reactions, was offered as a mechanism for the observed inhibitions.

IN A PREVIOUS report from this laboratory it was shown that potassium antimonyl tartarate (tartar emetic), which is used extensively for the treatment of bilharziasis both in the United Arab Republic and abroad, inhibited both kynureninase and kynurenine transaminase in normal mouse liver[1] and kidney homogenates.[2] This inhibition resulted in a change in the relative concentrations of the different metabolites in the formylkynurenine pathway of tryptophan metabolism. Since many of these metabolites are known bladder carcinogens,[3–5] this effect might be related to the development of bladder tumors in bilharzial patients treated with tartar emetic. It was of interest to study the effects of other antibilharzial agents on this pathway to determine whether they also produce similar effects. In the present study, the effects of Stibophen, lithium antimonyl thiomalate (LAT), and 5-(nitro-thiazolyl)-2-oxo-tetrahydroimidazole (NTOTI) were compared with the effects of tartar emetic. The results indicate that the observed effects on kynurenine metabolism are dependent on the antimony content of the various drugs. NTOTI produced no inhibition of either kynureninase or kynurenine transaminase *in vitro*.

MATERIALS AND METHODS

Animals. Adult albino mice weighing from 15 to 20 g fed *ad libitum* on a specially prepared diet containing all the necessary factors were used.

Materials. Kynurenic acid and kynurenine sulfate were purchased from Sigma Chemical Co. (St. Louis, Mo.). α-Ketoglutarate was supplied by L. Light and Co.,

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Ltd. (Colnbrook, England). Anthranilic acid was prepared and tested for purity in this laboratory. Pyridoxal phosphate was also prepared in this laboratory by using the method described by Beiler and Martin.[6] In some experiments, high quality pyridoxal phosphate from commercial sources was used (Grade 'A' Calbiochem, Los Angeles, Calif.). The results obtained with either sample of pyridoxal phosphate were statistically indistinguishable. Redistilled water from an all-glass still was used to make solutions.

Drugs. The following drugs were used: Stibophen, lithium antimonyl thiomalate (LAT), potassium antimonyl tartarate, and 5-(nitro-thiazolyl)-2-oxo-tetrahydro-imidazol (NTOTI). The latter was used as a solution in polyethylene glycol 400 prepared in the cold (100 mg/ml). A polyethylene glycol control was always included in each experiment where NTOTI was used. All other drugs were dissolved in the potassium phosphate buffer (0·05 M, pH 7·4) used as the incubation medium.

Preparation of the homogenates. The mice were killed by exsanguination after stunning by a blow on the head. The fresh livers were quickly removed and placed in ice-cold 0·25 M sucrose solution. Tissue homogenates (10 per cent based on the wet weight of the tissue) were prepared in the cold isotonic sucrose solution by using a Potter–Elvehjem homogenizer.

Incubations. Reaction mixtures (final volume, 4 ml) were incubated in 25-ml Erlenmeyer flasks shaken in a water bath kept at 37° with air as the gas phase. At the end of the 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 duplicate 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: DL-kynurenine sulfate, 5·0 μM; potassium phosphate buffer (pH 7·4), 0·05 M; α-ketoglutarate, 30·0 μM; calcium chloride, 0·005 M; magnesium sulfate, 0·001 M; pyridoxal phosphate, 40·0 μg; 10 per cent whole liver homogenate, 2·0 ml.

Quantitative estimation of metabolites. Kynurenine, anthranilic and kynurenic acids were determined by the method of Miller *et al.*[7] Kynurenic acid and anthranilic acid were also determined by the method of Mason and Berg.[8]

RESULTS

The effects of increasing the concentration of tartar emetic and inhibiting concentrations of both Stibophen and LAT and the highest attainable concentration of NTOTI on the production of kynurenic acid and anthranilic acid in the presence of varying concentrations of pyridoxal phosphate (PLP) were determined (Figs. 1 and 2).

As can be seen from Figs. 1 and 2, only partial counteraction of the inhibitory effects of the schistosomicidal drugs on the two enzyme systems can be produced with increasing concentrations of pyridoxal phosphate. The amounts of both kynurenic and anthranilic acids produced in the presence of these drugs were always lower than those produced in their absence. This indicates a noncompetitive type of inhibition of these drugs on kynureninase and kynurenine transaminase. The increase in the production of both kynurenic and anthranilic acids in response to increasing the concentrations of PLP added probably reflects stimulation of the uninhibited portion of the enzymes.

With higher concentrations of the schistosomicidal drugs, a greater portion of the enzymes are inhibited and lesser recovery is produced by the supplementation of PLP.

As postulated earlier,[1] the inhibitory effects of the antimony-containing schistosomicidal drugs might be due to a reduction of the functional PLP necessary for the action of both enzymes. This is probably due to complex formation between PLP and the antimony contained in these drugs. The resulting inactive complex would replace the active complex between PLP and polyvalent cations.

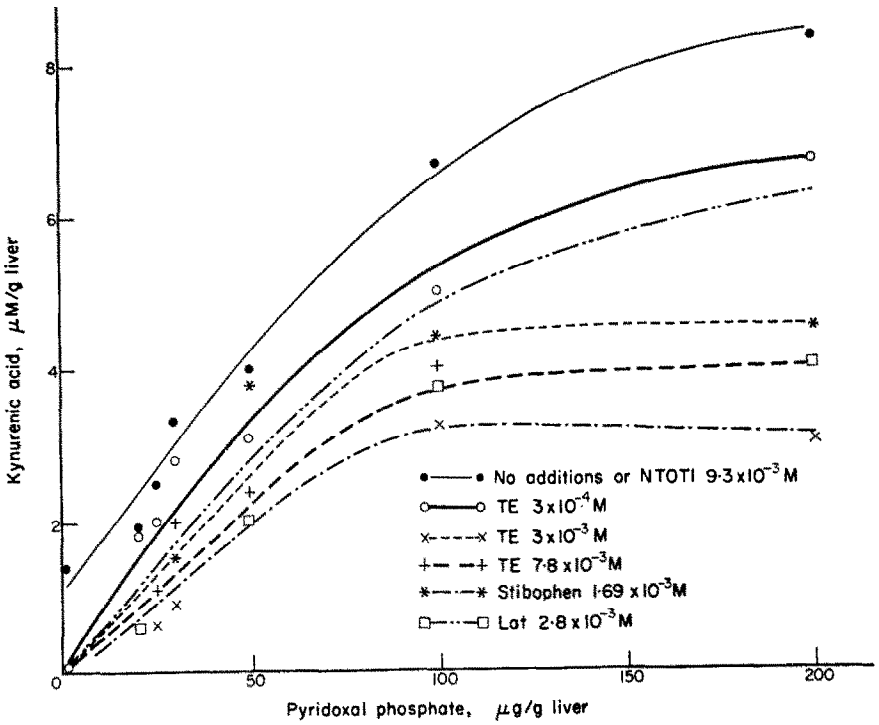

FIG. 1. Effect of three different concentrations of tartar emetic (TE) and inhibiting concentrations of both Stibophen and lithium antimonyl tartarate (LAT) and the maximal attainable concentration of 5-(nitro-thiazolyl)-2-oxo-tetrahydroimidazole (NTOTI) on the production of kynurenic acid from kynurenine by normal mouse liver homogenates in the presence of increasing concentrations of pyridoxal phosphate in the medium. The incubation medium (4 ml) contained 5 μM DL-kynurenine sulfate, 20μM α-ketoglutarate, 0·005 M calcium chloride, 0·001 M magnesium sulfate and 10 per cent whole liver homogenate (2 ml) in 0·05 M potassium phosphate buffer, pH 7·4. Incubations were carried out for 3 hr at 37°. The ordinate represents the amount of kynurenic acid produced (in μg/g liver) in 3 hr. The abscissa represents the concentration of pyridoxal phosphate.

To further test this hypothesis, the effects of high concentrations of calcium and magnesium on the inhibitory effects of tartar emetic were investigated. The results are shown in Table 1. From the table, it is clear that both calcium and magnesium can at least partially counteract the inhibitory effects of tartar emetic. This is possibly due to the competition with antimony for pyridoxal phosphate with the production of the active pyridoxal phosphate chelate. The mechanism of the inhibition produced by Stibophen and LAT seems to be similar to that of potassium antimonyl tartarate. However, Stibophen seems to be slightly more active than tartar emetic on a molar basis. It can also be seen from Figs. 1 and 2 that NTOTI, which is a very active anti-

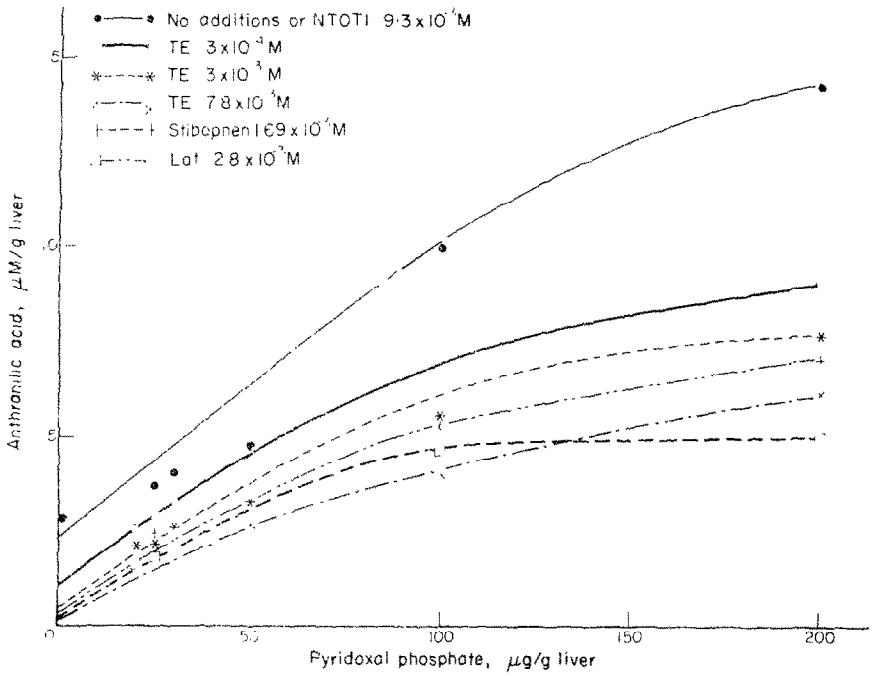

FIG. 2. Effect of three different concentrations of tartar emetic (TE) and inhibiting concentrations of both Stibophen and lithium antimonyl tartarate (LAT) and the maximal attainable concentration of 5-(nitro-thiazolyl)-2-oxo-tetrahydroimidazole (NTOTI) on the production of anthranilic acid from kynurenine by normal mouse liver homogenates in the presence of increasing concentrations of pyridoxal phosphate in the medium. The conditions are the same as in Fig. 1.

TABLE 1. EFFECTS OF INCREASING THE CONCENTRATION OF CALCIUM AND MAGNESIUM IONS ON THE INHIBITION BY TARTAR EMETIC (3×10^{-4} M) OF KYNURENINASE AND KYNURENINE TRANSAMINASE IN NORMAL MOUSE LIVER HOMOGENATES*

Ion concn M		Metabolites determined (μmoles/g liver)†		
Ca^{++}	Mg^{++}	Kynurenine utilized‡	Kynurenic acid	Anthranilic acid
		4·82 ± 0·53	1·31 ± 0·21	2·10 ± 0·31
0·005	0·001§	5·32 ± 0·23	1·46 ± 0·11	2·82 ± 0·14
0·005	0·001	5·01 ± 0·27	0·10 ± 0·02	3·7 ± 0·19
0·065	0·001	9·11 ± 0·87	2·3 ± 0·19	5·3 ± 0·48
0·013	0·005	8·32 ± 0·73	2·6 ± 0·23	4·0 ± 0·32

* The incubation medium (4 ml) contained 5 μM DL-kynurenine sulfate, 30 μM α-ketoglutarate, tartar emetic (3×10^{-4} M), 10 per cent liver homogenate (2 ml) in 0·05 M potassium phosphate buffer, pH 7·4. Incubations were carried out for 3 hr at 37°.

† Average 4–6 experiments ± S.E.

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

§ No tartar emetic added.

schistosomal drug that does not contain antimony, did not have any inhibitory effects on this system in the concentration used. Thus, it seems that the effects observed are more related to the antimony content of the drugs used. LAT also contains sulfur. It is not clear whether the sulfur content of LAT plays a role in its inhibitory action on the two enzyme systems. When the per cent inhibition observed for the two enzyme systems was plotted against the logarithm of molar concentration of the antimony

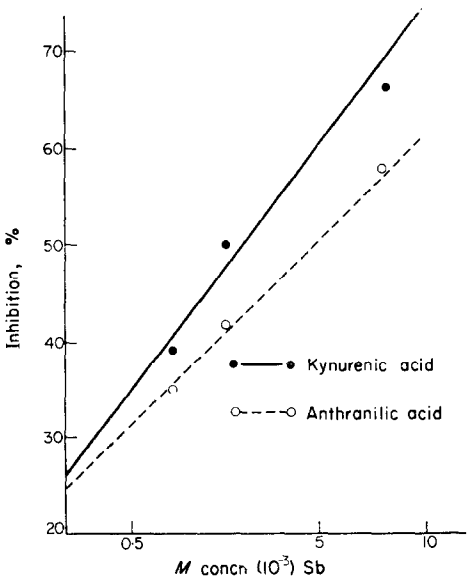

FIG. 3. Inhibition of the production of both anthranilic and kynurenic acids (as per cent of control) as a function of the molar concentration of antimony in schistosomicidal drugs.

present in the medium, a straight line was obtained (Fig. 3) for either enzyme. This indicates that the inhibition observed is directly related to the concentration of antimony in the medium. Therefore, it does not seem that the sulfur contained in LAT plays any significant role in its inhibitory effects on kynureninase or kynurenine transaminase in the mouse liver.

DISCUSSION

It is clear from the results already described that the antimony-containing antibilharzial agents used in the present study produce significant inhibitions of the two PLP-dependent reactions, viz. kynurenine transaminase and kynureninase. This inhibition seems to be due to a reduction of the functional pyridoxal phosphate concentration at the enzyme level, which was also observed in the livers of mice infested with *Schistosoma mansoni*.[9] The inhibition produced by antimony-containing drugs might be due to the formation of an inactive chelate between PLP and the antimony contained in these compounds. NTOTI, which is an antimony-free antibilharzial agent did not produce any observable inhibition of the two enzyme systems. It is interesting to note that Mg^{++} and Ca^{++} in high concentrations are able, at least partially, to reverse the inhibitory effects of tartar emetic. It would seem, therefore, that the stability constant of the PLP-antimony chelate is not very high and that it

could be dissociated in the presence of high concentrations of Ca^{++} and Mg^{++}. Studies of the stability constants of these chelates are under way.

From the results already described, it is clear that the antimony-containing anti-bilharzial drugs produce significant alteration of the relative concentrations of the different metabolites of the formylkynurenine pathway of tryptophan metabolism. This, combined with the similar effects produced by bilharzial infestation alone,[9] might at least partly explain the high incidence of bladder tumors in bilharzial patients treated with these drugs. No such effects were found under our conditions for the non-antimony-containing NTOTI. It would be interesting to see whether this is reflected in a lower incidence of bladder tumors in patients treated with this drug as compared with those treated with the older antimony-containing compounds.

Studies on the urinary levels of different tryptophan metabolites in bilharzial patients treated with these different compounds would answer this question and allow more adequate conclusions concerning the effects of these drugs when used in the treatment of bilharziasis in man.

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