Studies with Tryptophan Metabolites *in vitro*

KYNURENINE METABOLISM IN KIDNEYS OF MICE INFESTED WITH *SCHISTOSOMA MANSONI*

By M. SAMIR AMER,* M. H. ABDEL-DAIM AND G. A. ABDEL-TAWAB
*Department of Pharmacology, Faculty of Pharmacy and the Cancer Research Unit,
Medical Research Institute, Alexandria, U.A.R.*

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The conversion *in vitro* of kynurenine into kynurenic acid and anthranilic acid in both normal kidneys and those obtained from mice infested with *Schistosoma mansoni* was investigated. Normal mouse kidneys seem to possess an excess of functional pyridoxal phosphate over those obtained from infested mice. Kynureninase and kynurenine transaminase in the latter kidneys are more easily inhibited by deoxypyridoxal phosphate and tartar emetic, indicating low stores of active pyridoxal phosphate. The possible implication of these findings in relation to the role of the kidneys in producing abnormal patterns of tryptophan metabolism and possibly contributing to the production of bladder tumours in bilharzial patients is discussed.

Previous work from this Laboratory has indicated that *Schistosoma mansoni* infestation produces a deficiency of active pyridoxal in the infested mouse liver (Amer, Abdel-Daim & Abdel-Tawab, 1967a). It was also shown that treatment with tartar emetic (Amer, Abdel-Daim & Abdel-Tawab, 1967b) or with other antimonials (Amer, Abdel-Daim & Abdel-Tawab, 1968) produces a similar deficiency in pyridoxal phosphate. The latter is probably due to the formation of an inactive chelate between pyridoxal phosphate and the antimony contained in these drugs instead of the active chelate of pyridoxal phosphate with the proper multivalent cation. These effects are important in the possible aetiology of bladder tumours in bilharzial patients, particularly those treated with antimony-containing drugs. Decrease in the active concentrations of pyridoxal phosphate and the consequent inhibition of the two pyridoxal phosphate-requiring enzymes kynurenine transaminase and kynureninase result in altered metabolism of tryptophan. The latter would be manifested by the increased urinary excretion of certain tryptophan metabolites (Abdel-Tawab, Kelada, Abdel-Daim & Makhyou, 1966; Abdul-Fadl & Khalafallah, 1961; Khalafallah & Abdul-Fadl, 1964). Since some of these metabolites are carcinogenic (Boyland & Watson, 1956; Allen, Boyland, Dukes, Horning & Watson, 1957; Bryan, Brown & Price, 1964; Bryan, Morris & Brown, 1965), the possibility of a causal relationship to

the induction of bladder tumours exists (Boyland, 1963; Wallace & White, 1963; Alifano, Papa, Taurelli, Elicio & Quagliariello, 1964; Price & Brown, 1962). It was decided to study the effects of *S. mansoni* infestation together with the effect of antimonials on the metabolism of kynurenine by the kidney to determine whether that organ contributes to the altered rate of excretion of tryptophan metabolites in the urine. In the present study, the metabolism of kynurenine by homogenates of normal mouse kidneys and of those obtained from mice infested with *S. mansoni* was investigated. The results indicate that normal mouse kidneys seem to have an excess of pyridoxal phosphate, whereas there is no excess of pyridoxal phosphate in the infested mouse kidneys. Treatment with tartar emetic resulted in the inhibition of both kynureninase and kynurenine transaminase.

MATERIALS AND METHODS

Animals. Adult albino mice weighing 15–20 g. fed *ad libitum* on a specially prepared diet containing all the necessary factors were used. Exposure of mice to cercariae of *S. mansoni* was started 60 days before the experiments (Oliver & Stirewalt, 1952). The infested animals were used only after producing eggs for at least 1 week.

Materials. Kynurenine sulphate and kynurenic acid were purchased from Sigma Chemical Co. (St Louis, Mo., U.S.A.). α -Oxoglutarate was supplied by L. Light and Co. Ltd. (Colnbrook, Bucks.). Anthranilic acid was prepared in this Laboratory (m.p. 144–145°). Pyridoxal phosphate and deoxypyridoxine phosphate were also prepared in this

* Present address: 4221 South Western Boulevard, Chicago, Ill. 60609, U.S.A.

Laboratory by the method of Beiler & Martin (1947). In some experiments high-quality pyridoxal phosphate from commercial sources was used (grade A; Calbiochem, Los Angeles, Calif., U.S.A.). The results obtained with either sample of pyridoxal phosphate were statistically indistinguishable. Redistilled water from an all-glass still was used to make the solutions.

Preparation of the homogenates. The mice were killed by exsanguination after being stunned by a blow on the head. The fresh kidneys were quickly removed and placed in ice-cold 0.25 M-sucrose solution. Tissue homogenates (10%, wet wt./vol.) were prepared in the 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% (w/v) trichloroacetic acid was added to each flask and the mixture transferred to centrifuge tubes with 1 ml. of water. The precipitate was removed by centrifugation and the supernatants were frozen until analysed. 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 sulphate (5.0 μ M), CaCl₂ (5 mM), MgSO₄ (1 mM), potassium phosphate buffer, pH 7.4 (0.05 M), α -oxoglutarate (30.0 μ M), pyridoxal phosphate (40.0 μ g.), deoxypyridoxal phosphate (40.0 μ g.), 10% whole-kidney homogenate (2.0 ml.).

Quantitative determination of metabolites. Kynurenine, anthranilic acid and kynurenic acid were determined by the method of Miller, Tauchida & Adelberg (1953). Kynurenine and anthranilic acid were also determined by the method of Mason & Berg (1952).

RESULTS

The production of kynurenic acid and anthranilic acid from kynurenine was studied in both normal kidney homogenates and those obtained from *S. mansoni*-infested mice. The results are shown in Table 1. It is clear that, in the absence of any additions to the system, the kidneys obtained from infested mice metabolize kynurenine in a manner similar to that of normal mice (Expt. 1). When

exogenous pyridoxal phosphate is added (Expt. 2), no appreciable effect on the metabolism of kynurenine by either kidney is produced. However, the addition of deoxypyridoxal phosphate produces significant inhibitions in the production of both kynurenic acid and anthranilic acid by the infested mouse kidney, whereas the only effect on the normal kidney is a slight inhibition of kynureninase with a decreased production of anthranilic acid (Expt. 3). Tartar emetic, in the concentration used, appears to inhibit the two enzyme systems in both kidneys. However, the inhibition is more pronounced in the infested mouse kidney. The anthranilic acid/kynurenic acid ratio indicates that, in presence of tartar emetic, kynurenine transaminase is more inhibited relative to kynureninase in the infested mouse kidney, whereas a slightly opposite situation may exist in the presence of deoxypyridoxal phosphate in the normal kidney. The inhibition of the normal kidney by tartar emetic seems to be distributed evenly between both kynureninase and kynurenine transaminase with not much change in the anthranilic acid/kynurenic acid ratio. The amount of kynurenine utilized generally reflects the amount used in the formation of kynurenic acid and anthranilic acid. In most instances, more kynurenine is utilized than can be accounted for by the conversion into the two acids, which could be used in the formation of 3-hydroxykynurenine and other products not assayed in the present experiments. In the experiments with the kidneys obtained from *S. mansoni*-infested animals in the presence of tartar emetic, considerably more kynurenine was utilized than can be accounted for by the formation of kynurenic acid and anthranilic acid. The reason for this is not known.

DISCUSSION

It is evident from the present study that the *S. mansoni*-infested mouse kidney metabolizes kynurenine in a manner similar to that of the normal

Table 1. *Kynurenine metabolism in normal and S. mansoni-infested mouse kidney*

Incubations were carried out for 3 hr. at 37° in a total volume of 4 ml. containing 10% kidney homogenate (2 ml.). When present, the final concentrations were: CaCl₂ (5 mM), MgSO₄ (1 mM), pyridoxal phosphate (40 μ g.), deoxypyridoxal phosphate (40 μ g.), α -oxoglutarate (30 mM) and DL-kynurenine sulphate (5.0 mM). The results are calculated as μ moles/g. of kidney and are the means of four to eight experiments $\pm$ S.E.M. The values given for kynurenine utilized represent the difference between the kynurenine recovered and that originally present in the medium/g. of kidney. N, Normal kidney; I, kidney from infested animals.

Expt. no.	Addition to system	Metabolite determined								Anthranilic acid Kynurenic acid ratio	
		Kynurenine utilized		Kynurenic acid		Anthranilic acid				N	I
		N	I	N	I	N	I				
1	None	13.4 ± 1.2	12.5 ± 3.2	2.9 ± 0.3	2.5 ± 0.3	9.2 ± 0.9	10.8 ± 1.5	3.1	4.2		
2	Pyridoxal phosphate	15.3 ± 2.4	13.8 ± 2.4	3.2 ± 0.2	2.6 ± 0.3	10.6 ± 1.3	8.4 ± 1.0	3.3	3.2		
3	Deoxypyridoxal phosphate	10.0 ± 2.6	2.1 ± 0.2	3.2 ± 0.4	0.0 ± 0.0	7.0 ± 0.9	0.0 ± 0.0	2.2	0.0		
4	Tartar emetic (3mM)	9.55 ± 1.16	14.7 ± 2.0	2.1 ± 0.3	0.7 ± 0.1	7.0 ± 0.8	5.0 ± 0.6	3.3	6.9		

kidney. However, the normal kidney seems to possess excess of pyridoxal phosphate, which is not the case in the infested mouse kidney. The latter seems to have just enough pyridoxal phosphate to saturate both its enzyme systems. Additional pyridoxal phosphate does not affect the amounts of kynurenic acid or anthranilic acid produced, since pyridoxal phosphate appears to be already present in sufficient quantities in both kidneys. However, the addition of the pyridoxal phosphate anti-metabolite, deoxypyridoxal phosphate, produces complete inhibition of both enzyme systems in the infested kidney with no production of either kynurenic acid or anthranilic acid. The effect of deoxypyridoxal phosphate on kynurenine metabolism in the normal kidney is manifested only by a slight inhibition of kynureninase and a decreased production of anthranilic acid. The lack of inhibition in the normal kidney might be due to the presence of extra concentrations of pyridoxal phosphate that could counteract the added deoxypyridoxal phosphate. It is noteworthy that the normal kidney metabolizes kynurenine in a manner similar to the normal liver when the latter is supplemented with exogenous pyridoxal phosphate. In the latter case, the addition of deoxypyridoxal phosphate also resulted in a slight decrease in kynureninase activity (Amer *et al.* 1967a).

Tartar emetic, when present at a concentration comparable with that attainable *in vivo* (Otto & Maren, 1950), inhibits both kynureninase and kynurenine transaminase in both the normal and infested mouse kidneys. The inhibition is more pronounced on the infested mouse kidney and in particular on kynurenine transaminase. The preferential inhibition of kynurenine transaminase by tartar emetic was also observed in the mouse liver (Amer *et al.* 1967b). This might again be due to the higher sensitivity of kynurenine transaminase to pyridoxal phosphate lack. Different sensitivities of pyridoxal phosphate-requiring enzymes along the formylkynurenine pathway of tryptophan metabolism have been reported previously (Holtz & Palm, 1964). Though the kidney seems to metabolize kynurenine at a higher rate than the liver on a weight to weight basis under normal conditions, i.e. in the absence of supplemented pyridoxal phosphate, the actual contribution of kidney to the metabolism *in vivo* is expected to be small, owing to the relatively smaller size of that organ. The metabolism of kynurenine by the kidney seems to

be less readily affected by either *S. mansoni* infestation or tartar emetic than the metabolism by the liver. However, treatment with antimony-containing schistosomicidal agents might lead to altered tryptophan metabolism in the kidney. In the latter case, the kidney would then begin to contribute to the already abnormal pattern originating in the liver. This might contribute to the possible development of bladder tumours.

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