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Enzymic hydrolysis of malaoxon by mouse liver homogenates

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In a previous communication [1] we reported that malathion (*S*-(1,2-dicarbethoxyethyl) *O,O*-dimethyl phosphorodithioate) is hydrolysed by two types of esterases: malathion B-esterase which is predominently microsomal and acts at a pH between 7·4 and 7·6, and malathion A-esterase which is predominently in the cell-sap, acts at a pH of 8·8 and requires SH group activators such as 2-mercaptoethanol or reduced glutathione. The malathion esterase activity was determined by a newly developed colorimetric procedure [2].

Malaoxon (*S*-(1,2-dicarbethoxyethyl) *O,O*-dimethyl phosphorothioate) is the toxic oxygen analogue of malathion. It is thus an anti-cholinesterase (anti-ChE) agent and a carboxylesterase inhibitor. It has two carboxyethyl ester groups, and by analogy with malathion, it is considered that detoxification is effected by enzymatic hydrolysis of one of these ester groups by the non-specific B-esterases [3, 4]. There is, however, no definite evidence to show that malaoxon is, in fact, hydrolysed by the same enzymes which are also inhibited by it. The only work in which a carboxylesterase is implicated in the hydrolysis of malaoxon is that of Murphy and DuBois [5] who used a preparation of malaoxon obtained by the oxidation of malathion by bromine water. The enzymatic hydrolysis of their preparation was shown to be inhibited by EPNO (*O*-ethyl *O*-*p*-nitrophenyl phenylphosphonate, the oxygen analogue of EPN) which is a powerful B-esterase inhibitor. This is considered as evidence that malaoxon is hydrolysed by a B-type of esterase, and a dual role is assigned to malaoxon as an inhibitor as well as a substrate for such esterases [3, 4].

Recently we developed a method [6] for the determination of malaoxon in the presence of malathion. Using the method we analysed malaoxon preparations obtained by the procedure of Murphy and DuBois [5]. The results in Table 1 show that the amount of malaoxon in these preparations is low (about 7%) the remainder being malathion. To obtain higher yields more bromine water has to be used. Also, the technique used by Murphy and DuBois [5] and by Cohen and Murphy [7], to measure malaoxon esterase activity was a bio-assay in which the un-hydrolysed malaoxon was determined by its anti-ChE activity. This procedure, understandably, will not distinguish between a carboxylesterase of the B-type and a phosphotriesterase which belongs to the A-type according to the classification of Aldridge [8]. The question is thus open whether malaoxon is both an inhibitor and a substrate for non-specific carboxylesterases. In this connection it would be of interest to study which of the two malathion esterases [1] acts upon malaoxon.

The malaoxon used in our studies was obtained from American Cyanamid Co., Princeton, N.J. As determined by our procedure [6] it was quite pure, containing not more than 0·6% malathion. Malaoxon suspensions of

Table 1. Conversion of malathion to malaoxon by bromine water

Expt. No.	Vol. in ml of saturated bromine water (approx 0·1 M) added to 10 μmoles of malathion	Per cent conversion to malaoxon
1	0·024	7·3
2	0·20	22·4
3	0·40	30·2
4	0·60	60·4
5	0·80	76·2
6	1·00	100·0

Expt. 1 was performed according to Murphy and DuBois [5]. 5 ml of 1×10^{-3}M aqueous suspension of malathion was treated for 1 hr with 2 ml water and 3 ml of dilute bromine water (0·1 ml of saturated bromine water diluted to 25 ml). The volume of bromine water given in table is the equivalent in terms of saturated bromine water. In other experiments 1 ml (10 μmoles) of malathion in propylene glycol was treated with saturated bromine water as indicated. After a few minutes the mixture was diluted to 10 ml with water containing 0·1% Triton X-100, extracted with 10 ml cyclohexane and the solvent layer separated and clarified by anhydrous sodium sulphate. Aliquots of the solvent phase were leached with alkaline hydroxylamine to remove malaoxon and the residual malathion was assayed as described in Ref. 6. The malaoxon content was obtained by difference. The values are averages of 3 experiments.

1.5×10^{-3}M in 0·05 M Tris–HCl buffer pH 7·6 containing 0·1% Trition X-100 were incubated at 37° with 0·5–2·0% mouse liver homogenate. Aliquots of the digest were extracted at 5-min intervals up to 60 min with an equal volume of *iso*butanol–benzene (1:1), and the residual malaoxon was assayed after evaporation of the solvent as described previously [6]. It was found that the hydrolysis of malaoxon under these conditions was negligible. Malaoxon is obviously only an inhibitor of non-specific B-esterases.

In 0·05 M Tris–HCl buffer pH 8·8. the activity was low (about 4 μmoles of malaoxon hydrolysed/g of wet liver per hr) when no SH group activator was added. In the presence of 10^{-3}M 2-mercaptoethanol in the buffer and 10^{-3}M reduced glutathione in the digest the activity was 26·1 ± 6·1 (S.D.) μmoles of malaoxon/g liver per hr (8 determinations). The value was not significantly affected when the assay system contained 10^{-5}M DFP. It is therefore concluded that malaoxon esterase is an A-type esterase and the B-type of esterase is without effect on malaoxon.

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The effect of allylisothiocyanate and other antithyroid compounds on blood coagulation in rats

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The seeds of various plants of the Brassica family are goitrogenic when fed to rats [1, 2]. The active component is considered to be a mixture of esters of isothiocyanic acid in the form of glucosides. These esters commonly called essential oil of mustard, have a high content of allylisothiocyanate (AITC) which is also goitrogenic [3]. Ahmad *et al.* [4] showed that AITC reduces blood coagulation time in rats. Since blood phospholipids are involved in blood coagulation [5], it was of interest to see if there were any changes in the plasma phospholipids following the administration of allylisothiocyanate associated with reduced blood coagulation time. As AITC is an antithyroid compound we studied the effect of other antithyroid compounds such as thiourea and thiouracil on blood coagulation time and plasma phospholipids and observed their effect in combination with thyroxine (T4).

MATERIALS AND METHODS

Female Norwegian rats, 8–12 weeks old and weighing 110–180 g, were kept on a basal diet [4] for 1 week before the experiment. On the eighth day, the animals were divided into eight groups. One of the groups served as a control and the remaining groups were treated for 7 days with 2 mg AITC, 23·3 μg thyroxine, 0·5 mg thiourea or 0·5 mg thiouracil per 100 g body weight administered intraperitoneally in 0·5 ml of water, alone or in combination.

Blood was collected from the tail of the rats of each group and clotting time was determined according to Sabraze's capillary tube method [6].

Anaesthetized animals were bled to death. The blood was centrifuged for 10 min at 3000 rev/min. The plasma was extracted [7] using the Folch reagent [8]. An aliquot

Table 1. Effect of thyroxine and antithyroid compounds on blood coagulation time seven days after treatment

Groups of animals	No. of animals	Blood coagulation time (sec)	*P ≤
Control	18	98·0 ± 5·0	
Thyroxine treated	18	89·0 ± 3·0	NS
AITC treated	18	55·5 ± 6·0	0·001
Thiourea treated	18	68·0 ± 5·0	0·001
Thiouracil treated	18	60·5 ± 7·0	0·001
Thyroxine and AITC treated	18	78·0 ± 4·0	0·05
Thyroxine and Thiourea treated	18	85·0 ± 5·0	0·05
Thyroxine and Thiouracil treated	18	83·0 ± 3·0	NS

* P is the average of two tail areas for the values of *t*.