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The influence of oximes on the acetylthiocholine hydrolysis rate

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IN STUDIES of the reactivation of alkylphosphate-inhibited cholinesterase, using the method of Ellmann *et al.*,[1] we noticed that the presence of oximes in concentrations of 10^{-3}M or more accelerated the non-enzymic hydrolysis of acetylthiocholine. This observation was further established by using the Warburg technique. The simultaneous decomposition of acetylthiocholine by cholinesterase and oxime in reactivated samples therefore might give results showing reactivation exceeding 100 per cent measured by the technique of Ellmann *et al.*

Modification of the original procedure by replacement of enzyme with oxime resulted in a linear relationship between the rate of acetylthiocholine hydrolysis and the oxime concentration. This is demonstrated in Fig. 1. which shows the change in absorbance per minute with different concentrations of pyridine-2-aldoxime-N-methiodide.

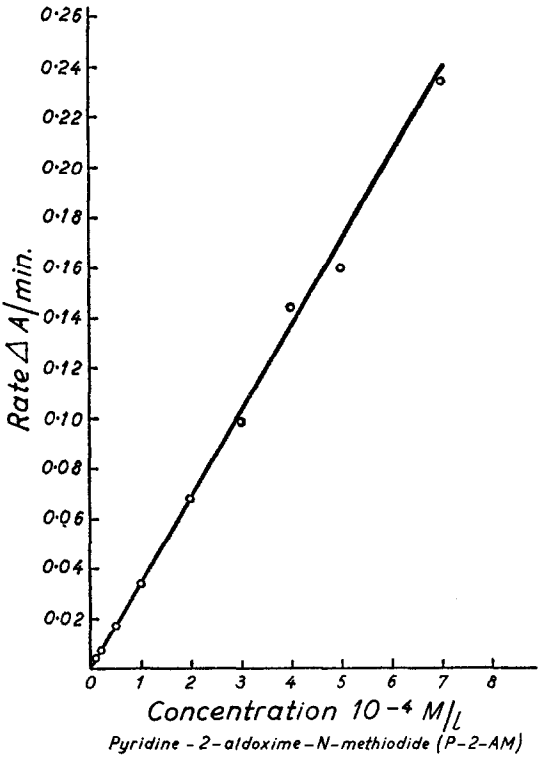

FIG. 1.

Similar standard curves recorded at 30°C could be obtained for a number of oximes and for hydroxylamine. The ratio

$$\frac{\Delta \text{ absorbance}}{\text{oxime conc.}} \times 10^{-2}$$

indicating the slope of the standard curve, is stated below for the various compounds.

Pyridine-2-aldoxime-N-methiodide (pralidoxime iodide, P-2-AM), 3·3.
Pyridine-2-aldoxime-N-methyl methansulphonate (P-2-S), 3·0.
Pyridine-2-aldoxime-N-methylchloride, 3·7.
Pyridine-2-aldoxime, 0·4.
Pyridine-4-aldoxime, 1·2.
Diacetylmonoxime (DAM), 1·8.
Trimethylen-bis-(pyridine-4-aldoxime) dibromide (TMB-4), 9·6.
Hydroxylamine, 1·0.

The reproducibility of the standard curves indicates a method for quantitative determination of oximes in aqueous solution by the acetyl-thiocholine method.

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The induction of aminoazo dye N-demethylase in nonhepatic tissues by 3-methylcholanthrene

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THE intraperitoneal injection of polycyclic hydrocarbons such as 3-methylcholanthrene (3-MC) and 3,4-benzpyrene (BP) markedly and rapidly increases the activity of enzyme systems in rat liver microsomes which N-demethylate 3-methyl-4-monomethylaminoazobenzene (3-methyl-MAB) and which metabolize other foreign compounds and drugs.[1–3] Similar treatment of rats with drugs such as phenobarbital, barbital, aminopyrine, phenylbutazone, or orphenadrine also results in such increases in activity.[4]

Our laboratory has utilized 3-methyl-MAB as a model substrate that appears to be metabolized by the same microsomal enzymes in liver that metabolize a variety of drugs. The oxidative N-demethylation of this substrate to 3-methyl-4-aminoazobenzene (3-methyl-AB) requires molecular oxygen, NADPH* and NADH for maximum activity,[5] and changes in the activity of the azo dye, N-demethylase are paralleled by changes in the activity of several other drug-metabolizing enzymes in liver microsomes. The present investigation was undertaken to determine whether the administration of 3-methylcholanthrene or phenobarbital could stimulate drug-metabolizing enzymes in nonhepatic tissues.

METHODS

Male Sprague–Dawley rats, weighing 50 to 70 g, were maintained on a 22% casein diet containing high levels of vitamins.[6] The rats were injected intraperitoneally with 1 mg of 3-MC in 0·25 ml of corn oil for 2 days. Control rats received injections of corn oil. The animals were killed by decapitation and

* NAD^+, $NADP^+$, NADH, and NADPH, refer to the oxidized and reduced forms of nicotinamide andenine dinucleotide and nicotinamide adenine dinucleotide phospate respectively.