period. Lysosomal activities and protein content of the kidney, as in the small intestine, did not show circadian fluctuations.

The results show that although some parameters in the kidneys, such as mitotic activity, show circadian dependence[16], others (enzymes playing an important role in tubular absorption) do not show significant fluctuations during the L/D cycle.

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Diethylmesoxalate hydrate, a new irreversible inhibitor of cholinesterases

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Summary. Bovine erythrocyte acetylcholinesterase and human plasma cholinesterase are irreversibly inhibited by diethylmesoxalate hydrate, the inhibition potency being comparable to that of certain insecticidal organophosphates and carbamates. Insect cholinesterases, however, appear to be much less affected by diethylmesoxalate hydrate. The compound was also found to inhibit the hydrolysis of paraoxon by rabbit plasma A-esterase, but in a reversible mode.

The inhibition of cholinesterases represents a very important and successful principle for insecticidal activity. Although this biochemical mode of action does not a priori lead to selective insect control agents, the pesticide industry has succeeded in identifying and developing a number of organophosphates and carbamates with a favourable mammal/insect toxicity ratio. New types of cholinesterase inhibiting compounds are, therefore, of great potential value.

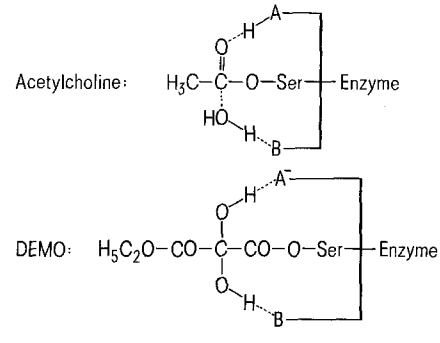
The hydrate of diethylmesoxalate (DEMO), $H_5C_2-O-CO-C(OH)_2-CO-OC_2H_5$, is an example for a new irreversible cholinesterase inhibitor. The inhibition characteristics of DEMO and its diisopropyl analogue (DIPMO) have been evaluated in some detail and will be described in the present communication.

Materials and methods. The following enzyme preparations and inhibitors were used: acetylcholinesterase (AChE) from bovine erythrocytes and electric eel (Sigma), from houseflies (homogenates of fly-heads), from *Spodoptera littoralis* (homogenates of first instar larvae); human plasma cholinesterase (ChE) and rabbit plasma A-esterase; diethylmesoxalate (Fluka), diisopropylmesoxalate (synthesized according to reference[1]); monocrotophos (dimethyl cis-1-methyl-2-methylcarbamoylvinyl phosphate), dioxacarb (2-(1,3-dioxolan-2-yl) phenyl-N-methyl carbamate) and paraoxon (diethyl 4-nitrophenyl phosphate) (Ciba-Geigy, analytical standards). An automated procedure[2] was used for most of the cholinesterase inhibition experiments.

Results. DEMO and DIPMO were found to inactivate cholinesterases from several sources in an irreversible mode, i.e. inhibition was found to be time-dependent similar to carbamates and organophosphates. Expressed in terms of I_{50}-values, the inhibition potencies of DEMO and DIPMO towards bovine AChE and human ChE were equal to or greater than those of dioxacarb and monocrotophos (table). Eel and housefly AChE were less readily inhibited by the mesoxalates, and AChE from *Spodoptera littoralis* was almost insensitive to DEMO and DIPMO.

Irreversibly inhibited cholinesterases, i.e. the carbamylated or phosphorylated enzymes, reactivate slowly. It was therefore interesting to see how fast the inhibition by DEMO would reverse. With bovine AChE at 37 °C and pH 8.0, the reactivation constant 'k_3' was found to be 0.017 min^{-1}. This figure is between the rate constants determined for the reactivation of dimethylphosphorylated and N-methylcarbamylated AChE. DEMO inhibited housefly AChE, however, was more rapidly reactivated, k_3 being 0.092 min^{-1}.

DEMO, by topical application, is only marginally toxic to insects. 3rd instar larvae of *Spodoptera littoralis* were not at all affected at a dosage of 1 mg/larva, and houseflies tolerated 10 µg/fly without showing symptoms. Houseflies



Binding of acyl serine to 2 catalytic groups (A and B) of a cholinesterase.

Inhibition potencies (I_{50}-values, M) of DEMO and DIPMO as compared with an organophosphate and a carbamate insecticide

	ChE Human plasma	AChE Bovine	Eel	Housefly	*Spodoptera*
DEMO	1.1×10^{-6}	7.2×10^{-6}	1.5×10^{-4}	$\sim10^{-5}$	6.0×10^{-4}
DIPMO	1.9×10^{-6}	6.6×10^{-6}	1.3×10^{-4}	$\sim10^{-4}$	$>10^{-3}$
Monocrotophos	1.4×10^{-6}	2.5×10^{-5}	1.4×10^{-5}	1.3×10^{-6}	4.0×10^{-6}
Dioxacarb	1.4×10^{-5}	5.4×10^{-6}	2.7×10^{-6}	6.7×10^{-7}	3.4×10^{-6}

caged in a 9-cm Petri dish, evenly coated with 20 mg DEMO, however, showed severe intoxication symptoms, and 100% mortality after 24 h. The acute LD_{50} for DEMO in mice was 160 mg/kg after i.v. injection. Intoxication symptoms were comparable to those observed after organophosphate poisoning.

In addition to cholinesterases, rabbit plasma A-esterase was also tested for its sensitivity towards DEMO and DIPMO, the substrate being paraoxon. Both were found to be reversible, competitive inhibitors, the inhibition constants K_I being 0.56 mM for DEMO and 0.04 mM for DIPMO.

Discussion. In contrast to esters of α-keto acids, which are known to be reversible inhibitors of cholinesterases[3], diesters of mesoxalic acid hydrate represent a new type of esterase inhibitors. Mammalian cholinesterases appear to be more sensitive than the corresponding insect enzymes. The insensitivity of the insect enzymes may explain the lack of sufficient insect toxicity. In addition, due to its high polarity, DEMO may not be able to penetrate the insect cuticle to a satisfactory extent.

At this time the mode of the irreversible inhibition of cholinesterases by DEMO and DIPMO is still unknown. However, the fact that cholinesterases and other B-esterases are irreversibly, A-esterases on the contrary reversibly inhibited, the I_{50}-values and the reactivation rates make them look like a carbamate or an organophosphate inhibitor. Therefore we suggest that DEMO and DIPMO acylate the cholinesterases, and that the slow reactivation, i.e. deacylation rates, are due to the gem. diol of the hydrates which bind to the active site as analogues of the tetrahedral transition state of the deacetylation reaction, as shown in the figure.

This hypothesis is only of speculative nature but is supported by the recent finding that trifluoroacetophenone hydrate and other stable hydrates are inhibitors of cholinesterases[4].

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Reversible inactivation of the nitrate reductase of rice plants

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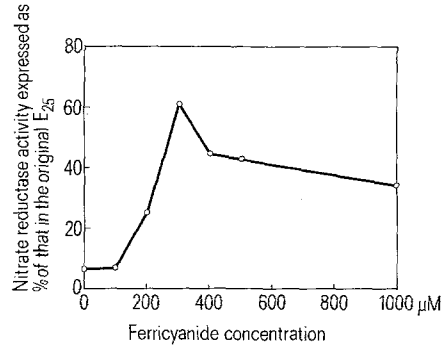
Summary. Studies of the inactivation of the rice nitrate reductase showed that the nitrate-reducing moiety and not the diaphorase moiety was reversibly inactivated by NADH and cyanide. Ferricyanide could reverse the inactivation, and nitrate could protect the enzyme against inactivation. Although the general characteristics of the reversible inactivation of rice nitrate reductase appeared similar to those of the algal nitrate reductase, it was found that the rice enzyme was automatically reactivated when NADH and cyanide were removed. Attempts to isolate inactivated nitrate reductase from ammonium-treated tissue were unsuccessful.

In the extracted state, nitrate reductase of algae exists in 2 forms, an active oxidized form and an inactive reduced form[1-5]. The enzyme was changed by reduced pyridine nucleotides and cyanide to the inactive form, which could be fully reactivated after reoxidation with ferricyanide[3,4]. Losada et al.[6] reported that reversible inactivation of nitrate reductase was induced in *Chlorella* cells by ammonium. Since Shen[7] has demonstrated the preferential assimilation of ammonium by rice seedlings, the purpose of this work was to see whether rice nitrate reductase is regulated by the same mechanism.

Materials and methods. Rice seedlings (*Oryza sativa*, L.var. IR8) were grown in culture solution[7]. Leaves of 12-day-old plants were harvested in a chilled medium containing 0.1 M phosphate buffer, pH 7.5, 1 mM cysteine hydrochloride and 5 µM FAD. The homogenate was filtered through cheesecloth and centrifuged at 20,000 × g for 20 min in a refrigerated centrifuge. The supernatant was passed over a Sephadex G-25 column. The eluate containing nitrate reductase was designated as E_{25}.



Reactivation of NADH-cyanide inactivated nitrate reductase by ferricyanide. E_{25} was inactivated by incubation with 200 µM-NADH and 10 µM-KCN in phosphate buffer (0.1 M, pH 7.5) at 24 °C for 10 min. Then the enzyme was incubated with ferricyanide of appropriate concentration at 24 °C for 10 min before nitrate reductase assay.