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SHORT COMMUNICATIONS

The anticholinesterase ability of diethyl *S*-*n*-propyl phosphorothiolate: errors caused by the presence of an active impurity

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'acha and O'Brien published a series of papers on the relationship between the toxicity potency towards acetylcholinesterase (EC 3.1.1.8.) of diethyl alkyl phosphorothiolates. itly had cause to repeat part of this work and find Bracha and O'Brien's results to be ticular compound re-examined. We attribute this error to traces of tetraethyl pyrophos- ent in the compound prepared by Bracha and O'Brien. The finding of TEPP as a reac- 'ganophosphorus compounds is not new and was studied in detail over 20 years ago 'avison.[4]

pyl phosphorothiolate (I) was the compound reexamined and was prepared by two oute A was to react *n*-propyl bromide with the sodium salt of diethyl phosphorothioic to react the sodium salt of *n*-propanethiol with freshly re-distilled diethyl phosphoro- B was identical to the technique used by Bracha and O'Brien.[1] Both routes gave com- cal physical characteristics. Intraperitoneal LD50's were determined using male albino 25 g). The compounds were dissolved in PEG 300 and administered at a dose volume ach dose was administered to randomised groups of 10 animals. First order rate coeffi- ition of bovine erythrocyte acetylcholinesterase (Sigma type I) were determined using e for monitoring the acid produced by the hydrolysis of acetylcholine.

for the inhibition experiments were acetylcholine $1{\cdot}46 \times 10^{-4}$ M, I = 0·1 M with AChE 0·2 μM units ml^{-1}, and $T = 37°$. Organophosphorous compounds in concen- solution were added in sufficient amount to produce first order rates of inhibition in sec^{-1}. The isopropanol in the reaction mixture did not exceed 2 per cent and had minimal ction rate. Apparent second order rate constants for inhibition were obtained from a ler rates against inhibitor concentration.

A) in dilute sodium hydroxide solution (*ca.* 0·002 N) for several hours caused no de- ory potency whereas similar treatment of I(B) caused an almost complete loss of activity. ibitory potency was observed on treating solutions of TEPP in a similar manner. It that this is proof only of the presence of an active impurity in I(B), not that the impurity the probability that it is TEPP is high as this is readily produced by the hydrolysis rochloridate.[5] Assuming that the impurity is TEPP, from a comparison of the rates he amount of TEPP present in I(B) can be estimated at *ca.* 0·3 per cent.

TABLE. 1. LD50's AND APPARENT SECOND ORDER RATE CONSTANTS OF INHIBITION OF CHOLINESTERASE BY I AND TEPP

Compound	LD50 (mg kg^{-1})	$k_i(M^{-1}sec^{-1})$
I (A)	88 (78-100)	0·25
I (B)	45 (42-48)	67
I (B) (ref. 2)	28 (23-34)	$1{\cdot}6 \times 10^{2}$*
TEPP	1·3 (1·2-1·4)	$2{\cdot}3 \times 10^{4}$

Numbers in brackets are 95 per cent confidence limits.
* Measured in the absence of substrate.

the compounds are also given in Table 1 and it is of interest to note that whereas the I(B) provide the main contribution to the inhibitory potency, they have only a small icity.

Because of the general use by Bracha and O'Brien of method B in the synthesis of their compounds, there must now arise doubts as to the validity of their results and of their conclusions as to the factors affecting the rates of inhibition and on the relationship between the rates of inhibition and the toxicities of their compounds.

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Effect of certain metabolic regulators on the activity of lysosomal enzymes in regenerating rat liver

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AFTER partial hepatectomy livers undergo numerous metabolic changes in order to maintain homeostasis, which is known to be associated with the process of rapid cell multiplication and DNA formation, along with some definite changes in RNA and protein synthesis.[1–3] Recently it has been noted that during liver regeneration, concentration of DNA in the liver is increased, while that of NAD is decreased and it is suggested that NAD inhibits the process of mitosis during liver regeneration.[3] Information is also available regarding the regulation of synthesis of several enzymes such as DNase, alkaline phosphatase and deoxythymidinekinase in the regenerating livers under the influence of certain metabolic regulators.[4–6] Administration of hydrocortisone is known to induce ornithine decarboxylase in resting liver but it has no effect on this enzyme in regenerating liver, although it inhibits the process of DNA synthesis during the process of liver regeneration.[7] This indicates that the mechanism of regulation of enzyme synthesis in regenerating liver is different from that of normal liver. In this communication, investigations on the effects of certain metabolic regulators such as ACTH, c-AMP and glucagon on the activities of several lysosomal enzymes during the process of liver regeneration have been presented.

Male albino rats weighing between 90–100 g were used as experimental animals. One third of the liver was removed from the experimental group as described by Ferris and Clark[3] and rats of all groups were then maintained on 2% glucose solution. The different groups of animals are mentioned in Table 1. ACTH (0·1 IU/100 g), glucagon (100 μg/100 g) and c-AMP (200 μg/100 g) were injected to the respective groups of animals 3 hr before sacrifice. The rats were sacrificed 18 hr after partial hepatectomy to collect the regenerated part of liver for biochemical assay. Lysosome rich mitochondrial fractions have been used as enzyme sources and these have been prepared according to the method as described earlier.[8] For the disruption of lysosomes, Triton X-100 (0·2 per cent, v/v) was used. The activity obtained after treatment with Triton X-100 is designated as total activity. Assays for β-glucuronidase, acid phosphatase and alkaline phosphatase in the lysosomal fractions were carried out according to Michell *et al.*[9] Protein contents of the enzymes were determined by the method of Lowry *et al.*[10]

It is noted from Table 1 that the activities of acid and alkaline phosphatase are increased in regenerating liver as compared to the normal; while studying β-glucuronidase it is noted that there is an increase in the total activity of this enzyme in the regenerated system. Administration of c-AMP to the regenerating group of rats markedly inhibits the activities of acid and alkaline phosphatase but ACTH administration