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Acute Toxicity of Aminocarb in Male Rats and Inhibition of Tissue Esterases

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The carbamic acid ester insecticide aminocarb (4-dimethylamino-3-methylphenyl N-methylcarbamate, MATACIL^R) has been used extensively by several provincial governments in Canada for the control of a lepidopterous forest pest, the spruce budworm (Choristoneura fumiferana (Clemens)). Little has been published in scientific journals about the acute and/or chronic toxicity of this chemical. The acute oral LD_{50} of aminocarb in rats is of the order of 30-40 mg/kg body weight (GAINES 1969; ANONYMOUS 1979). Degradation in mammals can proceed readily in vivo and in vitro by way of hydrolytic mechanisms with subsequent conjunction of the phenolic products as well as via oxidative N-demethylation by hepatic microsomal mono-oxygenases with one metabolite, 4-amino-3-methylphenyl N-methylcarbamate, being more potent than the parent compound (MENZIE 1969; OONNITHAN and CASIDA 1969). This report presents results of the inhibition of tissue cholinesterases and carboxylesterases in vivo following the administration of acute toxic doses of aminocarb to male rats.

MATERIALS AND METHODS

Solutions of technical aminocarb (MATACIL^R, 97.4% purity, obtained from the Mobay Chemical Corporation, Kansas City, Missouri) were prepared in a vehicle of ethanol:propylene glycol (25:75) to yield a concentration of 12.5 mg/ml. Male Sprague-Dawley rats (Charles River strain Crl:CD(SD)BR, 225-275 g body wt) were purchased from Charles River Canada Inc., St.-Constant, Quebec, and were acclimatized in our animal care facility for 7 days prior to use. The animals were fasted overnight, weighed and subdivided into groups of 5 animals, each rat receiving a single oral dose of aminocarb (25 mg/kg body wt) by oral intubation using a glass syringe fitted with a ball-tipped curved feeding needle. At suitable intervals after treatment (30, 60, 120, 180, 240, 300, 360, 480 min and at 24 hr), subgroups of 5 randomly-selected rats were lightly anesthetized by diethyl ether and killed by exsanguination via direct cardiac puncture, collecting the blood in heparinized syringes. The blood samples were centrifuged and the plasma and erythrocyte fractions were stored at -20°C until analyzed. All rats were necropsied and the livers and brains were removed, weighed and stored in air-tight glass bottles at -20°C until analyzed.

Samples of frozen rat liver were minced finely with scissors

and washed twice with cold 0.9% sodium chloride to remove residual blood. The minced tissue was homogenized with ice-cold 0.067M phosphate buffer pH 7.4 to produce a 20% w/v homogenate, using a glass Potter-Elvehjem homogenizer fitted with a motor-driven Teflon pestle. Twenty percent homogenates of frozen rat brain were prepared in the same manner.

The pseudocholinesterase (PChE) of rat plasma was determined spectrophotometrically by the method of ELLMAN et al (1961) using 10^{-3}M propionylthiocholine iodide as the optimal substrate at 37°C and pH 7.4. The brain acetylcholinesterase (AChE) activity was measured in diluted aliquots of whole homogenate in the same manner using 10^{-3} acetylthiocholine iodide as the substrate. The erythrocytic AChE activity was measured by a pH-stat titrimetric technique at 25°C and pH 8.0 (ECOBICHON and STEPHENS 1973). The hepatic nonspecific carboxylesterase (CE) activity was determined by a pH-stat titrimetric technique using 2 X 10^{-3}M triacetin (glyceryl triacetate) as the substrate at 25°C and pH 7.4 (ECOBICHON and ZELT 1979).

RESULTS AND DISCUSSION

Figure 1 shows the activities of the erythrocytic and brain AChE, plasma PChE and hepatic CE of subgroups of rats treated with aminocarb (25 mg/kg) and killed after treatment at the intervals shown. The enzymatic activities are presented as a percent of the mean activities of a vehicle-treated group (n=10) of control rats. The erythrocytic AChE was depressed by some 20% during the first two hours after treatment but was within normal range 180 min after treatment. The brain AChE was depressed by some 40%, this reduction persisting for up to 180 min after treatment. At subsequent intervals, the brain AChE activity was within the normal range. The PChE was sharply depressed to approximately 55% of control values within 30 min of treatment but returned to the normal activity range by 180 min. A subsequent slight reduction in PChE activity was observed at 240 and 300 min but activities were normal at 360 min and subsequent time intervals. A marked reduction in hepatic CE was observed which persisted out to 480 min after treatment but the CE had returned to normal within 24 hr of treatment.

Despite the fact that quite severe though transient signs of anticholinesterase poisoning were observed in the rats treated with this near-toxic dose of aminocarb, it was difficult to correlate the signs with the measured inhibition of tissue esterases. In vitro experiments have shown this insecticide to be a more potent inhibitor of erythrocytic AChE (I_{50}=8.8 X 10^{-6}M) than of plasma PChE (I_{50}=2.9 X 10^{-5}M) but, following acute oral dosing, the inhibition of rat erythrocytic AChE was found to be minimal and not useful as an index of toxicity. The absence of inhibition may be artifactual in that the erythrocytes must be lyzed by dilution (1:40) and titrated against a 10^{-3}M concentration of substrate for a period of 5-10 min, all 3 factors (dilution, substrate and time) contributing to the reversal of the inhibition. A more representative inhibition was obtained for the plasma PChE and the brain

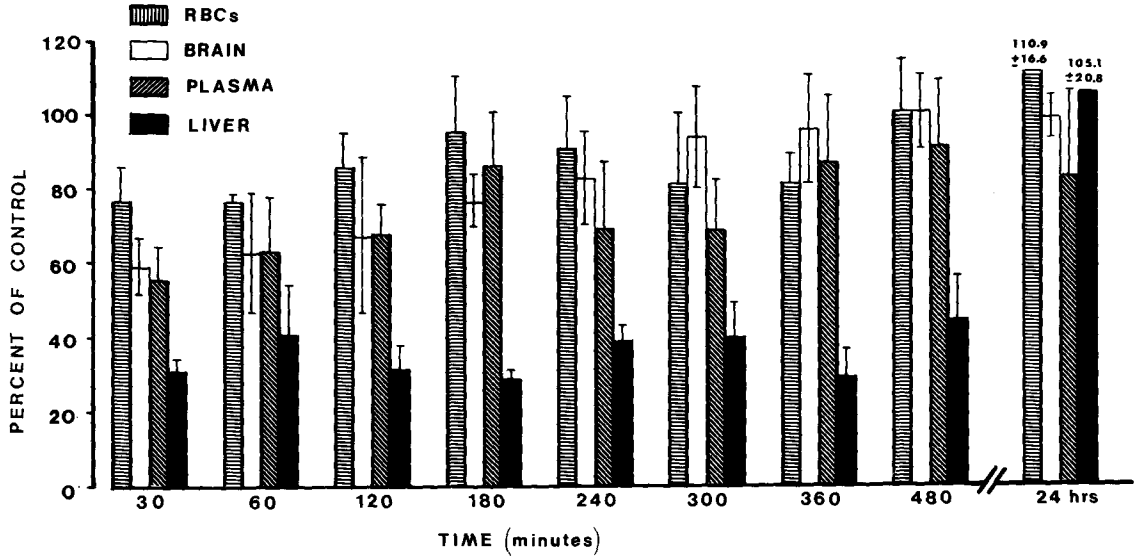

Figure 1. The erythrocytic and brain acetylcholinesterase, plasma pseudocholinesterase and hepatic non-specific carboxylesterase activities of groups (n=5) of young adult male rats following oral treatment with aminocarb (25 mg/kg body wt). The activities are expressed as a percentage of mean activities determined for a group (n=10) of vehicle-treated rats. The vertical bars represent the standard deviations of the mean percentage value.

AChE since, in the spectrophotometric technique used, the enzymes were only in contact with the substrate for a maximum period of 2 min. Significant signs of poisoning (exocrine secretions, chromodaccryohorea, piloerection, nasal congestion with rales, generalized muscular tremors) were observed in all animals for 60-120 min but subsided afterward.

The marked inhibition of hepatic CE by aminocarb was representative of what would be expected to occur following the oral administration of a reversible esterase-inhibiting insecticide that would be biotransformed into inactive products in the liver by these same carboxylesterases (ECOBICHON 1979). The rat rapidly degrades aminocarb by hydrolysis of the methyl carbamoyl group since, when the metabolism of ^{14}C-labeled aminocarb was studied, 79% of the ^{14}C was expired as CO_2 within 48 hr with urinary elimination accounting for 25% of the radiolabel and 4.0% being detected in the feces (KRISHNA and CASIDA 1966).

With *in vitro* studies using isolated microsomes from rat liver, 6 metabolites were formed, but it appeared that, under these conditions, hydrolytic degradation was of secondary importance to other pathways including oxidative N-demethylation to form 4-methylamino- and 4-amino-3-methylphenyl N-methylcarbamate as well as 4-dimethylamino-3-methylphenyl N-hydroxymethylcarbamate (OONNITHAN and CASIDA 1968, 1969). The first two metabolites were found to be more potent as anticholinesterase agents *in vitro* than was aminocarb (OONNITHAN and CASIDA 1968). Whether these potent metabolites are formed in sufficient quantities *in vivo* or persist for any length of time is not known but, since they have an intact carbamoyl ester group, they would be susceptible to hydrolytic degradation, giving rise to a number of biologically-inactive phenolic products (MENZIE 1969).

The present experiments revealed that the exposure of rats to toxic oral doses of aminocarb resulted in severe but transient inhibition of tissue esterases. The short duration of action was not surprising since it is known that rats efficiently degrade this agent by hydrolytic mechanisms, aminocarb acting somewhat as a poor substrate for cholinesterases and carboxylesterases.

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