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Recovery of Brain Cholinesterases of Brown-Headed Cowbirds from Organophosphorus Intoxication: Effect of Environmental Temperature

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Organosphosphorus (OPs) represent the major class of broad-spectrum pesticides currently sprayed on forests, farmlands and gardens (Gallo and Lawryk 1991). Non-target species including mammals, fishes and birds are frequently injured by OPs (Smith 1987). Their acute effects are attributed to their inhibition of cholinesterases, leading to an accumulation of acetylcholine (ACh) in the central and peripheral nervous systems (Corbett 1974). Measurements of brain or plasma cholinesterase activity has been widely used to document the acute toxicity of OPs (review: Mineau 1991).

It is well known that the toxicity of anticholinesterases is influenced by ambient temperature (Baejter and Smith 1956; Fuhrman and Fuhrman, 1961; Ahdaya et al. 1976). Maguire and Williams (1987) reported that a cold stress enhances brain acetylcholinesterase (AChE) inhibition of chlorpyrifos-exposed Northern bobwhites. Parathion toxicity also increased when Japanese quails *(Contournix japonica)* were exposed to cold temperatures for a single day (Rattner 1982). Brain AChE activity of European starlings measured 24 hrs after being exposed to dimethoate was more inhibited at temperatures lower than at 25 °C (McDuff 1995). Hypothermia was also reported as a consequence of OPs intoxication (McDuff 1995), and was suggested to contribute to increased toxicity of OPs in cold temperatures (Rattner and Franson 1984). Cold temperature was suggested to reduce the birds metabolism, thereby retarding detoxification and increasing brain AChE inhibition (Maguire and Williams 1987). However, other studies reported that brain AChE activity of parathion-treated quails was no more inhibited (Rattner and Grue 1990) or even less inhibited (Rattner et al., 1987) at cold ambient temperature. In an attempt to shed more light on the impact of ambient temperature on OPs toxicity, we monitored during 24 hrs the impact of a single dose of dimethoate on the brain AChE activity and body temperature of brown-headed cowbirds at 5 °C and 25 °C.

MATERIALS AND METHODS

A total of 192 adult male brown-headed cowbirds *(Molothrus ater*) were captured with a cannon net and kept in an indoor aviary (3 m x 5 m x 5 m) for at least one month before the experiments to allow acclimatization (AOU Committee 1988), but no longer than three months to avoid changes in basal metabolism and body temperature (Warkentin and West, 1990). During this period, they were fed *ad libitum* with cracked corn and mixed seeds commercially prepared for wild birds. Vitamins and minerals were added to drinking water. Feeding sites were distributed at different places and heights in the aviary to minimize competition over

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resources. Relative humidity was 50 ± 5%, temperature 15.0 ± 2.0 °C and illumination on a 12: 12 LD cycle (400:0 lux).

The impact of ambient temperature on dimethoate toxicity was assessed with four groups of 36 birds: a control group and a treated group exposed to 25 °C, a control group and a treated group exposed to 5 °C. All birds were equipped with an external thermosensitive transmitter fixed at the base of the neck on the jugular vein, after removing feathers and down (Brunet et al. 1996). They were then transferred to individual cages (0.15 x 0.3 x 0.6 m) within an experimental chamber 24 hrs before the experiment. Each cage was equipped with a perch, as well as food and water in sufficient amounts to last the whole experiment. Relative humidity was maintained at 50 ± 5%, illumination on a 12:12 LD cycle (400:0 lux) and ventilation at 1.5 l/mm The experiment started when the birds received either water or a sublethal dose of dimethoate injected directly into their crop by gavage with a canulated syringe. The pesticide concentration was 15.80 mg/kg BW, which corresponds to 1/2 of the LD_{50} estimated for European starlings (Schafer et al. 1983). Dilutions were prepared in water with dimethoate (Cygon 2E®) sold commercially as a 24.9 % active solution. Three birds were sacrificed every 2 hrs during 24 hrs to measure brain AChE activity, according to the technique of Hill and Fleming (1982) in µmoles/min/g of brain. Nonparametric single factor analysis of variance (Kruskal Wallis) followed by Mann-Withney tests were carried out to compare brain AChE activities and body temperatures through time and between ambient temperatures (Zar, 1984). Analyses were performed on the 144 birds that survived until they were sacrificed for brain AChE assays. Values of $p > 0.05$ were considered significant

RESULTS AND DISCUSSION

Brain AChE activity of the control brown-headed cowbirds did not change significantly over 24 hrs at 5 °C ($H = 12.642$ $p = 0.32$) and 25 °C ($H = 13.06$, $p = 0.29$) (Fig. 1A). Similar results were reported for brain AChE activity of Northern bobwhites (Rattner and Fairbrother 1991). We also report that mean AChE activity was significantly lower at 25 °C than at 5 °C ($Z = -2.46$, $p = 0.014$) (Fig. 2A). Such effect of ambient temperature on brain AChE activity was also reported for European starlings (McDuff 1995). Figure 1A also indicates large variations in AChE activity among birds, as reported by Rattner and Fairbrother (1991) and by McDuff (1995). This probably constitutes the most important factor of difficulty in using AChE activity to monitor exposure to OPs. For this reason, an exposure to OPs was considered significant for AChE inhibition ≥ 20% (Ludke et al. 1975).

Dimethoate exposure caused variations in brain AChE activity through times at 5 °C ($H = 27.00$, $p = 0.0046$) and 25 °C ($H = 19.86$ $p = 0.047$). At both temperatures, the birds experienced a gradual decrease in AChE activity during the first 6-7 hrs, which was followed by a recovery period that lasted at least 18 hrs (Fig. 1B). Mean brain AChE activity computed over the last 8 hrs of the experiment was significantly lower at 5 °C than at 25 °C (Mann-Withney test, $Z = -2.46$, $p = 0.014$) (Fig. 2B). Similar results were obtained at 10% and 90% relative humidity (data not shown). The skin-feather air layer might have isolated the birds from the cooling effect of humidity. Maguire and Williams (1987) also reported that brain AChE activity of Northern bobwhites was more inhibited at cold temperatures. Brain AChE activity of European starlings measured 24 hrs after being exposed to dimethoate was more

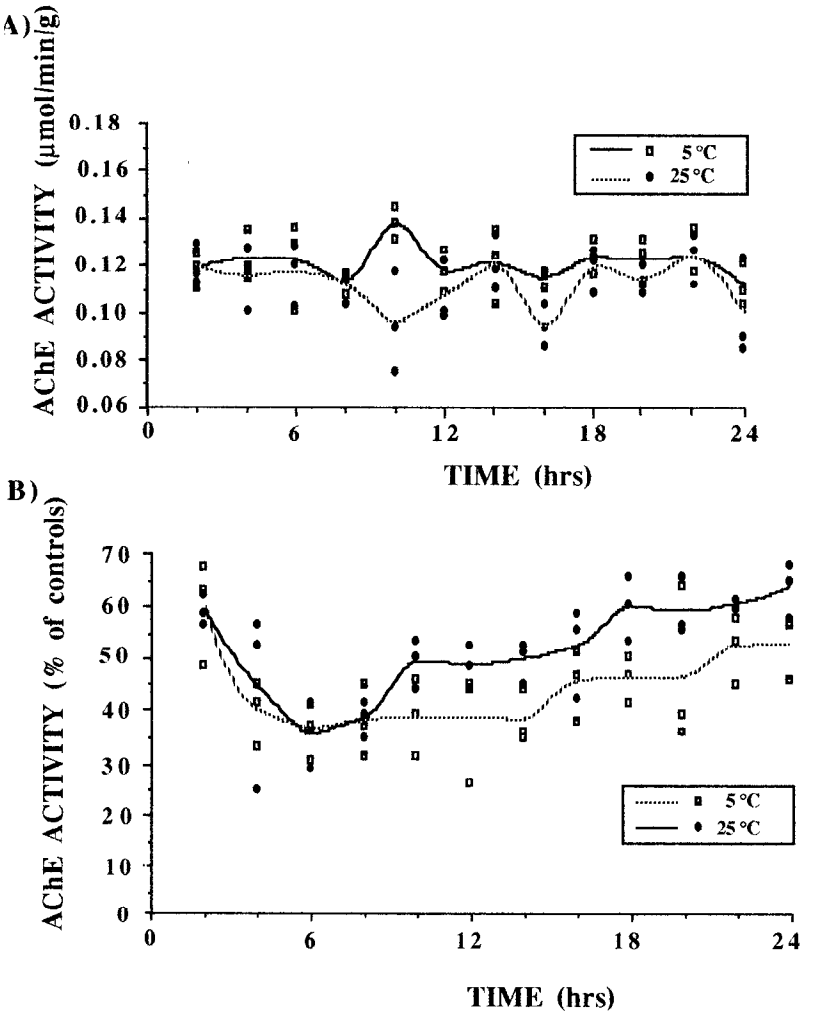

Figure 1. Impact of ambient temperature on daily variations in AChE activity of European Starlings. Each symbol represents a single measurement, expressed in percent of the mean AChE activity of the control birds over 24 hrs at corresponding ambient temperatures.

inhibited at low temperatures than at 25 °C (McDuff 1995). These results suggest that cold stress enhances brain AChE inhibition induced by OPs. However, continuous monitoring of AChE activity indicates that the initial decrease in AChE activity was not affected by ambient temperature, but that the birds exposed to 5 °C recovered more slowly than those at 25 °C (Fig. 1B). Therefore, ambient temperature affected the rate of recovery of brain AChE activity from dimethoate.

The brown-headed cowbirds displayed a typical circadian cycle of body temperature, with a day-night range of 2.5 °C and a period of 24 ± 1 hrs at an

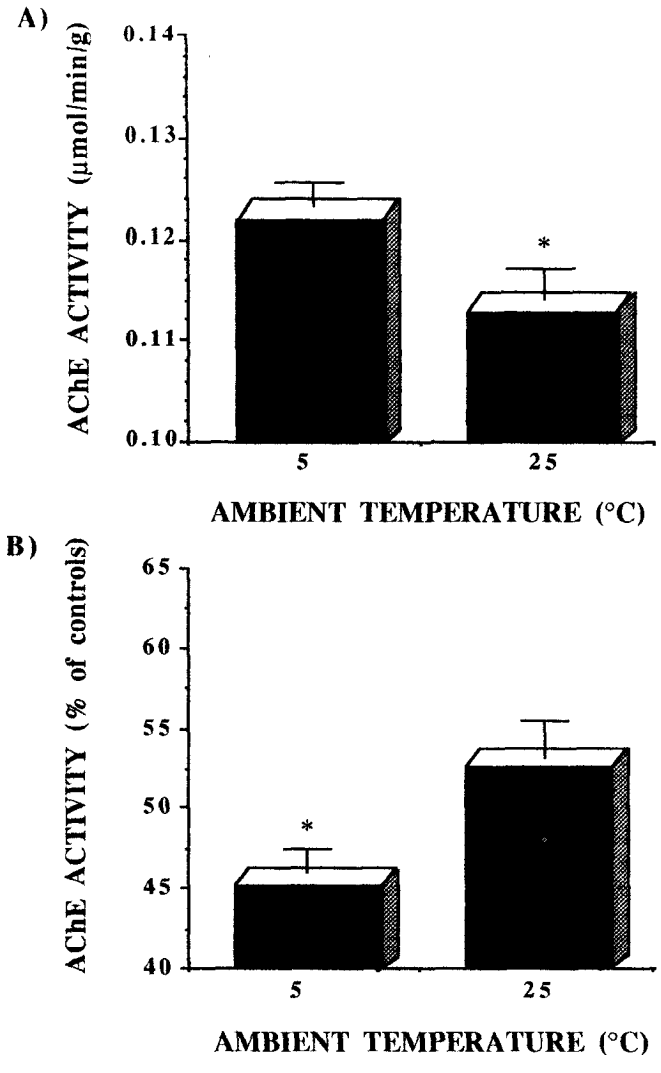

Figure 2. Impact of ambient temperature on mean AChE, activity of control (A) and treated (B) European Starlings. Values represent mean (± SEM) of 36 measurements. Mann-Withney tests indicated significant difference (* $P < 0.05$).

ambient temperature of 25 °C (data not shown). Figure 3 illustrates the effect of pesticide exposure on the birds temperature cycle at 5 °C and 25 °C. At both temperatures, the birds experienced hypothermia 2 to 6 hrs after pesticide ingestion. Methyl parathion also induced hypothermia in American kestrels *(Falco sparverius)* (Rattner and Franson 1984). We also report that mean body temperature, computed between 2:00 and 6:00, was significantly lower under cold stress ($U = 16.13$, $p = 0.0012$). This relationship between OPs-induced hypothermia and ambient temperature was also reported for European starlings (McDuff 1995). Close comparison of the patterns of AChE activity (Fig. 1A) and body temperature (Fig. 1B) resulting from dimethoate exposure indicates that hypothermia occurred earlier

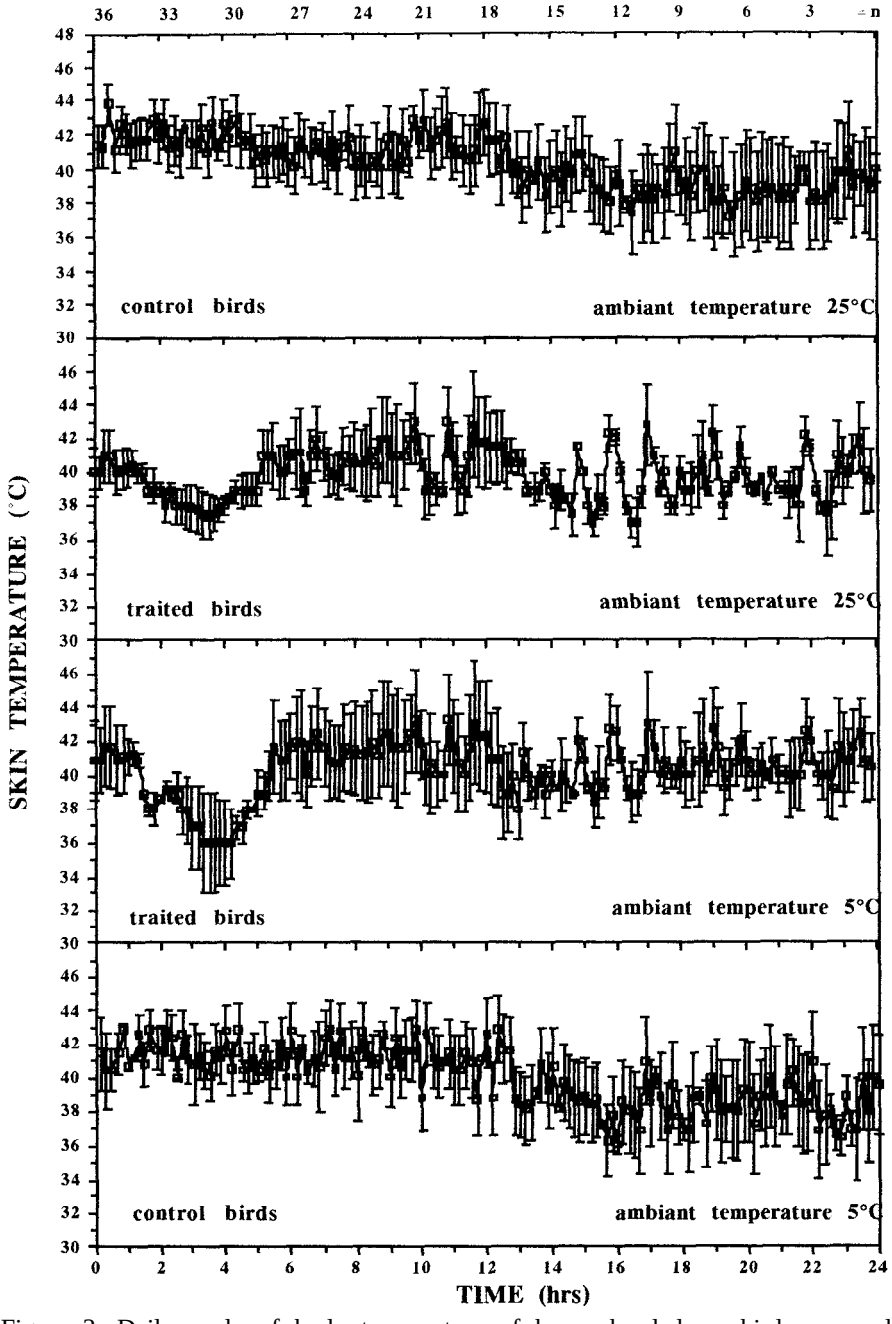

Figure 3. Daily cycle of body temperature of brown-headed cowbirds exposed to dimethoate at ambient temperatures of 25 °C and 5 °C. Values represent mean (± SD) temperature recorded every 10 min for all birds. Values of N above the figure indicates that the sample size decreased through time as three birds were sacrificed every 2 hrs.

than brain AChE inhibition. In fact, the birds had already restored normal body temperature when brain AChE activity was at its lowest level. Other studies have demonstrated that there does not appear to be a relationship between the degree of hypothermia and inhibition of brain AChE activity (Ahdaya et al. 1976; Rattner and Franson, 1984). The asynchrony between hypothermia and AChE inhibition could explain this lack of relationship. Dimethoate could be exerting hypothermic effects by acting at a specific region within the hypothalamus that would not be reflected as inhibition of whole brain AChE, or it could be acting peripherally.

In conclusion, we have demonstrated that, under these conditions, cold temperature does not increase brain AChE inhibition, but rather slows down the rate of recovery. We also provide evidence that cold temperature aggravates but does not prolong hypothermia. These results suggest that death occurring few hours after an exposure to an OPs, especially at cold ambient temperature. The death could be a consequence of unmanageable hypothermia. Whereas those encountered more than 8 hrs later could arise from a variety of symptoms associated with prolonged ACh accumulation in the nervous systems, including muscle incoordination and dehydration.

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