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BRIEF COMMUNICATION

Malathion Administration: Effects on Physiological and Physical Performance in the Heat[1]

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FRANCESCONI, R., R. HUBBARD AND M. MAGER. *Malathion administration: Effects on physiological and physical performance in the heat.* PHARMACOL BIOCHEM BEHAV **19**(6) 1031–1035, 1983.—To determine the effects of low-dosage organophosphate administration on exercise in a hot environment, malathion (7.5 mg/day, 4 days) was administered IP to rats, and effected a 35% ($p<0.01$) reduction in plasma cholinesterase levels. Treadmill endurance (9.14 m/min, no incline, 35°C ambient) was unaffected when the animals were exercised to hyperthermic exhaustion (Tre ~43°C). While rates of heat gain were similar between groups, malathion-treated rats displayed higher Tsk ($p<0.05$) at a number of sampling times during the treadmill run. While creatine phosphokinase levels were unaffected by either cholinesterase inhibition or exercise in the heat, lactate dehydrogenase activities were increased ($p<0.01$) in both groups following hyperthermic exhaustion. Although plasma levels of lactate, potassium, urea nitrogen, and creatinine were all significantly ($p<0.01$) increased as a result of exercise in the heat, these increments were not exacerbated by cholinesterase inhibition. Results generally indicated that at this moderate level cholinesterase inhibition, malathion administration did not adversely affect physiological, physical, or thermoregulatory efficacy.

Cholinesterase inhibition Hyperthermic exhaustion Heat injury Clinical chemical indices

WHILE the behavioral and mental performance decrements induced by administration of organophosphates to a variety of laboratory animals have been well documented and reviewed [5], very little data have been presented regarding effects on physical performance or thermoregulation. Meeter [22] had reported that organophosphate intoxication usually elicited rapid decreases in rectal temperature of rats caused by increased heat loss via vasodilation in the tail. Ahdaya *et al.* [1] demonstrated that parathion was effective in reducing rectal temperatures in mice, and that there was a correlation between the degree of cholinesterase inhibition and the magnitude of the temperature decrement. However, no reports are available concerning the effects of organophosphate poisoning on thermoregulatory or physiological responses during exercise in the heat.

This was of particular interest to us since atropine, widely recognized as a very effective antidote for organophosphate poisoning [12,13], also affects heat-loss mechanisms [3,7], largely through its anticholinergic action [2,17]. In a recent communication [15] we have demonstrated that atropinized rats displayed greatly increased heating rates when passively exposed to a hot environmental temperature (41.5°C) due to the absence of saliva- and urine-spreading behavior in the atropinized rats. While we have demonstrated [10] that chronic chlorpromazine administration compromises the ability to work in the heat, the effects of atropine singly or in combination with organophosphate poisoning, on work in the heat have not been reported. Ultimately, we wish to examine the effects of several intensities of cholinesterase inhibition with and without anticholinergic antidotes on the ability to work and thermoregulate in the heat.

To approach this problem systematically, we initally wished to investigate the physiological and physical decrements induced by an organophosphate poison which effected moderate levels of plasma cholinesterase inhibition. Malathion is used extensively as an organophosphate inhibitor of brain, red cell, and plasma cholinesterase activity [24,29]. Further, modulation of the dosage level is effective in achieving predetermined levels of cholinesterase inhibition [6,18]. Consequently, we could attain a moderate level of cholines-

[1]The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as an official US Department of the Army position, policy or decision unless so designated by other official documentation. In conducting the research described in this report, the investigators, adhered to the "Guide for the Care and Use of Laboratory Animals," as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

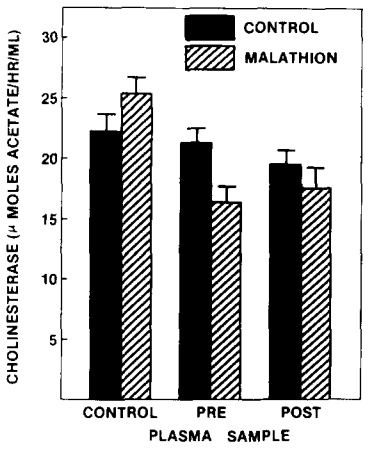

FIG. 1. Effects of malathion administration and exercise in the heat on plasma levels of cholinesterase activity. Malathion treated rats (n=12) received 7.5 mg malathion/day for 4 consecutive days; controls (n=12) received the diluent only. Rats ran at 9.14 m/min, level treadmill at 35°C until hyperthermic exhaustion ensued (Tre=43°C). The control blood sample was removed before the initial injection of malathion; the pre and post blood samples were removed immediately prior and subsequent to exercise in the heat to hyperthermic exhaustion.

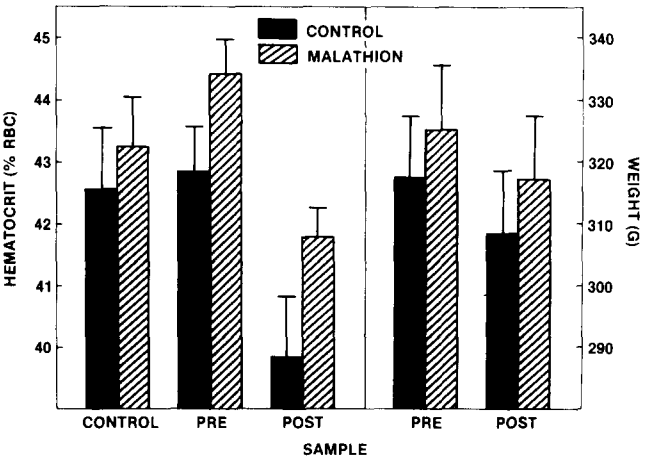

FIG. 2. Effects of malathion administration and exercise in the heat to hyperthermic exhaustion on hematocrit ratios and body weights. All conditions are as noted under Fig. 1.

terase inhibition and quantitate its effects on the physical work capacity and thermoregulatory responses of a heat-exposed, exercising rat model.

METHOD

Adult, male rats (Sprague-Dawley, 310–325 g, 2.5 months, Charles River Breeding Laboratories) were divided into two groups (n=12/group), one of which served as a control. Rats were held in a windowless room maintained at 22°C±1.0°C, one animal per cage, with free access to food (Charles River Laboratory Chow) and water. An automatic timing device controlled fluorescent lighting (on, 0600–1800 hr daily) to aid in establishing and standardizing diurnal/nocturnal periodicities of body temperature. No preconditioning to heat or exercise was necessary since no adaptational effects were under consideration, and naive animals will readily exercise at the slow treadmill speed selected.

Malathion (95%, technical grade, Analabs, Inc., New Haven, CT) was diluted with a mixture of acetone, 70% ethyl alcohol, physiological saline (1:4.2:6.2) aseptically to achieve a final concentration of 7.5 mg malathion/1.0 ml diluent. One ml (7.5 mg) was thus administered intraperitoneally to the experimental group each day (mean initial wt=330 g) for four consecutive days (mean final wt =325 g) to attain a total dosage of 30 mg (92.3 mg/kg). Prior to the first injection each rat (experimental and control) was fitted with an indwelling catheter inserted permanently into the exterior jugular vein for blood sampling. Upon recovery from this minor surgery and immediately before the first injection, a 1.0 ml blood sample was taken (control or time 0), the hematocrit measured, and the blood was centrifuged (10000 g, 4°C) and the plasma removed and frozen (−20°C) for subsequent analysis. Control rats were treated identically save for the injection of 1.0 ml diluent only on each of 4 consecutive days (mean initial wt=322 g, mean final wt=317 g).

Approximately 10 min after the final injection a second blood sample was obtained and treated exactly as the first; this was the pre-run blood sample. Before initiating exercise, a rectal probe (model No. 701, Yellow Spring Instr. Co., Yellow Springs, OH) was inserted to a depth of 6 cm, and a surface probe (Yellow Springs No. 709) was affixed mid-length on the tail. Both probes were securely attached with plastic adhesive tape so as to be unaffected positionally by the subsequent exercise. Rectal (Tre) and tail-skin (Tsk) temperatures were monitored on a minute-by-minute basis during exercise (9.14 m/min, level treadmill) in a hot (35°C, 25% RH) environmental chamber until hyperthermic exhaustion (Tre=43°C) ensued. Immediately upon completion of the treadmill run (i.e., at the time when rats became hyperthermically exhausted) a post-run blood smaple was removed and treated as the previous, and the rats were then returned to the holding room.

In addition to assaying all plasma samples for the commonly reported indices of heat/exercise injury [14,16], each sample was also analyzed for cholinesterase activity to determine the efficacy of malathion inhibition. Cholinesterase levels were quantitated using kits supplied by Sigma Chemical Co. (St. Louis, MO) after procedures described in their technical bulletin (No. 420). This method monitors a decrement in color of m-nitrophenol caused by the reduction in pH as acetic acid is generated from acetylcholine; the absorption was measured at 400 nm. Glucose was quantitated using Worthington Statzyme test kits while plasma lactates were measured by means of Sigma test kits, each according to standard procedures outlined in the respective technical bulletins. Potassium (K^+) and sodium (Na^+) levels were analyzed by flame photometry (Radiometer, Copenhagen). Creatine phosphokinase (CPK), lactic acid dehydrogenase (LDH), urea nitrogen (UN), and creatinine levels were estimated using Worthington test kits by methods described in the appropriate technical bulletins. Spectrophotometric procedures were carried out on a Gilford Stasar III automated spectrophotometer, except cholinesterase levels were determined on a Zeiss PM QII research spectrophotometer.

Statistical analyses were made by analysis of variance [21] followed by Tukey's t test [20] for establishing statistical significance; minimal critical differences in mean values are calculated in this test, and all pairs can be compared. The

TABLE 1

EFFECTS OF MALATHION ADMINISTRATION AND EXERCISE IN THE HEAT TO HYPERTHERMIC EXHAUSTION ON SELECTED CLINICAL CHEMICAL INDICES OF HEAT EXERCISE INJURY

	Control			Malathion-Treated		
	CATH*	Pre-run	Post-run	CATH	Pre-run	Post-run
Glucose (mg/100 ml)	168.1 ±4.8	167.1 ±7.6	145.7 ±12.8	168.8 ±5.9	163.5 ±3.9	160.8 ±10.4
Lactate (mg/100 ml)	19.3 ±1.5	17.9 ±2.4	42.6 ±6.1	22.2 ±1.7	18.7 ±2.4	45.1 ±7.8
Sodium (meq/l)	146.1 ±1.7	147.4 ±1.3	150.0 ±1.9	147.6 ±1.8	148.2 ±1.5	150.3 ±1.4
Potassium (meq/l)	4.2 ±0.1	4.4 ±0.1	5.2 ±0.3	4.3 ±0.1	4.8 ±0.2	5.6 ±0.3
Urea Nitrogen (mg/100 ml)	15.4 ±0.7	16.3 ±0.9	21.7 ±1.0	16.5 ±0.7	16.2 ±0.7	21.4 ±0.7
Creatinine (mg/100 ml)	0.55 ±0.01	0.64 ±0.02	1.03 ±0.09	0.52 ±0.02	0.61 ±0.02	1.06 ±0.05
Lactate Dehydrogenase (u/l)	46.8 ±3.6	68.0 ±7.5	191.2 ±20.5	68.3 ±12.1	66.4 ±7.0	177.6 ±24.7
Creatine Phosphokinase (u/l)	116.7 ±21.1	107.7 ±31.2	228.2 ±76.2	200.4 ±49.9	69.4 ±8.5	148.0 ±34.9

Mean values ± standard error of the means are noted for n=12 in both groups.
*Sample taken at the time of catherization.

paired and non-paired t test were also used where appropriate. The null hypothesis was rejected at $p<0.05$.

RESULTS

The malathion administration achieved a significant inhibition of plasma cholinesterase is depicted in Fig. 1. In the first (control, malathion) plasma sample of the group treated with malathion, the mean specific activity of cholinesterase was 25.47±1.70 (mean±S.E.M.) μmoles of acetate formed per hour per ml of plasma. After 4 consecutive days of malathion administration this activity declined significantly to 16.48±1.42 ($p<0.01$), an inhibition of 35.4%. It is also of interest to note that exercise in the heat to hyperthermic exhaustion (pre vs. post samples both groups) had no effect ($p>0.05$) on plasma cholinesterase levels (minimal critical difference for significance=5.9 units, Tukey's t test).

During exercise in the heat to hyperthermic exhaustion, Tre was unaffected by malathion administration. However, despite virtually identical starting Tsk (mean=23.06°C, control; mean=23.00°C, malathion), Tsk was statistically significantly ($p<0.05$, unpaired t test) elevated from 10–22 min of exercise, in the malathion-treated group. The mean endurance capacity was identical for both groups ($p>0.05$, unpaired t test; mean=37.0 min, control; mean=37.06 min, malathion), thus totally unaffected by this dosage level of malathion. Figure 2 demonstrates that average weight (water) loss during exercise in the heat was not different ($p>0.05$, unpaired t test) between groups (mean=9.06 g, control; mean=8.0 g, malathion). Hematocrit ratios were unaffected ($p>0.05$) by pretreatment with malathion; the hemodilution observed in the post-exercise samples ($p<0.02$, control; $p<0.001$, malathion) might be partially attributable to the initial blood withdrawal although under similar conditions, we have observed hemodilution even when much smaller volumes (0.3 ml) have been taken.

Table 1 summarizes the effects of malathion administration and exercise in the heat to hyperthermic exhaustion on circulating levels of several clinical chemical indices of heat/exercise injury. The results indicate that lactate, postassium, urea nitrogen, creatinine, and lactate dehydrogenase are all increased significantly by exercise in the heat to hyperthermic exhaustion, but unaffected by malathion administraion. Several rats in the malathion-treated group had CPK levels greater than 300 U, thus contributing to the high mean level of activity in the cath sample. The apparent increment in pre-run to post-run samples in both groups is actually not statistically significant (minimal critical difference of the means necessary for significance=148 U, Tukey's t test).

DISCUSSION

The design of the present experiment may have permitted the experimental animals to adapt slightly to the effects of malathion administration. It is unknown currently whether a single dose of malathion sufficient to reduce circulating cholinesterase by 35% could have been tolerated equally well in terms of thermoregulatory capacity and work performance. It is well known that experimental animals gradually develop decreased sensitivity to consecutive doses of toxic agents with the acquisition of tolerance probably dependent upon the dosage, specific poison, frequency, and experimental agent [5]. In the present experiments the daily dose (23.5 mg/kg) is approximately 7% [26] of the LD 50 and is administered over a 72 hr time period, probably indicating

that the adaptational process does not play a major role in the functional normality of the malathion-treated rats. This hypothesis is supported by the observation that following catheterization and during the interval of malathion and diluent administration, control and malathion-treated rats display similar fluctations in body weight and normal body temperature. No unusual behavioral characteristics were observed. The small daily volumes of acetone (<0.1 ml) and ethanol (<0.3 ml) had no effects on performance or thermoregulation when these rats were compared with others subjected to identical exercise and environmental conditions [9–11].

Generally, the results of the present investigation demonstrated that malathion administration, at a dosage level sufficient to induce moderate inhibition of circulating cholinesterase levels, had no debilitating effects on physical performance in a hot environment. By contrast, Kurtz [18], using Sprague-Dawley rats, had reported that avoidance performance was affected 1 hour after injection of malathion although blood and brain cholinesterases remained at 90% of control levels. Thus, while behavior may be affected by organophosphate intoxication [5,19], there are several reports indicating that physiological factors may be more resistant although it is necessary to acknowledge differences in doses, rates of administration etc., between the behavioral and physiological experiments. Chakraborty *et al.* [4] demonstrated in rats that neither malathion nor the more potent insecticide, parathion, had effects on hemoglobin concentration or kidney and liver organ weight to body weight ratios. Paul *et al.* [26] reported that an oral dose of 100 mg/kg malathion to rats induced no respiratory distress symptomatology. Villeneuve *et al.* [30] reported, also in rats, that consumption of up to 5 ppm parathion in food for 42 days had no significant effects on the organ to body weight ratios of heart, brain, liver, kidney, spleen or thymus, although body weights were reduced. Since the LD50 of orally administered malathion is in the order of 340 mg/kg [26], it may not be surprising that a total dosage of less than 100 mg/kg over 3 experimental days did not affect physical work capacity.

While Meeter and Wolthuis [23] demonstrated generalized hypothermia as a result of administration of organophosphate anticholinesterases, they also reported that increasing the ambient temperature reduced the magnitude of this hypothermic response. Meeter [22] later showed that greater heat loss through the tail may be responsible for the generalized hypothermia. In the present experiments the initial Tre and Tsk between groups are essentially identical, and indicate no chronic effects on thermoregulation of repeated low-dosage malathion administration. The increased Tsk during exercise in the heat in the malathion-treated rats is of statistical significance and may be related to the original observation of Meeter [22] concerning increased tail-skin heat loss after pesticide administration. However, this observation appears to be of no physiological importance since heating rates, manifested in increments in Tre during exercise in the heat, are virtually identical between groups.

While the behavioral [5,19] and clinical [8,27] manifestations of organophosphate intoxication have been extensively reported, fewer publications have addressed the clinical chemical alterations subsequent to pesticide exposure. Namba *et al.* [25] have noted that accidental or deliberate ingestion of pesticides can elicit circulatory hyperglycemia, slightly elevated blood urea nitrogen, and no effects on glutamate-oxaloacetate (GOT) or glutamate-pyruvate (GPT) transaminases. Sidell [28], in reviewing several cases of accidental exposure to much stronger anticholinesterases, reported no acute effects on hematocrit, blood urea nitrogen, creatinine, GOT, Na^+ or K^+. Paul *et al.* [26] also observed marked hyperglycemia as one of the few pathological sequelae to malathion administration. Evidently, the intensity of malathion intoxication achieved in the present investigation was insufficient to induce significant effects on circulating glucose levels. In fact, the incurrence of heat/exercise injury, while producing anticipated increments of the appropriate clinical chemical indices, had no more effect on the malathion-treated rats than controls.

We concluded from these studies that organophosphate administration, sufficient to induce moderate cholinesterase inhibition, did not have deleterious effects on endurance in a hot environment. While tail-skin temperature was increased, body heat gain was unaffected in the malathion treated groups. Exercise in the heat to hyperthermic exhaustion caused significant increments in plasma levels of lactate, LDH, K^+, UN, and creatinine, but these effects were not exacerbated by malathion administration. Further experiments are planned to examine the effects of more severe intoxication, in the presence and absence of atropine prophylaxis, on physiological responses to exercise in the heat.

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