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# SELECTIVE INHIBITION OF THERMOGENESIS BY TRIBUTYL *S,S,S,*-PHOSPHOROTRITHIOATE (DEF)

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**1** Rats and mice given tributyl *S,S,S,*-phosphorotrithioate (DEF) showed large dose-related falls in body temperature which lasted from several hours to several days at environmental temperatures below thermoneutrality (30 to 31°C).

**2** DEF produced only mild sedation and a remarkable degree of motor control was retained even when body temperatures fell below 30°C. At the dose producing maximal hypothermia only 2% of rats died within the first 24 h, although prolonged hypothermia was usually lethal.

**3** Hypothermia was associated with a complete block of cold-induced thermogenesis, with relatively little effect on basal metabolism at thermoneutrality.

**4** Heat conservation mechanisms (peripheral vasoconstriction and piloerection) appeared to be unaffected by DEF and retained their usual temperature thresholds.

**5** Adrenal catecholamine secretion in response to handling or acute cold exposure was normal in DEF-treated rats but the fall in body temperature could be markedly reduced by large intraperitoneal injections of noradrenaline, although not atropine. The increase in oxygen consumption produced by injected catecholamines was also unaffected by DEF treatment.

**6** It is concluded that the block of cold-induced thermogenesis probably results from a lack of catecholamine release at the tissue level. That this is likely to be mediated at a peripheral site is suggested by the lack of effect of intracerebroventricular DEF.

## Introduction

Tributyl *S,S,S,*-phosphorotrithioate (DEF) is an organophosphorus ester used commercially as a defoliant. It is a poor inhibitor of acetylcholinesterase, but has found biochemical application as a general carboxyesterase inhibitor (Casida, Baron, Eto & Engel, 1963; Silver & Murphy, 1978) at 2 to 20 mg/kg. Symptoms of acetylcholinesterase inhibition are not seen below 400 mg/kg. Intermediate doses of DEF produce a novel effect, namely a profound and long-lasting hypothermia accompanied by remarkably little behavioural depression. This action has been briefly described in a communication (Little & Ray, 1979) and the present paper gives the results of a further investigation of the factors involved in DEF hypothermia.

## Methods

DEF was a gift from the Chemagro Corporation, and was available as technical grade (88% pure) or analytical standard grade (99.8% pure). Following preliminary trials in which the analytical and technical samples produced essentially similar results, the technical grade was used for all experiments. DEF is a clear liquid, immiscible with water and was administered as a 200 mg/ml solution in glycerol formal (Fluka) for all experiments except the 150 and 200 mg/kg intravenous injections for which a 500 mg/ml solution was used, and the intracerebroventricular injections for which undiluted DEF was used.

Adrenaline and noradrenaline were injected in 50 mg/ml ascorbic acid solutions at pH 6.5 to 7.0. Adrenaline and noradrenaline concentrations in plasma were measured fluorometrically by the method of Diamant & Byers (1975) using an excitation wavelength of 400 nm and an emission wavelength of 500 nm with 10 nm slit-widths. Blood was obtained by collecting the first 2 ml after decapitation, and blood from pairs of rats was combined for each assay. The collection procedure was completed in not more than 3 s after decapitation.

Rats were 250 to 300 g female LAC Porton derived Wistar, mice were 20 to 22 g male LAC A, and rabbits were 2.5 kg male New Zealand Whites.

Oxygen consumption was measured by the method of Stock (1975), rats being housed in perspex chambers (18 × 9 × 16 cm) provided with thermostats and with internal circulation of gases. Oxygen

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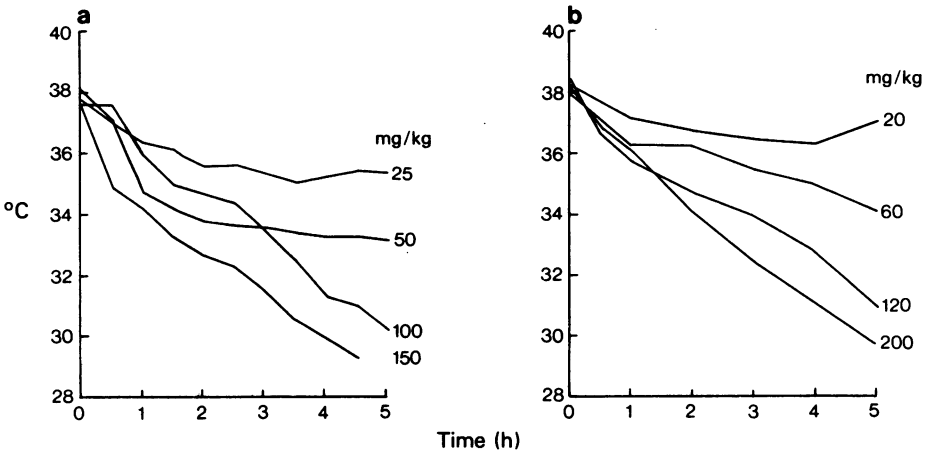


**Figure 1** The effect of varying doses of DEF on the rectal temperatures of individual rats at 20 to 22°C ambient temperature. Rats were injected at time zero by the intravenous (a) or intraperitoneal (b) routes.

was supplied in 2.5 ml units by an automatic injection system triggered by a fall in chamber pressure. Oxygen consumption was calculated from 6 min counts, and an approximate activity index calculated as the modulus of the difference between sequential 2 min counts.

The rat tail vascular response was monitored by two 4 mm diameter disc thermistors lightly clipped to the tail 2 to 3 cm from its end. Rats were placed in a cylindrical wire mesh restrainer under a heating lamp 60 to 70 cm distant with their tails in a shaded environment at 20 to 23°C. The lamp was adjusted initially to produce about 50% tail vasodilatation, and rats so placed were able to regulate their core temperatures by variation in tail blood flow. The degree of tail dilatation was indicated by the elevation of tail surface temperature above ambient.

Injections into the lateral ventricles were made in conscious rats via a 30 gauge needle inserted into a previously implanted supradural guide tube according to the method of Goodrich, Greehey, Miller & Pappenheimer (1969). Injection volumes were 4 to 7 µl, and the siting of the needle tip was verified histologically after injection of Indian ink. Arterial blood pressure was recorded from conscious rats via an implanted femoral arterial cannula, and the electroencephalogram (EEG) recorded from implanted stainless steel supradural electrodes.

Results are expressed as mean ± s.e. mean.

## Results

### *Hypothermia*

In environmental temperatures below approximately 30°C, administration of DEF to rats by the oral, intraperitoneal or intravenous routes produced a large and protracted fall in body temperature. The mean rectal temperature of 19 rats given 200 mg/kg intraperitoneally at 20 to 22°C was 33.0 ± 0.4°C at 2.5 h and 28.1 ± 0.5°C after 24 h, solvent-injected rats remaining at 37.7 ± 0.1°C. The falls in rectal temperature produced by a range of intravenous and intraperitoneal doses in rats at 20 to 22°C ambient are shown in Figure 1. These represent the approximate range of effective values, since increase beyond the dose shown produced no further decrease in temperature. Oral DEF was slightly less effective than intraperitoneal DEF. Injection directly into a lateral ventricle at 10, 20 and 50 mg/kg (the latter divided over 1 h) produced no significant changes in rectal temperature in rats.

The fall in body temperature produced by DEF persisted for several days after intraperitoneal injections in excess of 100 mg/kg, rats surviving for 2 to 3 days at severely subnormal temperatures. Although rats died within the first 2 to 5 h after intravenous injections of 150 mg/kg and above, only one death was observed within the first 24 h after intraperitoneal injection of 200 mg/kg DEF in a total of 56 rats. The duration of DEF action was estimated by maintaining rats given 120 mg/kg (i.p.) at normal body temperatures in a 30°C environment, and measuring the initial rate of cooling on transfer to a 19 to 20°C environment. Solvent-injected rats showed little fluctuation in temperature, but Figure 2 shows that DEF-injected rats continued to cool for up to 48 h after injection. Once allowed to cool beyond 30°C, few rats showed recovery of body temperature.

Mice showed a similar response to that of rats after intraperitoneal injection of DEF over the range 50 to

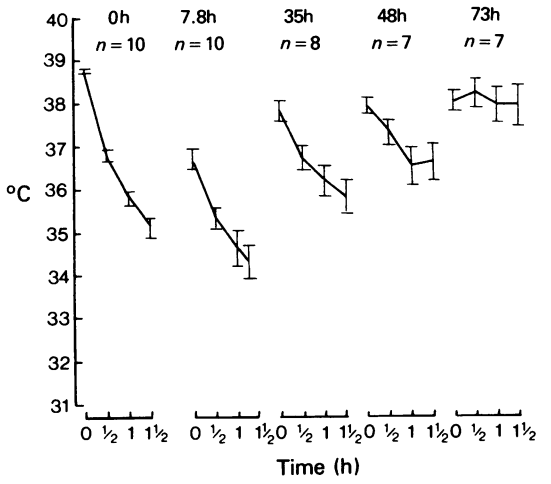


**Figure 2** Fall in rectal temperature of rats dosed with 120 mg/kg of DEF intraperitoneally on exposure to a 19 to 20°C environment at various times after dosing.

200 mg/kg except that falls in rectal temperature were more rapid and that somewhat lower values were reached (see Figure 4). Rabbits showed no fall in rectal temperature over 5 h following intraperitoneal DEF at 200 and 400 mg/kg. The higher dose caused erratic respiration, salivation, and death at 5 h.

*Behaviour*

With body temperature maintained in a 30°C environment DEF-injected rats showed little change in behaviour. On placing them in a 20 to 22°C environment, DEF-treated rats appeared to show normal vasoconstriction but did not show increased spontaneous activity as did normal rats. Although reacting normally to external stimuli, DEF-treated rats tended to move about the cage for a relatively short period after handling and then lie still, much as normal rats in a hot environment. As body temperature fell, rats showed piloerection and became increasingly sluggish, squeaking if handled, but were able to move about even at rectal temperatures below 32°C. Mice in particular retained a remarkable degree of motor control, even at rectal temperatures as low as 25°C. EEG records were obtained from a single rat given 200 mg/kg DEF (i.p.) and showed normal cortical activation on alerting the animal. Records were consistent with mild sedation as long as rectal temperature was maintained within the normal range. The activity index constructed from oxygen consumption records tended to confirm these observations. Six solvent-injected rats showed an increase from $0.65 \pm 0.05$ to $0.89 \pm 0.08$ units on changing chamber temperature from 30 to 15°C, whereas 6 rats given 200 mg/kg DEF (i.p.) showed a decrease from $0.67 \pm 0.06$ to $0.52 \pm 0.06$ units. Thus DEF produced no change in activity index at 30°C ambient temperature.

*Metabolism*

Oxygen consumption was measured in rats 4 to 6 h after intraperitoneal injection of 200 mg/kg DEF. At 30°C ambient the mean oxygen consumption rates of 14 rats injected with DEF and 14 rats injected with solvent were $1.04 \pm 0.04$ and $1.24 \pm 0.07$ l $kg^{-1}$ $h^{-1}$ respectively. Rectal temperatures were fairly well maintained under these conditions, DEF-injected rats having a mean of $36.1 \pm 0.3$°C and solvent-injected rats $37.2 \pm 0.2$°C. Five solvent-injected rats maintained at 30°C for the first 4 h and then tested at 15°C, increased their oxygen consumption rates to $2.46 \pm 0.06$ l $kg^{-1}$ $h^{-1}$ and maintained their rectal temperatures at $37.8 \pm 0.1$°C. In contrast 5 DEF-injected rats failed to increase their oxygen consumption, remaining at $1.01 \pm 0.11$ l $kg^{-1}$ $h^{-1}$ when cooled to $27.8 \pm 9.7$°C. These values represent the means over the 2 h test period, but in the DEF-treated animals, oxygen consumption showed a tendency to fall during cold exposure as rectal temperature fell.

*Cardiovascular actions*

Arterial blood pressure was unchanged in 2 urethane anaesthetized rats after 200 mg/kg DEF (i.p.) or after 80 mg/kg (i.v.). Three conscious rats also failed to show changes in mean blood pressure after 200 mg/kg (i.p.) rectal temperature being maintained. Heart rate fell from pre-injection values of 400–450 beats/min to 200 to 280 beats/min within 40 min after injection. Although these rats continued to show a startle response to loud noises etc., heart rate did not increase to pre-injection values even transiently over a 2 h period.

Tail vascular response was monitored before and after DEF in 6 conscious rats. The elevation of tail surface temperature above ambient varied from 0 to 10°C in individual animals both before and after DEF injection at 200 mg/kg (i.p.). Values were taken at 6 min intervals, and the curves in Figure 3 were constructed to show the relationship between tail and rectal temperatures. The range of rectal temperatures of individual rats before injection varied between 0.4 and 1.3°C, although a wider range was shown by the group as a whole. Tail temperature was well correlated with rectal temperature, but in individual animals it could be seen that factors such as degree of activity, and the rate and direction of change in rectal temperature were also important.

Following DEF injection, rats showed little change in the relationship between tail and rectal tempera-

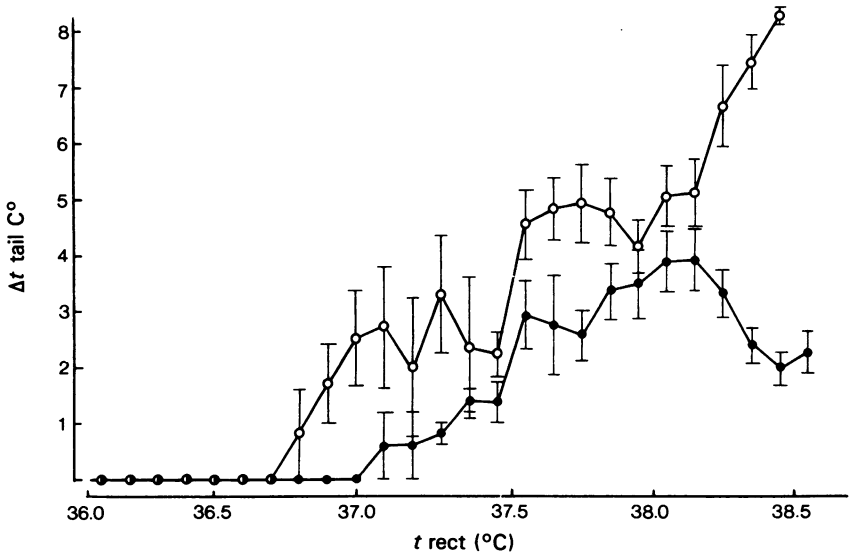


**Figure 3** Relationship between rectal temperature (*t* rect) and the elevation of tail temperature (Δ*t* tail) above ambient in solvent-(○) and DEF (●)-injected rats (200 mg/kg i.p.).

ture. The curve in Figure 3 was constructed over the second to fourth hour after DEF, and rats regulated their rectal temperatures at 38.0 ± 0.17°C during this period, a value not significantly different from their pre-injection mean of 37.8 ± 0.15°C. Some rats showed a rather sluggish vasodilatation response towards the end of this period and allowed their rectal temperatures to rise to 38.5 to 39.0°C before dilating. This is reflected in the downturn in the curve for DEF-treated rats at higher temperatures. The mean elevation of tail above ambient temperature was 5.2 ± 0.4°C before DEF and 2.8 ± 0.2°C after DEF, rats maintaining body temperature by a decrease in tail blood flow. On moving the heating lamp away, the DEF-treated rats showed a brisk fall in tail temperature to ambient as before injection. This was maintained down to rectal temperatures of at least 30°C. Three rats were tested 24 h after DEF injection, their body temperatures having been maintained in a 30°C environment. These regulated about rectal temperatures of 38, 38.5 and 39°C under the lamp, and also showed sustained falls in tail temperature to ambient as soon as they were exposed to cold.

*Effects of cholinoceptor blockade and catecholamines*

The effect of cholinoceptor blockade on DEF hypothermia was investigated in mice, 11 of which were given 200 mg/kg DEF (i.p.) without pretreatment, 7 of which were given 100 mg/kg atropine (i.p.) 40 min before DEF, and 5 of which were given atropine alone, all at 22°C ambient (see Figure 4). The high dose of atropine was itself sufficient to produce a transient fall in temperature, but the mice recovered from this within an hour. However, this pretreatment failed to prevent the DEF-induced hypothermia, both atropine-pretreated and normal mice reaching similar low temperatures at 3.7 h. In contrast intraperitoneal injection of noradrenaline markedly reduced DEF-induced hypothermia. Figure 5 shows the effect of repeated injections of 1.5 mg/kg noradrenaline on the rate of cooling of 10 rats given 200 mg/kg DEF 4 h previously. When compared to that of 5 rats given ascorbate solvent only, the rats given noradrenaline increased their rectal temperatures after each injection. The cooling curve of the noradrenaline injected rats from 3.8 h was similar to that shown by the solvent-injected animals but delayed by the period over which the injections were given, and the 24 h values were closely similar in both groups.

The effect of DEF pretreatment on the response to catecholamines was investigated in more detail using the elevation in oxygen consumption following intraperitoneal injection as an index in rats maintained near to normal body temperature in a 30°C environment. Noradrenaline at 1.5 mg/kg produced a maximal response in normal rats and was effective over a period of approximately 1 h (Figure 6). Six DEF pretreated rats and 6 solvent-injected rats showed increases of 1.35 ± 0.18 and 1.13 ± 0.09 l/kg over their respective baselines, the DEF pretreatment producing no significant change in response. The same was true of a lower dose of 0.25 mg/kg noradrenaline, which produced increases of 0.44 ± 0.07 and 0.53 ± 0.1 l/kg

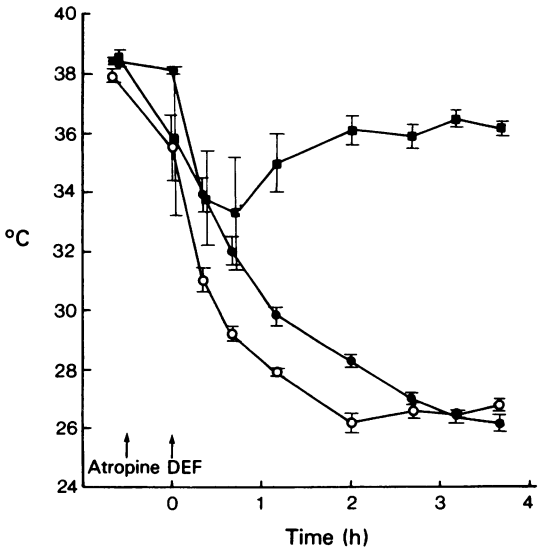


**Figure 4** Falls in rectal temperature of mice injected with 100 mg/kg atropine at −40 min (■), 100 mg/kg atropine at −40 min plus 200 mg/kg DEF at 0 min (○), and 200 mg/kg DEF only at 0 min (●). Environmental temperature was 22°C.

in 6 DEF and 6 solvent-treated rats. DEF pretreatment did not significantly alter the response to 0.5 mg/kg adrenaline (i.p.) either, 7 DEF- and 7 solvent-treated rats increasing their oxygen consumption by 0.36 ± 0.04 and 0.42 ± 0.08 l/kg respectively.

The rise in blood pressure produced by 2 μg intravenous injections of noradrenaline was also found to be normal when measured in two urethane-anaesthetized rats preinjected with 200 mg/kg DEF.

Plasma adrenaline and noradrenaline were measured in 4 pairs of rats maintained at 30°C ambient for 2 h after i.p. injection of 200 mg/kg DEF and found to be 3.65 ± 0.30 and 1.24 ± 0.28 ng/ml. The corresponding values from 4 pairs of solvent-injected rats were 2.00 ± 0.16 and 1.14 ± 0.18 ng/ml, adrenaline levels being significantly elevated in DEF-injected rats ($P < 0.01$). Subsequent exposure to a 4°C environment increased these values in both DEF- and solvent-injected rats (see Figure 7) and caused a rapid fall in rectal temperature in the DEF-injected animals. The combined values for 10, 20 and 30 min of cold exposure showed adrenaline and noradrenaline levels to rise to 7.49 ± 0.84 and 4.23 ± 0.69 ng/ml in DEF-injected rats. The corresponding concentrations in solvent-injected rats were 4.56 ± 0.18 and 2.35 ± 0.15 ng/ml, all values being significantly elevated above those obtained before cold exposure ($P < 0.01$) and DEF-injected rats showing similar increases to those of solvent-injected rats.

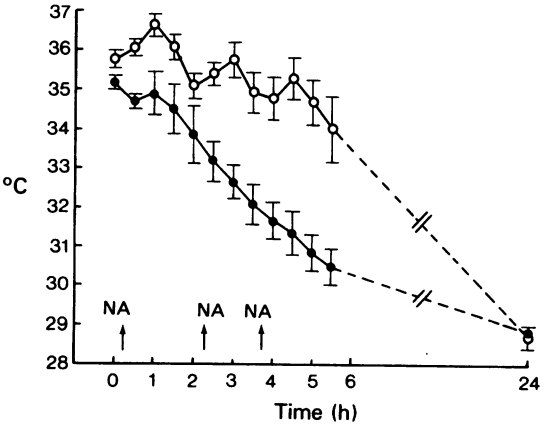


**Figure 5** Rectal temperature of rats given 200 mg/kg DEF i.p. at −4 h, maintained at 30°C ambient, and transferred to 22°C ambient at 0 h. At the times marked, rats were given intraperitoneal injection of solvent (●) or 1.5 mg/kg noradrenaline (NA) (○).

Increasing the effective stress of the blood collection procedure by collecting the larger volume of 7 ml of blood over a correspondingly longer period increased catecholamines in both DEF and solvent injected rats by 250–500% at 30°C ambient.

## Discussion

The degree of hypothermia produced in rats or mice increased with dose over the range 20 to 200 mg/kg, the somewhat more pronounced effects seen in mice being presumably due to their greater surface area to body weight ratio. The responses to intravenous and intraperitoneal administration in rats were closely similar, except for a somewhat greater acute toxicity via the intravenous route, this similarity suggesting that DEF equilibrates fairly rapidly within the body. In contrast, the long lasting nature of the DEF action suggested a relatively slow removal from the body. The carboxylesterase-inhibitory action of DEF reported by Casida *et al.* (1963) for mice lasted 3 to 5 days, a similar value to the 2 to 3 day duration of the rat hypothermia seen in the present study.

Since DEF-treated rats regulated about normal or slightly elevated body temperatures when provided with a supplementary heat source, it would appear that the hypothermia did not result from an active attempt to reduce body temperature but rather from an inability to maintain it. The lack of increase in oxygen consumption (and hence heat production) in cool environments seen in DEF-treated animals was presumably the major reason for their failure to maintain normal body temperature since heat conserva-

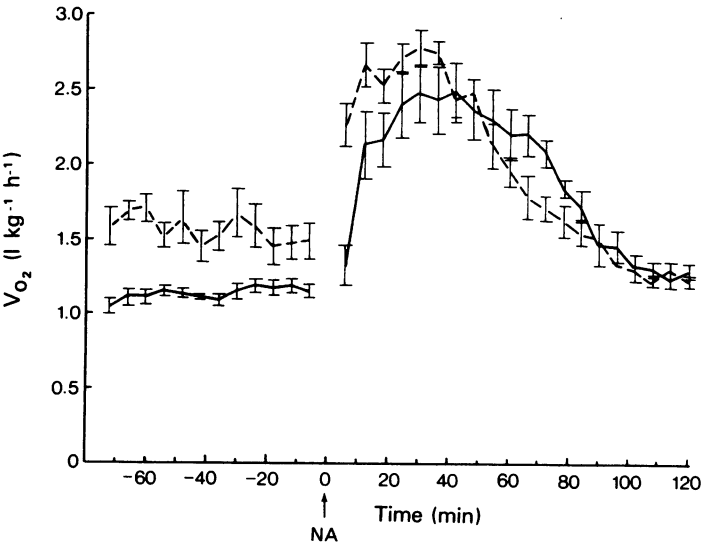


**Figure 6** Oxygen consumption rate of 6 rats given 200 mg/kg DEF (—) or solvent (---) intraperitoneally at −4 h followed by noradrenaline (NA, 1.5 mg/kg i.p.) at 0 min.

tion, as indicated by tail vasoconstriction and piloerection, appeared to be normal. This is in contrast to the hypothermia produced by ganglion-blocking agents, which is associated with a marked increase in heat loss. The loss of thermogenic capacity appeared to be largely due to an inability to increase oxygen consumption over the basal rate, rather than an overall depressive effect, since although there was a significant decrease in oxygen consumption in the 30°C environment this was only 16% (compared to a fall of 59% at 15°C), and was associated with a fall of 1.1°C in body temperature. The lack of any distinct behavioural action at 30°C ambient, and the unchanged activity index also suggested that it was specifically the cold-induced heat production that was inhibited by DEF. The hypothermia resembled that produced by chlorpromazine in that mild sedation was seen at 20 to 22°C ambient. However, chlorpromazine hypothermia appears to be brought about by a less selective action, being accompanied by large increases in tail blood flow and a loss of piloerection (Kollias & Bullard, 1964).

The mechanism of DEF-induced hypothermia would appear to be quite distinct from that produced by cholinomimetics such as oxytremorine or anticholinesterases such as soman since these are both blocked by atropine (Maikel, 1970; Meeter & Walthius, 1968). The finding that the falls in body temperature could be largely prevented by large intraperitoneal injections of noradrenaline, and that DEF-treated animals could increase their oxygen consumption normally in response to such injections, clearly suggested that the inhibition of thermogenesis was not due to lack of metabolic capacity.

The large intraperitoneal noradrenaline and adrenaline injections probably produced their effects directly by elevation of tissue catecholamine concentrations, and the responses obtained showed that apparently normal thermogenesis could occur in response to exogenous catecholamines. Although the doses used were high compared to intravenous ones, they were comparable with those used by other investigators (Banet & Hensel, 1976) and showed normal responses both at the maximal and 47% maximal response levels.

Adrenal medullary function appeared to be normal in DEF-treated rats, since plasma levels at 30°C were comparable with those found by other investigators for handled animals (Popper, Chuang & Kopin, 1977). Furthermore in DEF-injected rats, plasma catecholamines increased by at least as much as those of solvent-injected animals on acute cold exposure or after additional stress. DEF pretreatment did not inhibit the response of rat vas deferens to nerve stimulation (personal communication Jean Bradbury) and this together with the normal tail vascular tone, blood pressure, and cardiovascular responses to noradrenaline, suggested that DEF had no generalized $\alpha$- or $\beta$-adrenoceptor blocking action. A specific action on the sympathetic nerve endings mediating the increase in metabolic rate in fat pad and other tissues is a possibility; this would also be consistent with the high

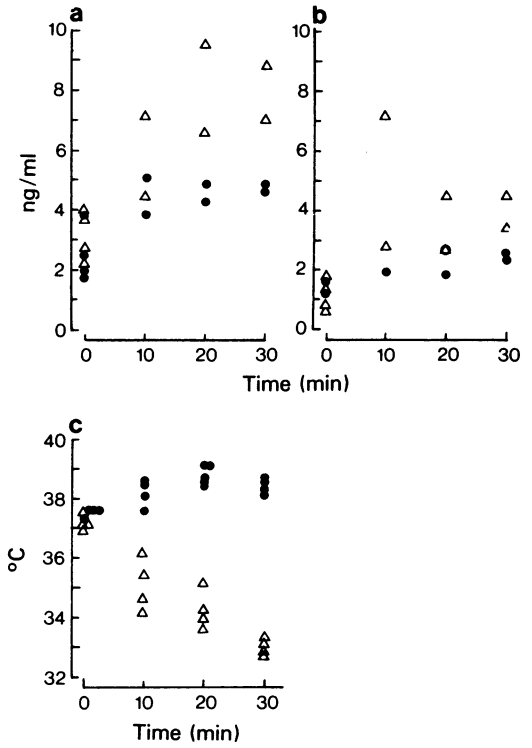


**Figure 7** Plasma adrenaline (a), plasma noradrenaline (b), and rectal temperature (c) of individual rats injected with 200 mg/kg DEF (△) or solvent (●) 4 h previously. Rats were maintained at 30 C following injection, and transferred to 4 C ambient at time = 0.

lipid solubility of DEF and would explain the selective blockade of the thermogenic response to cold together with the normal thermogenic response to exogenous catecholamines. Such an action could be brought about at a central or peripheral site, but since DEF is ineffective by the intracerebroventricular route a central site of action is unlikely. This would also appear to be supported by the species specificity of DEF which does not fit with the known response to intracerebroventricular transmitters. The rat (Myers & Yaksh, 1969) and rabbit (Cooper, Cranston & Honour, 1965) but not mouse (Brittain & Handley, 1967) show similar responses to noradrenaline while 5-hydroxytryptamine produces a uniform response, but DEF is effective in rat and mouse but not rabbit. The specificity of action of DEF in this case may rather be a reflection of the greater reliance on thermogenesis of the smaller species to maintain body temperature.

Although the specific site of action of DEF is not yet known, its remarkably selective action upon thermogenesis makes it potentially a very useful tool in the investigation of body temperature control; and in addition its ability readily to produce marked hypothermia with minimal CNS or cardiovascular effects should prove useful in investigating the temperature-dependence of many other body processes.

I should like to thank Mr T. Lister for carrying out the catecholamine assays and for general technical assistance; also Jean Bradbury for supplying the vas deferens results and Mr R.U.E. Gwilliam for constructing the oxygen consumption chambers.

## References

BANET, M. & HENSEL, H. (1977). The control of shivering and non-shivering thermogenesis in the rat. *J. Physiol.*, **169**, 669–676.

BRITTAIN, R.T. & HANDLEY, S.L. (1967). Temperature changes produced by the injection of catecholamines and 5-hydroxytryptamine into the cerebral ventricles of the conscious mouse. *J. Physiol.*, **192**, 805–813.

CASIDA, J.E., BARON, R.L., ETO, M. & ENGEL, J.L. (1963). Potentiation and neurotoxicity induced by certain organophosphates. *Biochem. Pharmac.*, **12**, 73–83.

COOPER, K.E., CRANSTON, W.I. & HONOUR, A.J. (1965). Effects of intraventricular and intrahypothalamic injection of noradrenaline and 5-hydroxytryptamine on body temperature in conscious rabbits. *J. Physiol.*, **181**, 852–864.

GOODRICH, C.A., GREEHEY, B., MILLER, T. B. & PAPPENHEIMER, J.R. (1969). Cerebral ventricular infusions in unrestrained rats. *J. appl. Physiol.*, **26**, 137–140.

KOLLIAS, J. & BULLARD, R.N. (1964). The influence of chlorpromazine on physical and chemical mechanisms of temperature regulation in the rat. *J. Pharmac. exp. Ther.*, **145**, 373–381.

LITTLE, R.A. & RAY, D.E. (1979). Tributyl *S,S,S,*-phosphotrithioate (DEF), a potential tool in thermoregulation research. *Br. J. Pharmac.*, **66**, 438P.

MAICKEL, R.P. (1970). Interaction of drugs with autonomic nervous function and thermoregulation. *Fedn Proc.*, **29**, 1973–1979.

MEETER, E. & WALTHIUS, O.L. (1968). Effects of cholinesterase inhibitors on the body temperature of the rat. *Eur. J. Pharmac.*, **4**, 18–34.

MYERS, R.D. & YAKSH, T.L. (1968). Feeding and temperature responses in the unrestrained rat after injections of cholinergic and aminergic systems into the cerebral ventricles. *Physiol. Behav.*, **3**, 917–928.

POPPER, C.W., CHUANG, C.C. & KOPIN, I.J. (1977). Plasma

catecholamine concentrations in unanaesthetised rats during sleep, wakefulness, immobilisation and after decapitation. *J. Pharmac. exp. Ther.*, **202**, 144–148.

SILVER, E.H. & MURPHY, S.D. (1978). Effect of carboxylesterase inhibitors on the acute hepatotoxicity of esters of allyl alcohol. *Tox. appl. Pharmac.*, **45**, 377–389.

STOCK, M. J. (1975). An automatic, closed circuit oxygen consumption apparatus for small animals. *J. appl. Physiol.*, **39**, 849–850.

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