(2 h) by 49% whereas salicylic acid applied in DMSO–glycerol caused less than 21% inhibition.

These two rat strains differ not only in their sensitivity and rate of response to individual oedemogens but also most probably in their skin permeability (facilitated by DMSO). This latter difference may be particularly critical in comparing systemic effects of topically applied agents, e.g. components of the Dermcusals, over the limited time period of oedema development.

The zymosan oedema is considered to be more complement-dependent and perhaps less sensitive to salicylates than the familiar carrageenan model of induced inflammation (Gemmell et al 1979).

When tested on DA × Lewis rats with established polyarthritis, both DCS–A and DCS–E applied dorsally for four consecutive days (5 ml kg^{-1} day^{-1}) appreciably reduced swelling of the tail and all four paws (Table 2) with no adverse effects, e.g., gross weight change, loss of agility and alertness. Double doses profoundly arrested the increase in paw/tail swelling and were also well tolerated, apart from causing some subsequent loosening of the skin at the site of application (notably without inflammation). Equivalent formulations without copper were devoid of anti-arthritic activity, when likewise applied dermally.

At 4 ml kg^{-1} given topically with glycerol, DMSO was not a significant anti-oedemic/anti-arthritic agent in our rats. Many reports suggest that DMSO does exert an anti-inflammatory effect (e.g. Brown 1971; Herschler & Jacob 1978). Only at much higher dose (12 ml kg^{-1}) did topically applied DMSO suppress the carrageenan oedema.

In the Dermcusal formulation, the primary role of the DMSO is to solubilize and promote the permeation of copper salicylate into the stratum corneum. Replacing the copper with iron (III) in this formulation did not increase the anti-oedemic activity above that shown by salicylic acid.

The copper acetate–ethyl salicylate formulation (DCS–E) falls within the pH range accepted as promoting hydration of the stratum corneum (Katz 1973), which in turn is reported to facilitate dermal absorption (Wepierre & Marty 1979).

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Core temperature changes following administration of naloxone and naltrexone to rats exposed to hot and cold ambient temperatures. Evidence for the physiological role of endorphins in hot and cold acclimatization

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It has been reported (Holaday et al 1978) that peripheral administration of naloxone to rats, after acute and chronic heat exposure, precipitates an increase in colonic temperature above that seen in control animals given 0·9% NaCl (saline). It was suggested that naloxone mediated its responses by antagonism of pituitary endorphins, since the drug's effect was diminished when tested in hypophysectomized rats. From that study, endorphins were proposed to possibly play a physiological role in heat adaptation.

Naloxone, however, has also been shown to cause small, but significant changes in the core temperature responses of rats kept at 22 °C (Blasig et al 1978; Thornhill et al 1978) or when exposed to the cold (Goldstein & Lowery 1975). Thus we wanted to see what effect narcotic antagonists, in relatively high but non-toxic doses used previously (Holaday et al 1978; Goldstein & Lowery 1975), would have on the core temperature of rats acclimitizing to severe changes in ambient temperature. To do this, core temperature responses of rats were measured following a single subcutaneous injection of either of two narcotic antagonists of varying durations of action—naloxone and naltrexone HCl—to groups of animals exposed to 4 °C or 38 °C for different periods of time.

Male, Sprague-Dawley rats, appoximately 300 g, were maintained at an ambient temperature (Ta) of 22 °C until the experiments began. Rats were then kept

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in a controlled environmental chamber (Ta preset at either 4 or 38 °C, relative humidity approximately 40% and this room had a 12 h on/off lighting schedule) for 1, 4, 12, 24, 24, or 48 h before drug injections were given. Naloxone and naltrexone HCl were administered (10 mg kg^{-1}, s.c.) in a volume of 0·25 ml/100 g body weight to groups of rats. Both drugs, in concentrations used previously (Holaday et al 1978; Goldstein & Lowery 1975) were tested to determine if the duration of the thermoregulatory changes evoked by these compounds correlated with their known difference in biological half-life (Blumberg & Dayton 1973; Resnick 1974). Subcutaneous injections of saline were administered concurrently, in similar volumes, to other groups of control rats. Core temperatures were measured at intervals from 30 min before and for 4 h post-administration with a precalibrated rectal probe inserted 6 cm beyond the anus.

A thermal response index (TRI) was calculated for the temperature response of each rat to saline, naloxone HCl or naltrexone HCl from the mean of 3 pre-injection control readings based on the method of Clark & Cumby (1978). The TRI's (areas under the temperature-time curve after 1, 2 and 4 h post-administration) were then averaged for each experimental group. Statistical analyses of the TRI were performed between the treatment groups using the Student's *t*-test for unpaired data.

Fig. 1 shows the changes in core temperature, from baseline control readings, of groups of rats given a s.c. injection of saline, aloxone HCl or naltrexone HCl following exposure of 1 or 4 h to a Ta of either 4 °C or 38 °C. Graph (a) of Fig 1 indicates that the core temperatures of both the naloxone or naltrexone HCl groups rose from between 1·0 to 1·5 °C above baseline control readings within the first 30 min post-administration and remained within this range over the rest of the 4 h period. Table 1 shows that the TRI from the core temperature responses over the whole 4 h period of both groups given a narcotic antagonist (Fig. 1a) were significantly increased from that of the control group given saline under the same experimental conditions. Core temperature changes to naloxone or naltrexone HCl after 4 h of exposure to 38 °C (Fig. 1b) were not as marked as those seen in groups in Fig 1a. Core tempera-

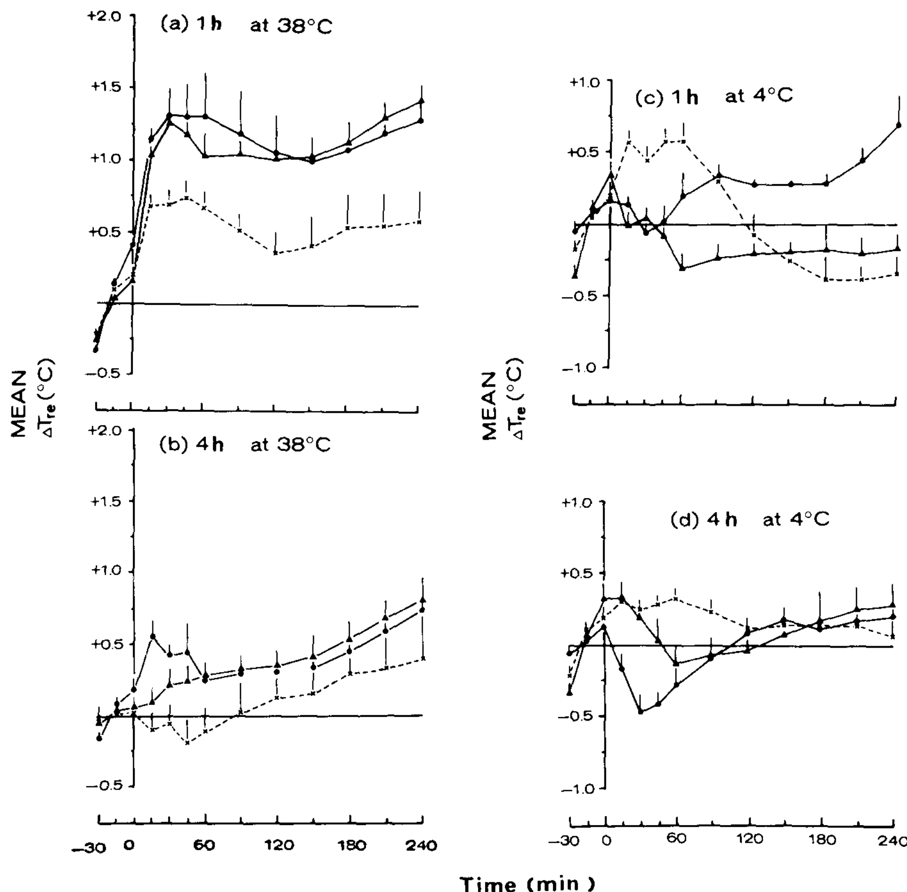

FIG. 1. Mean changes in rectal temperature (°C ± s.e.m.) from pre-injection control readings, are shown for groups (n = 4) of naive rats exposed to 38 or 4 °C for 1 or 4 h and then given a subcutaneous injection of saline (× - - - ×), 10 mg kg^{-1} naloxone HCl (●——●) or naltrexone HCl (▲——▲).

Table 1. Thermal Response Indices (TRI, °C × h ± s.e.m.) after 1, 2 and 4 h post-injection of groups of rats exposed for 1 or 4 h to 4 or 38 °C and given saline (I), naloxone HCl (II) or naltrexone HCl (III) (10 mg kg^{-1}).

Group	After 1 h	After 2 h	After 4 h
		Ta = 38 °C; 1 h exposure period	
I	0·488 ± 0·08	0·95 ± 0·21	1·81 ± 0·61
II	1·00 ± 0·15*	2·19 ± 0·45*	4·45 ± 0·90*
III	0·92 ± 0·09*	1·96 ± 0·17*	4·34 ± 0·36*
		Ta = 38 °C; 4 h exposure period	
I	−0·03 ± 0·10	−0·02 ± 0·35	0·50 ± 1·00
II	0·26 ± 0·06*	0·53 ± 0·18	1·44 ± 0·35
III	0·16 ± 0·04*	0·43 ± 0·12	1·56 ± 0·28
		Ta = 4 °C; 1 h exposure period	
I	0·37 ± 0·08	0·66 ± 0·17	0·13 ± 0·40
II	0·04 ± 0·07*	0·32 ± 0·15	1·06 ± 0·15*
III	−0·04 ± 0·03*	−0·28 ± 0·09*	−0·68 ± 0·25
		Ta = 4 °C; 4 h exposure period	
I	0·19 ± 0·05	0·40 ± 0·09	0·66 ± 0·41
II	−0·31 ± 0·07*	−0·41 ± 0·15*	−0·14 ± 0·09
III	0·10 ± 0·07	0·11 ± 0·14*	0·36 ± 0·48

* Significant changes ($P < 0·05$; $P < 0·01$) in TRI from that of respective saline control groups over the same period.

tures did rise significantly over the first hour post-administration in both groups given a narcotic antagonist (Table 1) compared with the saline control group. Animals exposed to the heat (38 °C for 1 or 4 h) showed signs of increased heat dissipation, such as minimal locomotor activity, lying prostrate on their backs with their ventral surfaces exposed to the air, copious salivation, reddened scrota and descended testicles. These signs were evident in all of the experimental groups. Animals given either narcotic antagonist after a longer period of exposure to 38 °C (i.e. >4 h) failed to show any significant temperature changes from those of rats given saline after similar periods of exposure.

The TRI from core temperature responses over the first hour post-administration of both groups given a narcotic antagonist after 1 h exposure to the cold (4 °C) were significantly lower from that of the saline control group (Fig 1c and Table 1). In fact, core temperatures of the naltrexone-treated rats were still significantly decreased after 2 h post-injection from those of the saline control group. By this time, core temperatures of the naloxone group had rebounded (i.e. had risen) and continued to increase over the rest of the experiment such that their TRI over the whole 4 h period was significantly above that of the saline control group. Fig 1d shows that after 4 h of exposure to 4 °C core temperatures again decreased in those animals given naloxone and naltrexone such that their TRI were significantly below that of the controls 2 h post-injection. No differences in behaviour were seen between the saline, naloxone or naltrexone groups on exposure to 4 °C for 1 or 4 h; animals within each group showed shivering, piloerection and blanched ears and tail. Although not shown, significant decreases in TRI over the first hour post-administration also were seen in groups of naloxone- and naltrexone-treated animals exposed to the cold (4 °C) for 12 and 24 h when compared with those of saline control groups.

The results show that narcotic antagonists can alter the temperature responses of rats acutely exposed to extreme changes in ambient temperature of hot or cold. Naloxone and naltrexone caused rats' core temperatures to decrease significantly from controls when exposed to the cold (4 °C) and to be elevated above control animals on exposure to extreme hot temperatures (38 °C). Smaller doses of both narcotic antagonists have been tested on hot and cold exposed rats. The changes in core temperature response were similar qualitatively to those reported here but less pronounced. In the present study, 10 mg kg^{-1} doses of both antagonists evoked a similar magnitude of core temperature change, yet the duration of these changes was markedly prolonged by naltrexone in only one of the four exposure periods (1 h at 4 °C). This was unexpected in view of the differences in biological half-life known to exist from pharmacokinetic studies between naloxone and naltrexone (Blumberg & Dayton 1973). However, the temperature changes evoked by either drug did appear to be more marked in both the heat and cold-stressed rats as the exposure period to the extreme Ta was shortened.

This study suggests that blockade of brain and pituitary endorphins by narcotic antagonists causes an acute disturbance in the rat's ability to acclimatize

to severe changes in ambient temperature. It also infers that endogenous opioid peptides may have a physiological role in regulating the body temperature of rats undergoing changes in environmental temperature. Whether endorphins principally affect metabolic, cardiovascular or behavioral components of this acclimation process is not yet known.

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Decrease of noradrenaline *O*-methylation in rat brain induced by L-dopa. Reversal effect of *S*-adenosyl-L-methionine

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S-Adenosyl-L-methionine (SAMe) is the main methyl donor in many biochemical reactions (Axelrod 1966; Salvatore et al 1977; Usdin et al 1979). In the central nervous system SAMe takes part in catecholamine metabolism (Stramentinoli & Maffei 1978) and in the synthesis of biologically important compounds, such as melatonin (Wurtman & Axelrod 1968) and phosphatidylcholine (Mozzi & Porcellati 1979; Blusztajn et al 1979).

The SAMe concentration may be depleted by such compounds as L-dopa, which may act as methyl group acceptors (Wurtman et al 1970; Ordonez & Wurtman 1973).

If SAMe concentration is critical, treatment with L-dopa may affect normal transmethylating reactions. In fact, previous studies by Chalmers et al (1971) demonstrated that acute L-dopa administration to rats intracisternally injected with [^{3}H]noradrenaline caused a depletion of brain SAMe as well as a reduction in *O*-methylated [^{3}H]noradrenaline derivatives.

Our aim has been to demonstrate that the decreased *O*-methylation of brain noradrenaline following the depletion of brain SAMe after L-dopa administration can be reversed by exogenous treatment with SAMe.

S-[Methyl-^{14}C] Adenosyl-L-methionine($^{14}CH_3$-SAMe), 55 mCi mmol^{-1}, was obtained from the Radiochemical Centre, Amersham, England; L-dopa was from Merck, Darmstadt, Germany, and SAMe as disulphate-di-*p*-toluenesulphonate was obtained from BioResearch Co., Milan, Italy.

Male CD-COBS albino rats (Charles River, Italy), 175–200 g were injected with L-dopa (100 mg kg^{-1}, i.p.) or with L-dopa plus SAMe (100 mg kg^{-1}, i.m., 5 min after L-dopa).

L-Dopa was suspended in 2% gum arabic solution kept continuously stirred and injected in a volume of 4 ml kg^{-1}.

SAMe was dissolved in phosphate buffer 0·17 M to reach a final pH of 7·0 and injected in a volume of 2·0 ml kg^{-1}. The control rats received the corresponding vehicles; the phosphate buffer was also brought to pH 7·0 using 1 M HCl.

The animals were decapitated 45 min after SAMe injection. The brains were rapidly removed and the cortex dissected, washed carefully in chilled saline, blotted on filter paper and frozen on dry ice and kept at −30 °C until assayed (within 24 h).

The cerebral tissue was then homogenized in 10% TCA dissolved in 0·05 M HCl 1:5 (w/v). After centrifugation at 3000 *g* at 4 °C for 10 min, 1-ml aliquots of the clear supernatant were mixed with 20 μl of [methyl-^{14}C]-SAMe (10 nCi). The solution was then washed three times with 2 vol of peroxide-free ether previously saturated with 0·05 M HCl.

SAMe concentration was then assayed according to the radioenzymatic method of Baldessarini & Kopin (1966), and 3-methoxy-4-hydroxyphenylglycol-SO_4 (MHPG-SO_4), the main methoxylated metabolite of brain noradrenaline, was determined spectrofluorimetrically in cortex extracts after chromatographic separation on DEAE Sephadex A-25 columns, following the procedure of Meek & Neff (1972).

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