J. Physiol. (1974), **238**, *pp.* 657–670
With 5 text-figures
Printed in Great Britain

INHIBITION OF THERMOREGULATORY NON-SHIVERING THERMOGENESIS BY TRAUMA IN COLD-ACCLIMATED RATS

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(*Received 3 December 1973*)

SUMMARY

1. Heat production and blood flow in the interscapular brown adipose tissue of 3° C acclimated rats have been measured by the heated thermocouple technique.

2. When the environmental temperature (T_a) was reduced from 30 to 3° C heat production by the brown adipose tissue began to increase at the lower limit of the thermoneutral zone and then increased linearly.

3. Blood flow also increased when T_a was reduced but was not so well correlated with T_a. There was however a good positive correlation between blood flow and heat production.

4. During the first hour after a 4 hr period of bilateral hind-limb ischaemia in a 20° C environment the percentage of rats not producing heat in the interscapular brown adipose tissue increased and the tissue blood flow fell.

5. By varying the T_a of the injured rat it was found that the T_a at which heat transfer from the interscapular brown adipose tissue commenced was significantly lower than in the controls although the slope of the regression lines relating heat production and T_a was unaltered.

6. Blood flow also increased in the injured rat when T_a was lowered but the increase in the tissue blood flow per unit increase in heat production was less than in the controls.

7. In a 5° C environment heat production in the interscapular brown adipose tissue of the injured rat was further increased by the S.C. injection of L-isoprenaline or L-noradrenaline.

8. It is concluded that the central control of thermoregulatory non-shivering thermogenesis is inhibited after injury in the rat.

INTRODUCTION

The effect of limb ischaemia on heat production by brown adipose tissue in cold-acclimated rats has been examined as part of a continuing investigation of the effects of trauma on thermoregulation. The present experiments were undertaken to obtain more direct proof than hitherto of the inhibition of thermoregulatory non-shivering thermogenesis after injury (Stoner, 1969) in a way which would reveal something about the mechanism of this inhibition. The interscapular brown adipose tissue was chosen for this study since its main, if not only, function is to produce heat under central nervous control.

Brown adipose tissue's heat production and blood flow were measured with heated thermocouples (Grayson, 1952) which others have used in this tissue (Brück & Wünnenberg, 1965). Linzell (1953) has emphasized the shortcomings of this technique in an unhomogeneous tissue but the enlarged interscapular fat pads of the cold-acclimated rat seemed suitable for the improved probes described by Grayson, Coulson & Winchester (1971) and proved so in practice. Exposure to an ambient temperature of 3° C for 2–3 weeks is sufficient to produce acclimation to that temperature (Fregly, 1953; Cameron & Smith, 1964) without altering the resistance of the rat to limb ischaemia (Stoner, 1965). Observations were made after the period of limb ischaemia since this is when inhibition of non-shivering thermogenesis is thought to occur (Stoner, 1969). While the circulation to the hind limbs is occluded the rat can increase non-shivering thermogenesis in response to a demand for heat (Stoner & Marshall, 1971) and usually maintains its core temperature (Stoner, 1961*a*). The results supported the view that trauma causes central inhibition of thermoregulatory non-shivering thermogenesis and have also led to some refinement of a neuronal model (Bligh, 1972) which could be applied to thermoregulation in the rat.

METHODS

Male albino rats of the Porton strain fed on M.R.C. diet 41B (Bruce & Parkes, 1956) were used. They were cold-acclimated (for definition of acclimation see Hart, 1961) at 3° C in groups of five (Stoner, 1965). The lighting of the cold-room was controlled to give 12 hr light per day (7.0 a.m. to 7.0 p.m.). The rats were used after 10–25 days (mean 17) at 3° C when they weighed 230 ± 20 g (mean $\pm$ S.D.). At this time the combined weight of the two lobes of the interscapular brown adipose tissue was $0{\cdot}54 \pm 0{\cdot}14$ g (mean $\pm$ S.D.).

The heated thermocouples have been described (Stoner, 1973). The reference thermocouples were also as before but mounted in PE 10 Clay Adams Intramedic polyethylene tubing (Becton, Dickinson & Co.) with Kleber Tixo K4 adhesive (Tenant Trading Co., London). Under ether anaesthesia the reference thermocouple was introduced into the lower third of the thoracic aorta via the left carotid artery. The interscapular brown adipose tissue was exposed through a longitudinal skin incision. The cephalic end of one lobe was freed and the heated thermo-

couple was inserted along its long axis following a track made with a needle. This ensured that an adequate amount of tissue surrounded the thermocouple. The thermocouple was held in place with a suture and with isobutyl 2-cyanoacrylate tissue adhesive (Ethicon Ltd, Edinburgh). The wires were brought out through the skin of the dorsum. At the end of the operation each rat was injected I.M. with 10 mg oxytetracycline (Terramycin injectable solution; Pfizer, Sandwich, Kent). They were then kept in a 20° C environment for 24 hr before being returned to the cold-room at 3° C. This period at 20° C would not be expected to affect the degree of cold-acclimation significantly (Fregly, 1953).

The experiments were carried out 2–6 days (mean 4) after the operation in the environmental chamber described previously (Stoner, 1971). The temperature of the air (T_a) in the chamber was measured with a thermocouple. Core temperature (T_c) was measured in the colon with a thermocouple inserted 6–8 cm from the anus. The injury used was a 4 hr period of bilateral hind-limb ischaemia produced with rubber tourniquets applied during a short period (approx. 3 min) of ether anaesthesia by Rosenthal's (1943) method. The constantan wires of the reference and heated thermocouples were soldered together outside the rat. The cycle of heating currents and the apparatus for applying it to the heater coil were described earlier (Stoner, 1973). Because of the slow return of the temperature to the base line at the end of the cycle continuous recordings were not made. Instead, 1–3 cycles were recorded at the end of the equilibrium periods. As far as possible, the temperature difference (ΔT) between the aortic blood and the brown adipose tissue corrected for blood flow, the I^2 equivalent of the heat production of the brown adipose tissue, and the increase in the thermal conductivity (Δk) of the brown adipose tissue due to blood flow were calculated (Grayson *et al.* 1971) from the first cycle.

L-Isoprenaline (John Wyeth & Brother, Ltd, Maidenhead, Berks.) and L-noradrenaline (Koch-Light Laboratories Ltd, Colnbrook, Bucks.) were injected S.C. as the bitartrates. The former was dissolved in 0·9 % NaCl and the latter in 0·9 % NaCl containing 50 μg L-ascorbic acid/ml. immediately before injection. Doses are expressed as the free base.

At the end of the experiment the rat was killed with sodium pentobarbitone (Veterinary Nembutal; Abbott) and further recordings were made at least 15 min after death. The positions of the thermocouples were then determined. They were removed from the body and embedded in 5 % gelatin for the calibration of the heated thermocouple (Haigh, 1954). The thermal conductivity (k) of dead brown adipose tissue was calculated from the recordings made in the dead rat using the calibration factor. The value of k in the control cold-acclimated rats was $9{\cdot}97 \pm 1{\cdot}62 \times 10^{-4}$ c.g.s. units (mean $\pm$ S.D.; $n = 13$) and $9{\cdot}54 \pm 1{\cdot}57 \times 10^{-4}$ ($n = 23$) in the injured. These values are not significantly different. The individual values were used in the calculation of Δk except in two rats where the heated thermocouple was damaged during removal from the rat and in which the appropriate mean value was used instead.

Where possible results have been expressed as means $\pm$ S.D. or S.E. Means have been compared by Student's t test as modified by Fisher (1934) for small samples. Other calculations follow the methods of Snedecor & Cochran (1967).

RESULTS

Effect of environmental temperature on heat production by brown adipose tissue in 3° C acclimated rats

The pattern of the results in an individual rat is shown in Fig. 1. When the rat was introduced into the chamber, T_a was kept at 30° C for 1–1·5 hr

to induce a resting state in the brown adipose tissue (Stoner & Little, 1969). T_a was then lowered to between 3 and 5° C in steps of about 5° C. After a period at this low temperature T_a was raised stepwise to 30° C. The time spent at any T_a below 30° C was sufficiently long for a steady state to be

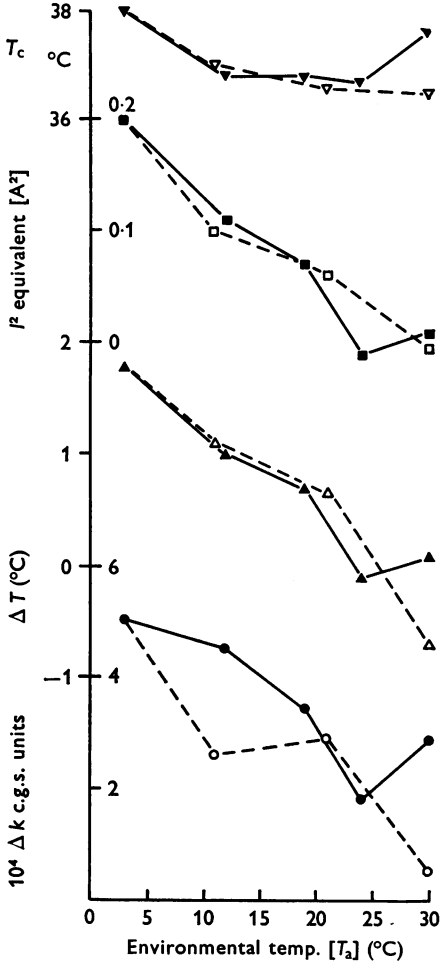

Fig. 1. The responses of interscapular brown adipose tissue to changes in T_a in a rat after acclimation at 3° C for a total of 21 days. Corrected temperature difference between aortic blood and brown adipose tissue, ΔT (▲); I^2 equivalent of heat production (■); change in thermal conductivity due to blood flow, Δk (●). T_c (▼) is also shown. Solid symbols joined by continuous lines indicate observations made during the reduction of T_a from 30 to 3° C. Open symbols joined by interrupted lines indicate observations made during the return of T_a from 3 to 30° C.

reached (Depocas, Hart & Héroux, 1957; Imai, Horwitz & Smith, 1968). In 60 % of cases the exposure period was 25–35 min (range 12–73 min).

As T_a fell T_c rose. The mean ($\pm$S.E.) increase in T_c (Fig. 2) during the reduction of T_a from 30 to 5° C was 0·92 $\pm$ 0·18° C (n = 13) which was significantly less (P < 0·01) than the corresponding value of 1·81 $\pm$ 0·17° C (n = 9) in 20° C acclimated rats studied earlier (Stoner, 1971).

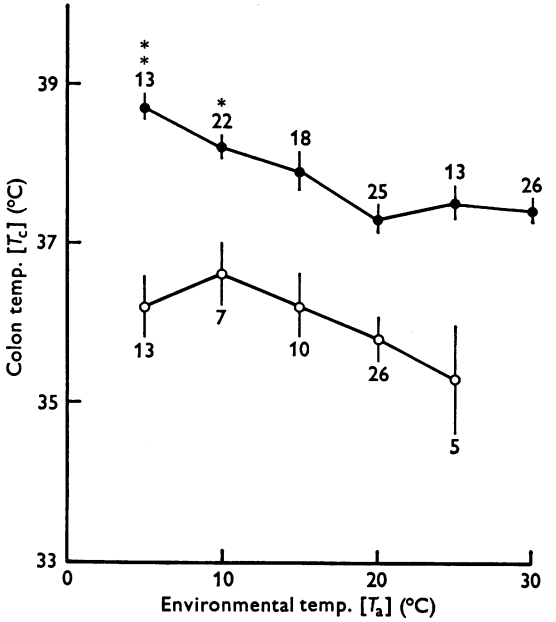

Fig. 2. Mean colon temperatures in control (●) and injured (○) 3° C acclimated rats at 5° C intervals of T_a between 5 and 30° C. Observations at intermediate T_c's added to those at the nearest fifth °C. The injured rats were studied 1–3 hr after 4 hr bilateral hind-limb ischaemia. The vertical line through each point indicates the S.E. and the number attached to it the number of observations. In the controls, one asterisk indicates that the difference from the value at T_a = 30° C is significant at P < 0·01, two asterisks that the difference is significant at P < 0·001. There were no significant differences between the values of T_c in the injured rats.

As T_a was reduced from the thermoneutral environment of 30° C there was a progressive increase in the corrected temperature difference (ΔT) between the aortic blood and the interscapular brown adipose tissue with a similar increase in the I^2 equivalent of the heat produced by the brown adipose tissue (Fig. 1). There was usually little difference in the values of ΔT and I^2 whether T_a was being lowered or raised. Hence in each rat all the values (7–11) were used to calculate the regression lines for their relationship with T_a, over the range 3–30° C, by the method of least squares (Fig. 3). In

three rats the responses were unaffected by a 3 min period of ether anaesthesia 5 hr before testing and the results from these rats have been included in this group. In all rats the values closely fitted linear regression lines, the slopes being significantly different from zero at $P < 0{\cdot}01$ except

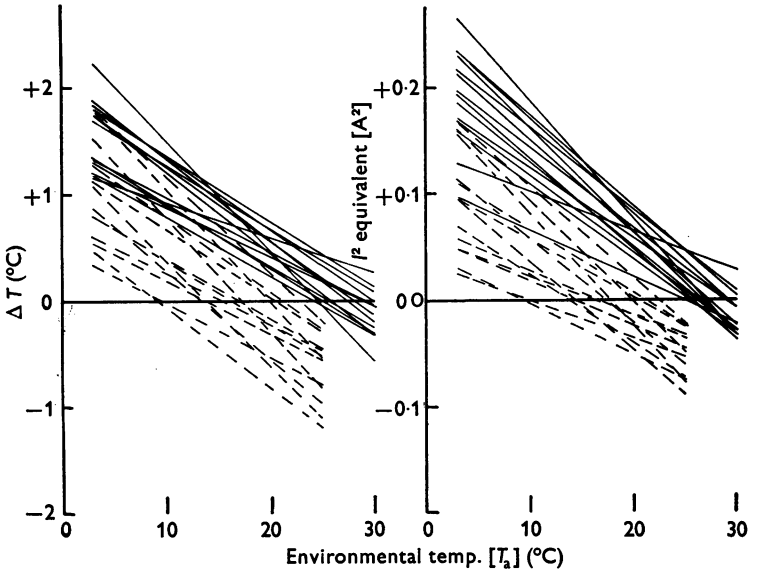

Fig. 3. Regression lines for the relationships between the corrected temperature difference between aortic blood and interscapular brown adipose tissue (ΔT° C) and the I^2 equivalent of heat production in the brown adipose tissue and the environmental temperature (T_a° C) in control (continuous lines) and injured (interrupted lines) 3° C acclimated rats. Each line refers to a single rat.

	$\Delta T/T_a$		I^2 equivalent/T_a	
	Slope	Intercept on T_a axis	Slope	Intercept on T_a axis
	Mean ± S.E.		Mean ± S.E.	
Controls ($n = 13$)	−0·063 ± 0·0048	28·6 ± 1·08° C	−0·0075 ± 0·0005	28·4 ± 0·9° C
Injured ($n = 13$)	−0·073 ± 0·0058	16·3 ± 1·17° C*	−0·0064 ± 0·0007	16·4 ± 1·13° C*

* Difference significant at $P < 0{\cdot}001$.

in one case where $P < 0{\cdot}05$. The mean (± S.E.) T_a at which there was no difference between the temperature of the aortic blood and of the interscapular brown adipose tissue was 28·6 ± 1·1° C ($n = 13$). Similarly the T_a at which, according to the I^2 equivalent determinations, this brown

adipose tissue commenced to produce heat for thermoregulation was $28{\cdot}4 \pm 0{\cdot}9°$ C (mean $\pm$ S.E.; $n = 13$). The T_a for peak metabolism in this tissue was not determined but would appear to be $< 3°$ C in these rats.

Effect of environmental temperature on blood flow (Δk) through brown adipose tissue

The blood flow through the interscapular brown adipose tissue was usually minimal in a 30° C environment and increased when T_a was reduced (Fig. 1). In the T_a range 3–30° C the maximum flow was nearly always at the lowest T_a tested (3–5° C) but the size of the increase was very variable. In thirteen rats there was an average ($\pm$ S.E.) $5{\cdot}7 \pm 1{\cdot}6$-fold increase when T_a was reduced from 30° C to 3–5° C but the range was 1·7–22·5-fold. The regression of blood flow on T_a was not as good as for the other two parameters. Of the thirteen regression lines the slope was only significantly different from zero ($P < 0{\cdot}05$) in 7. The situation was not improved by using $\log \Delta k$. However, Δk could be correlated with the I^2 equivalent of the heat produced by the brown adipose tissue and the regression lines for this correlation are shown in Fig. 4. In the thirteen rats the correlation coefficient was significant at the 1 % level in eight and at the 5 % level in four.

Effect of limb ischaemia on the responses of brown adipose tissue

The general effects of limb ischaemia in rats have been described elsewhere (e.g. Stoner, 1961*a*, *b*). Cold acclimation as used here does not alter the lethality of 4 hr bilateral hind-limb ischaemia in a 20° C environment but it slows the rate of the early fall in T_c when the tourniquets are removed (Stoner, 1965). The 3° C acclimated rats were kept in a 20° C environment during the 4 hr period of limb ischaemia and for the subsequent hour. During that hour the mean T_c ($\pm$ S.E.; $n = 22$) fell $0{\cdot}43 \pm 0{\cdot}14°$ C, about a third of the fall expected in 20° C acclimated rats (Stoner, 1958, 1965). Also the percentage of rats not exporting heat from the interscapular brown adipose tissue rose from 24 to 81 % during that hour and the blood flow through this tissue fell in all except three of twenty-one rats. The ratio (mean $\pm$ S.E.) of the blood flow before removal of the tourniquets to that 1 hr later was $4{\cdot}11 \pm 0{\cdot}82$ ($n = 21$).

T_a was altered 1 hr after removal of the tourniquets. Because of the deleterious effects of high environmental temperatures after injury T_a was not raised above 25° C and because of the low T_c and the progressive deterioration to be expected in the rat's clinical condition, particularly if exposed to a low T_a for a long period, the number of T_a's at which observations were made was reduced from 7–11 to 3–6 and the lowest T_a's were in the range 5–7° C. The time spent at each temperature was 20–30 min in

82 % of exposures (range 16–72 min). In the injured rats lowering T_a did not cause a significant rise in T_c (Fig. 2). However, the values of T_c at the different T_a's showed that the injured 3° C acclimated rats resisted a fall in T_a better than injured 20° C acclimated rats. Compare Fig. 2 with Stoner (1971, Fig. 5).

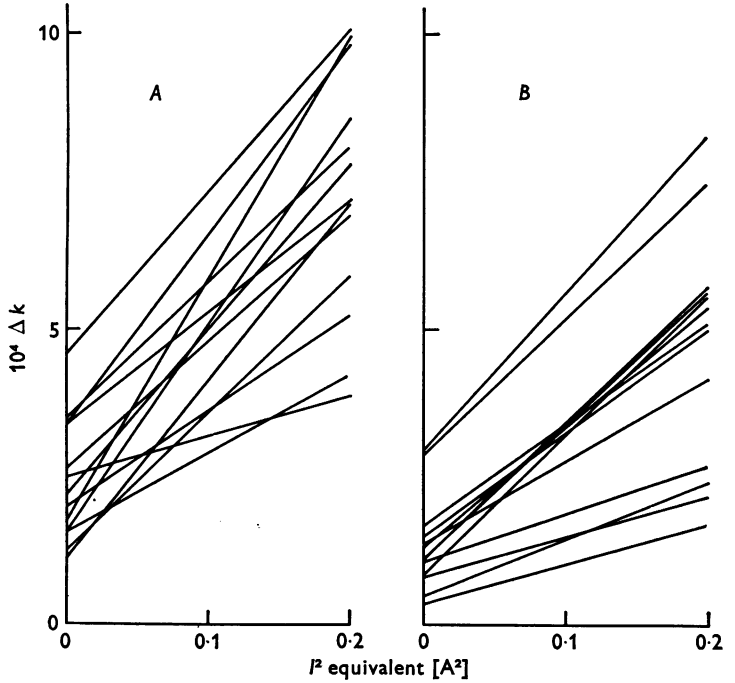

Fig. 4. Relationship between the change in thermal conductivity due to blood flow (Δk; c.g.s. units) and the I^2 equivalent of heat production in the interscapular brown adipose tissue of control (*A*) and injured (*B*) 3° C acclimated rats. Each line refers to a single rat.

As T_a was reduced the corrected temperature difference between the aortic blood and the interscapular brown adipose tissue increased as did the I^2 equivalent of the heat produced by this tissue. There was no indication that the peak response had been produced by lowering T_a to 5–7° C. Regression lines were calculated as before (Fig. 3). Analysis of variance showed that the I^2 equivalent values for the injured rats fitted straight lines as well as for the control rats but that the fit of ΔT values in the injured rats was not as good as in the controls, differing significantly ($P < 0{\cdot}01$) in an F-test. Further comparison between injured and control rats was therefore made on the regressions of the I^2 equivalent values on T_a. There was no significant difference between the mean slope in the controls and the injured. However, in the thirteen injured rats the mean ($\pm$ S.E.) T_a

at which the interscapular brown adipose tissue commenced to produce heat for thermoregulation was $16{\cdot}4 \pm 1{\cdot}1°$ C, very much lower ($P < 0{\cdot}001$) than in the controls.

When T_a was reduced the blood flow (Δk) increased in twelve out of thirteen rats. The regression lines for the correlation between Δk and I^2 were calculated as for the controls (Fig. 4). Comparison of the variances showed that they were at least as straight as in the controls. The slope of the regression lines for the injured rats, $16{\cdot}99 \pm 1{\cdot}99$ (mean $\pm$ S.E., $n = 13$) was significantly ($P < 0{\cdot}05$) lower than in the controls, $24{\cdot}49 \pm 2{\cdot}61$ ($n = 13$) so that in the injured rats comparable heat production was achieved at lower rates of blood flow.

Response to catecholamines after limb ischaemia

In a 20° C environment the temperature of the interscapular brown adipose tissue rises in 3 °C acclimated rats after the S.C. injection of isoprenaline or noradrenaline 15 min before or 40–70 min after removal of the tourniquets at the end of 4 hr bilateral hind-limb ischaemia (Stoner & Little, 1969). It was confirmed by the present techniques that 75 μg L-isoprenaline/kg body wt. S.C. 1 hr after removal of the tourniquets increased heat production by the brown adipose tissue when $T_a = 20°$ C. To test the thermogenic reserve of the brown adipose tissue in the injured rat this experiment was repeated in three rats at $T_a = 5°$ C. A typical result is shown in Fig. 5. About 1 hr after removal of the tourniquets T_a was lowered from 20 to 5° C. This was followed by an increase in T_c, the I^2 equivalent of the heat production and Δk. When a fairly steady state had been reached L-isoprenaline was injected S.C. causing a further rise in heat production (Fig. 5). There was a similar response to the S.C. injection of 100 μg L-noradrenaline/kg.

DISCUSSION

The increase in heat production by the interscapular brown adipose tissue on exposing the rat to cold is well known. The suggestion of Smith & Roberts (1964) that with a fall in T_a from thermoneutrality to 6° C the temperature of the blood leaving the tissue could be raised about 1° C was borne out by the present results (Fig. 3). The associated increase in blood flow was, on average, greater than that reported by Smith & Roberts (1964) or Jansky & Hart (1968). Although the techniques used here did not allow heat production or blood flow to be measured in absolute terms they are useful in showing the linear increase in heat production as T_a was reduced from thermoneutrality to the acclimation temperature, 3° C, and the direct linear relationship between heat production and blood flow

(Figs. 3, 4). These relationships do not seem to have been clearly demonstrated before.

According to Brück & Schwennicke (1971) non-shivering thermogenesis is controlled by thermal receptors in both the skin and the hypothalamus. The temperature of the latter is approximately the same as T_c (Stoner, 1972). The linearity of the relationship between heat production and T_a over the T_a range 30–3° C, despite an increase in T_c with a fall in T_a, suggests that the peripheral thermoreceptors are the main determinants of the response.

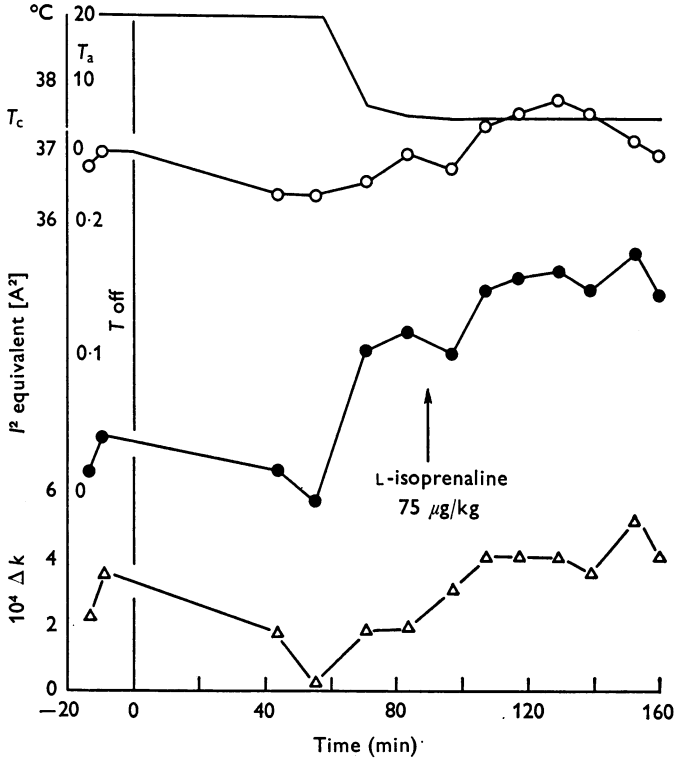

Fig. 5. The responses of interscapular brown adipose tissue (I^2 equivalent of heat production, ●—●; Δk (c.g.s. units), Δ—Δ) to changes in T_a(—) and L-isoprenaline after 4 hr bilateral hind-limb ischaemia in a rat after acclimation at 3° C for a total of 21 days. T_c also shown ○—○. Tourniquets removed at zero time.

An important feature of the regression lines of the controls in Fig. 3 is that the mean intercepts on the T_a-axis correspond to the lower limit of the thermoneutral zone in the rat (Stoner & Marshall, 1971). When T_a fell below this zone non-shivering thermogenesis increased progressively and

in the 3° C acclimated rat was continuously variable over the T_a range studied. Unlike shivering (Stoner, 1971) there was no gap between the lower end of the thermoneutral zone and the onset of heat production by brown adipose tissue.

The rat has not been much used by those building models for the study of thermoregulation. These have usually been developed with larger mammals in mind. As body size increases thermoregulatory non-shivering thermogenesis becomes less important (Heldmaier, 1971) and the dissipation of heat is a greater problem than its production. Consequently, in the family of models developed by Bligh (see Fig. 7; Bligh, 1972) there is a continuous variable, vasomotor tone, to allow thermoregulation in the zone between the onset of shivering on one side and of the specific heat-loss mechanisms, such as panting and sweating, on the other. The rat has little exposed skin for thermoregulation except for parts like the tail which form part of a specific heat-loss mechanism operating through arteriovenous anastomoses which open at threshold temperatures. In this species the continuous control is exerted over non-shivering thermogenesis as exemplified by the changes in heat production by brown adipose tissue. This form of heat production which is not confined to brown adipose tissue (Donhoffer, 1971; Stoner, 1973) fills the 'gap' between the lower limit of the thermoneutral zone and the onset of shivering at about the acclimation temperature and occupies the position of the continuous variable in Bligh's (1972) model when adapted for the rat.

Heat production by brown adipose tissue at T_a's below thermoneutral is clearly depressed during the 'ebb' (Cuthbertson, 1942) phase after a usually fatal period of hind-limb ischaemia (Fig. 3). Since it is unlikely that the hypothalamus is wrongly informed of the rat's environment (Stoner, 1971, 1972), the main question is whether or not this effect is due to peripheral or central changes. Heat production in brown adipose tissue is very sensitive to O_2 lack (Heim & Hull, 1966*a*). After an injury which causes local fluid loss and haemoconcentration a decrease in the O_2 supply to the brown adipose tissue could occur although during the period under investigation arterial oxygenation is normal (Stoner, 1958; R. A. Little & C. J. Threlfall, personal communication) and the mean arterial blood pressureis about 100 mmHg (Stoner & Little, 1969). In fact, three features of the results show that O_2 lack is not the reason for the impaired thermogenic response to a fall in T_a.

(1) Although the mean intercept of the regression lines for the injured rats in Fig. 3 was significantly displaced to the left there was no difference between the mean slope of the lines for the injured and control rats, i.e. once heat commenced to be exported from the interscapular brown adipose tissue the amount per unit fall in T_a was the same as in the controls.

(2) In the injured rats comparable heat production to that in controls occurred at lower rates of blood flow (Fig. 4).

(3) In a 5° C environment heat production by interscapular brown adipose tissue in injured rats was further stimulated by the injection of L-isoprenaline or L-noradrenaline. This was accompanied by an increase in blood flow (Fig. 5). This vascular response is considered to be secondary to the metabolic response (Heim & Hull, 1966*b*) but even if it was the initiator of the effect the experiment would still show that the brown adipose tissue was capable of further stimulation in the injured rat at 5 °C.

The results in the injured rats therefore provide further evidence that trauma interferes with the central control of thermoregulatory non-shivering thermogenesis. Since trauma also impairs shivering thermogenesis (Stoner, 1971, 1972) this failure of non-shivering thermogenesis cannot be replaced by shivering so that the results are important for small mammals and the neonates of larger species, including man. Taking these results with previous ones (Stoner, 1969, 1971, 1972; Stoner & Marshall, 1971) the overall effects of limb ischaemia on thermoregulation in the rat before the stage of necrobiosis (Stoner, 1961*b*) can be summarized as follows.

During the period of limb ischaemia the inhibition of the shivering and heat-loss pathways is increased, lowering the threshold temperatures for the onset of shivering and raising those for the opening of the arteriovenous anastomoses. When the tourniquets are removed and fluid loss occurs inhibition is also applied to the pathway for non-shivering thermogenesis.

Much remains to be investigated but two further points may be mentioned. Neither in the present results nor in the previous ones has there been any indication that inhibition was produced by a change in gain. Since the depression of non-shivering thermogenesis occurred at a time when the plasma volume was falling the afferent impulses concerned in the inhibition may arise from volume receptors. The arguments against trauma lowering the set-point have been given in a previous paper (Stoner, 1972).

My thanks are due to Mrs H. W. Marshall for her careful technical assistance with these experiments and to the Instrument Shop, M.R.C. Laboratories, Carshalton, for making the thermocouples. I also wish to thank Mr I. Capperauld, F.R.C.S. (Ed.) of Ethicon Ltd, Edinburgh for a supply of Isobutyl 2-Cyanocrylate Tissue Adhesive and Messrs John Wyeth and Brother Ltd for a sample of L-isoprenaline bitartrate.

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