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METABOLIC, RESPIRATORY AND VASOMOTOR RESPONSES TO HEATING THE SCROTUM OF THE RAM

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SUMMARY

1. Oxygen consumption, respiratory frequency, and temperatures of the rectum, common carotid artery, external auditory meatus, and skin on the ears, near the distal end of the metacarpus and metatarsus, upper thigh and mid-side of the body, were measured in five rams before, during and after heating the scrotum. Effects on the woolly animal exposed to ambient temperatures of 17–19° C, and on the shorn animal exposed to ambient temperatures of 5·6–24·5° C, were determined.

2. In eight experiments on woolly animals scrotal heating resulted in vigorous panting and a lowering of deep body temperatures by 0·3–1·1° C within 50 min. There was no significant change in oxygen consumption or body skin and upper thigh temperature. Changes in skin temperature of the extremities indicated, on average, a vasodilator response to heating. However, individual responses were unpredictable, there sometimes being dilation and constriction in different extremities of the same animal.

3. In twelve experiments on shorn animals in environments below 20° C, oxygen consumption before scrotal heating was higher than when the animal was woolly. Scrotal heating resulted in a fall in oxygen consumption and a lowering of deep body temperatures by 0·2–0·9° C within 50 min. There was no change in respiratory frequency or body skin and upper thigh temperature. As in the woolly animal, vasomotor responses were disorganized, although there was a tendency towards vasodilation.

4. In three shorn animals in environments of 20·5 and 24·5° C, oxygen consumption before scrotal heating was only slightly higher than when the animal was woolly. In two of these animals (in 20·5 and 24·5° C) scrotal heating resulted in a slight lowering of oxygen consumption and mild panting, and deep body temperature fell by 0·9° C in 50 min. In the third animal (in 24·5° C) there was no change in oxygen consumption but vigorous panting and vasodilation, and deep body temperature fell by 1·3° C in 50 min.

5. In a number of experiments on shorn animals scrotal heating was continued for more than 100 min. Deep body temperature was controlled at a new low level after the initial fall, and the possibility of a resetting of the 'set-point' temperature is discussed.

6. The previously unexplained fall in body temperature of the shorn ram in which panting did not follow scrotal heating may now be mainly ascribed to a reduction in metabolic rate. Also, the possible role of cutaneous evaporation should not be discounted. The inhibition of panting in the shorn sheep at environmental temperatures below 20° C remains unexplained.

7. Body heating alone, by covering the shorn animal with a sheep skin coat in an environment of 19° C, elicited well organized vasomotor changes in the ears and lower legs.

8. As a result of the present study and previous work by others, it is clear that all thermoregulatory mechanisms, with the exception of vasomotor changes, may be influenced in a predictable manner by temperature changes of the scrotum.

INTRODUCTION

Thermoregulatory mechanisms may be influenced by the temperature of the brain, of extra-cerebral deep body tissues or of skin (Bligh, 1966; Hammel, 1968; Benzinger, 1969). For example, panting, sweating, metabolic rate, shivering and vasomotor activity may all be altered as a result of manipulation of the temperature of the hypothalamus (Hammel, Hardy & Fusco, 1960; Fusco, Hardy & Hammel, 1961; Ingram, McLean & Whittow, 1963), or of the spinal cord (Simon, Rautenberg, Thauer & Iriki, 1964; Jessen, 1967; Hales & Jessen, 1969). However, these responses to changes in hypothalamic or spinal cord temperature are also influenced by skin temperature. Panting starts on heat exposure before there is any change in deep body temperature (rectal or carotid artery) (Sihler, 1879; Bligh, 1957, 1959). Although this has been interpreted as being due to the stimulation of peripheral temperature receptors, it need not necessarily be so because of possible rapid changes in hypothalamic temperature which are independent of rectal or carotid arterial blood temperature. This has become apparent since the demonstration by Baker & Hayward (1968*a*, *b*) of a very efficient heat exchange system in the carotid rete of the sheep. Here, cool or warm blood draining the ears or nasal mucosa may rapidly lower or raise the temperature of blood on its way to the brain. Nevertheless, Waites (1961, 1962) has demonstrated a most striking example of the possible influence of peripheral stimulation by heating the scrotum of the ram and eliciting marked panting, which continued even though rectal and carotid temperature fell by up to 2° C. Hypothalamic temperature

would certainly have fallen under these conditions. Also, Hales, Kao, Mei, Wang & Gretenstein (1970) have shown that panting could be elicited in the recipient of a pair of cross-circulated dogs by heating the body while its head was perfused with blood from an unheated donor; there were only neural, and no vascular, connexions between the body and head of the recipient.

Heating the scrotum will also result in sweating (Waites & Voglmayr, 1962, 1963), but effects on other thermoregulatory mechanisms are unknown. Further, Waites (1962) observed that if the ram was shorn, the panting response was absent but deep body temperature was still lowered. Sweat discharges over the scrotum were still present, but whether discharges over the body or whether changes in metabolic rate or vasomotor activity were responsible for the decline in body temperature was not established.

The present study was undertaken, (*a*) to extend Waites' observations to the effect of scrotal heating on metabolic rate and vasomotor function, and thus elucidate their contribution to the lowering of body temperature in the shorn animal and (*b*) to find out whether a constant central temperature would be attained if heating were continued over a longer period than in Waites' experiments. The results illustrate the complementary relationship existing between the various mechanisms concerned with the regulation of body temperature.

METHODS

Animals. Four Merino rams and one cross-bred ram 2–6 yr old and weighing 35–50 kg were used. They were accustomed to the experimental procedure. Fleece depth was 2·5–8 cm until they were sheared for later experiments. Their food consisted of sufficient oat grain and lucerne chaff (equal parts by weight) to maintain a stable body weight. For all experiments on rams 4 and 5, the last experiment on ram 3 and the last two experiments on ram 1, half feed was given about 2 hr before beginning the experiment and the other half after completion. For all other experiments, all feed was given on the afternoon preceding the experiment.

Temperature. The temperature of the surface of the skin of the scrotum was manipulated by circulating water from a temperature controlled bath through a polyethylene chamber enclosing the scrotum.

Temperatures were determined to within $\pm 0{\cdot}1^{\circ}$ C with 38 s.w.g. copper-constantan thermocouples, recording on a multi-point potentiometric recorder. Rectal temperature (T_{re}) was measured with a thermocouple mounted on the tip of a plastic rod 0·5 cm o.d. and inserted 10 cm into the rectum. In some experiments central arterial blood temperature (T_{ar}) was measured using a thermocouple mounted in the tip of a 20 s.w.g. hypodermic needle and inserted 4 cm into the common carotid artery which had been surgically prepared in a subcutaneous position (Hales & Webster, 1967). Temperature of the external auditory meatus (T_{me}) was also measured in some experiments, by means of a thermocouple mounted in the tip of a polyethylene tube 3 mm o.d. and inserted to within approximately 1 cm of the tympanic membrane; the canal was then plugged with cotton wool. For

skin temperatures, thermocouples were stuck on to the skin surface with Nobecutane (Evans). Sites of measurement were the posterior surface of the scrotum, mid-dorsal surface of the ears, midside of the body, upper thigh and lower leg (near the distal end of the metacarpus and metatarsus). Two to five days before an experiment the wool was closely clipped from the site, approximately 3×3 cm on the body and $3 \times 1{\cdot}5$ cm on the leg.

Vasomotor activity. Since ambient temperature during an experiment was constant, an increase in skin surface temperature was taken to represent vasodilation and a decrease in temperature was taken to represent vasoconstriction. The temperature of extremities referred to as 'constricted' was within 0–6·5° C of ambient temperature. Extremities are called 'intermediate' if the difference between their temperature and ambient was 5–8° C with ambient temperatures of 20·5 and 24·5° C, or 6–11° C, with ambient temperatures of 19° C or less. Extremities are referred to as 'dilated' if their temperature exceeded ambient by at least 8·5° C with ambient temperatures of 20·5 and 24·5° C, or by at least 11° C with ambient temperatures of 19° C or less; the maximum difference between ambient and skin temperature recorded was 22·4° C. The responses obtained were so erratic that a '*vasomotor score*' was derived to simplify the presentation of a 'mean' effect. The vasomotor score is derived by giving 100 points for a dilated extremity, 50 points for an intermediate and 0 points for a constricted extremity, and dividing the total number of points by the number of extremities involved. For example, it may be seen in Table 2 that, before scrotal heating of the woolly animals, there were eighteen extremities dilated, six intermediate and ten constricted. The vasomotor score is therefore $[(18 \times 100) + (6 \times 50) + (10 \times 0)]/34 = 62$ (to the nearest whole number), which is shown in Fig. 3.

Respiratory frequency. This was determined by recording the pressure changes in a pneumograph strapped around the lower thoracic margin.

Respiratory gas exchange. The animal wore a face mask through which room air was drawn at approximately 70 l./min. The air was dried by passing through calcium chloride, and the volume measured with two calibrated dry gas meters (Smith Dry Test Meter, U.G.I. Meters Ltd., London). Gas temperature was measured with a mercury in glass thermometer and gas pressure with a water manometer, both mounted at the exit from the gas meters. The mixed expired and room air leaving the gas meters was continuously sampled and passed through continuous gas analysers, the outputs of which were recorded on a potentiometric recorder. The percentage of oxygen present was measured paramagnetically (analyser Model F3, Beckman, U.S.A.) and the carbon dioxide and methane by infra red absorption (IRGA, Grubb Parsons, England). Both analysers were calibrated with gases of composition determined on the Haldane apparatus.

In five experiments methane production was followed for a short period with the infra red gas analyser which was capable of analysing for either carbon dioxide or methane but not both together. The effect of the methane on the calculation of oxygen consumption was found to be negligible, and therefore methane was neglected in the final calculations. Carbon dioxide output and oxygen consumption were calculated as described in the *Handbook of Respiration* (Altman, Gibson & Wang, 1958), and corrected to s.t.p.d.

Experimental procedure. The animal stood quietly, held by a yoke about the neck, in a climatic room. Respiratory frequency, oxygen consumption, carbon dioxide output, skin temperatures, rectal temperature, and in some experiments arterial blood and external auditory meatus temperatures, were recorded continuously. After measurement of scrotal skin temperature in air, water at the same temperature was perfused through the scrotal chamber so as to maintain a constant skin temperature. Control measurements of all parameters were then taken until steady

values were obtained. Scrotal heating, by perfusion with water at 40° C, usually began about 2½ hr after the animal entered the room, and continued for 1–3 hr; this was followed by cooling with water at 15–30° C for ½–1½ hr. In early experiments scrotal heating was discontinued when a definite response had been obtained, but in later experiments, heating was continued until rectal temperature had attained a new constant level. The cooling period was generally relatively short, ceasing soon after a definite response was observed.

RESULTS

Twenty-eight experiments were performed on the five rams (Table 1). Of these, eight involved scrotal heating in the woolly animal, fifteen in the shorn animal, and one on a shorn animal wearing a sheep skin coat. In

TABLE 1. Experiments performed, in chronological order for each animal

Ram no.	Fleece depth (cm)	Ambient temp. (° C)	Duration (min)	
			Heating scrotum	Cooling scrotum
1	6	18·5	65	27
1	6	18·5	45	34
1	Shorn	16·8	60	40
1	Shorn	16·8	68	40
1	Shorn	16·5	140	32
1	Shorn	20·5	105	35
1	Coat*	19·0	0	0
1	Coat*	19·0	0	0
2	8	16·8	64	28
2	8	16·8	64	38
2	Shorn	16·8	70	35
2	Shorn	5·6	62	26
3	8	17·5	69	40
3	8	17·0	60	24
3	Shorn	18·2	56	40
3	Shorn	11·6	68	40
3	Shorn	8·4	88	56
3	Shorn	19·0	174	32
4	2·5	19·0	108	58
4	Shorn	19·0	138	76
4	Shorn	24·5	135	55
4	Coat*	19·0	125	83
4	Shorn	10·0	136	84
4	Coat*	19·0	0	0
4	Coat*	19·0	0	0
5	3·3	19·2	130	35
5	Shorn	19·0	150	30
5	Shorn	24·5	135	45

* Shorn animal wearing a sheep's skin with fleece 3·5 cm deep.

the remaining four experiments the vasomotor responses to the wearing of a coat by a shorn animal were observed and the scrotum was not heated. Ambient dry bulb temperatures of from 5·6 to 24·5° C were employed in the experiments with shorn animals, to help elucidate the role of vasomotor and metabolic responses. In experiments with woolly animals the ambient temperature was 16·8 to 19·2° C.

Effects of heating the scrotum of the woolly animal

Examples of the results obtained are presented in Figs. 1 and 2.

Rectal temperature. When the scrotum was heated T_{re} usually began to fall immediately, although there was a latent period of approximately 10 min in three experiments. The fall averaged 0·6° C (range 0·3–1·1° C) over the first 50 min of heating. In two experiments where heating was continued for 108 and 130 min, the fall was 2·0 and 1·7° C respectively, falling very slowly towards the end of heating.

When the scrotal skin was cooled to its pre-heating temperature, or below this, T_{re} usually increased slowly. This part of each experiment was not continued for very long and the average rise in T_{re} in 25 min was 0·06° C (range $-0·1$ to $+0·2$° C).

Carotid and meatus temperatures. Temperature in the common carotid artery was measured in only one experiment (Fig. 2), and of the external auditory meatus in three experiments. T_{ar} and T_{me} were generally 0·4–0·7° C below T_{re}, but the pattern of changes in all three temperatures during scrotal heating and cooling was similar (Fig. 2). Thus, during the first 50 min heating there was a fall of 0·8–1·3° C. In the two experiments where heating was continued for 108 and 130 min, T_{me} fell by 2·2 and 1·8° C respectively; during 130 min heating T_{ar} fell by 1·7° C. Towards the end of heating in these two longer experiments body temperature was falling very slowly.

On cooling the scrotum T_{ar} and T_{me} increased by 0·1–0·2° C in 25 min.

Skin temperatures. Midside and upper leg temperatures were measured in seven experiments involving all animals. Neither heating the scrotum nor subsequent cooling had any significant effect on these temperatures (Figs. 1 and 2) which varied either up or down by 0·5–2·0° C throughout the entire experiment.

Ear temperatures were measured in six experiments involving four animals, and changes of up to 16° C were recorded. The skin temperature of the lower leg was measured in all eight experiments, but in some not all four legs were used; changes of up to 13° C were recorded. The responses were apparently erratic. Detailed results may be examined in Table 2; however, the 'mean' response is illustrated in Fig. 3 by the vasomotor score. Thus, during the control period the extremities were considerably

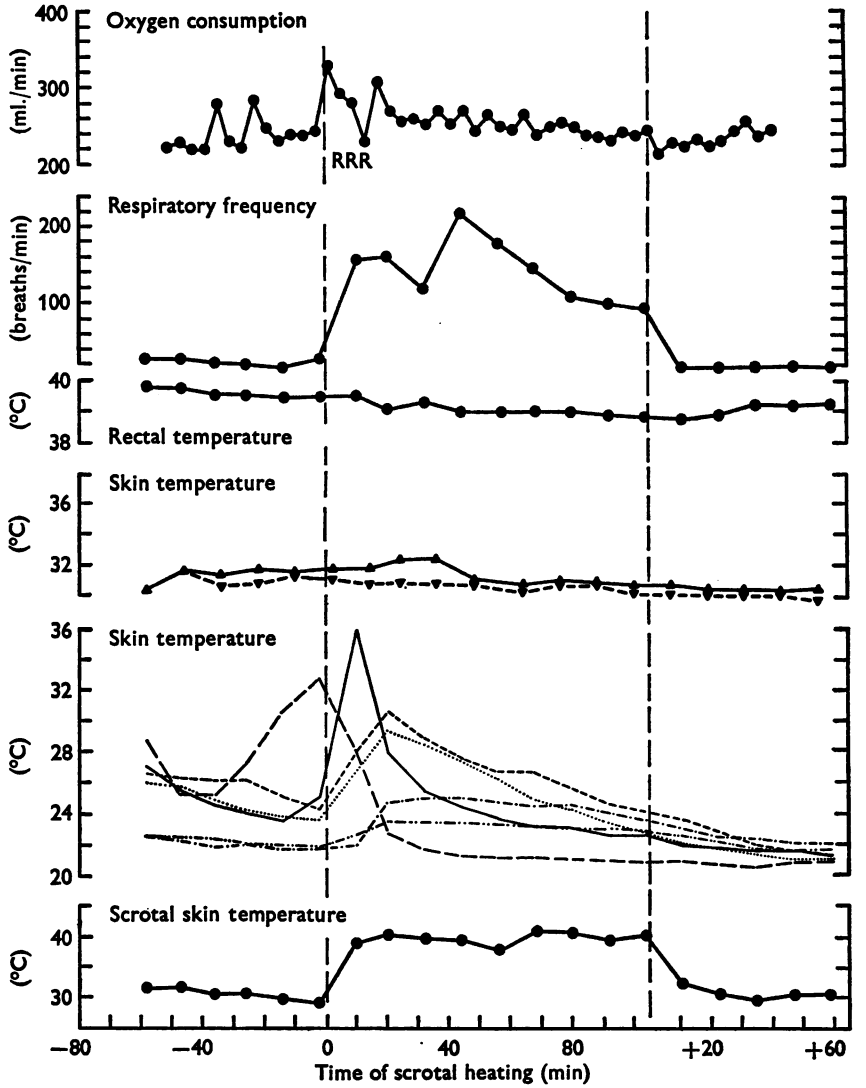

Fig. 1. Effect of heating the scrotum of ram No. 4, on oxygen consumption, respiratory frequency, deep body and skin surface temperatures. Fleece depth 2·5 cm, ambient temperature 19° C. The time is in relation to the start of scrotal heating. R: restless. Skin temperatures: mid-side of body (▲—▲), high thigh (▼ – –▼), right ear (— —), left ear (– – –), right low foreleg (–·–·–), left low foreleg (–··–··–), right low hind leg (····), left low hind leg (- - - - -).

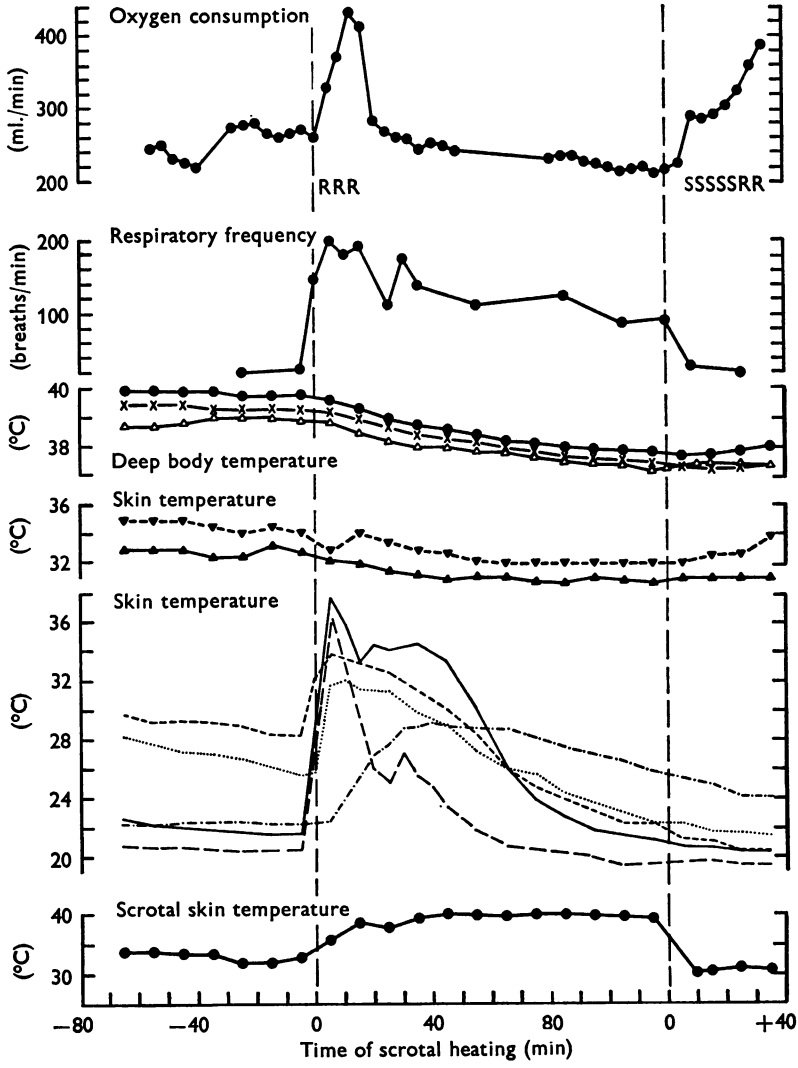

Fig. 2. Effect of heating the scrotum of ram No. 5, on oxygen consumption, respiratory frequency, deep body and skin surface temperatures. Fleece depth 3·3 cm, ambient temperature 19° C. The time is in relation to the start of scrotal heating. R: restless; S: shivering. Deep body temperatures: rectum (●—●), arterial blood (△—△), external auditory meatus (×—×). Skin temperatures: mid-side of body (▲—▲), high thigh (▼ – – ▼), right ear (——), left ear (– – –), right low foreleg (–·–·–), right low hind leg (·····), left low hind leg (- - - - -).

TABLE 2. Vasomotor changes on heating the scrotum of woolly and shorn animals with water, or the body of shorn animals by covering with a coat. The figures are the number of extremities observed in each vasomotor state

		Number of extremities								
		Dilated (D)			Intermediate (I)			Constricted (C)		
Woolly 16·8–19·2° C*	State before heating	18			6			10		
		D	I	C	D	I	C	D	I	C
	Heating (1)†	10	6	2	6	0	0	4	3	3
	(2)†	9	5	5	0	4	2	1	3	5
	State before cooling	10			12			12		
		D	I	C	D	I	C	D	I	C
	Cooling	7	2	1	0	6	6	0	0	12
Shorn 5·6–11·6° C*	State before heating	2			3			16		
		D	I	C	D	I	C	D	I	C
	Heating (1)	2	0	0	1	2	0	0	0	16
	(2)	2	0	0	0	0	3	1	1	14
	State before cooling	3			1			17		
		D	I	C	D	I	C	D	I	C
	Cooling	0	1	2	0	0	1	0	0	17
Shorn 16·5–19·8° C*	State before heating	1			3			31		
		D	I	C	D	I	C	D	I	C
	Heating (1)	1	0	0	2	1	0	6	2	23
	(2)	1	0	0	2	1	0	4	4	23
	State before cooling	7			5			23		
		D	I	C	D	I	C	D	I	C
	Cooling	4	1	2	0	5	0	0	0	23
Shorn 20·5–24·5° C*	State before heating	3			9			6		
		D	I	C	D	I	C	D	I	C
	Heating (1)	3	0	0	1	8	0	2	2	2
	(2)	2	1	0	0	3	6	0	1	5
	State before cooling	2			5			11		
		D	I	C	D	I	C	D	I	C
	Cooling	2	0	0	0	4	1	0	0	11
Coat 19·0° C*	State before coating	0			6			18		
		D	I	C	D	I	C	D	I	C
	Heating (1)	0	0	0	1	5	0	1	1	16
	(2)	0	0	0	5	1	0	8	4	6
	State before removing coat	13			1			4		
		D	I	C	D	I	C	D	I	C
	Coat off	0	8	5	0	1	0	0	0	4

* Ambient temperature.

† Heating is arbitrarily divided into two periods, (1) and (2).

dilated; scrotal heating initially caused a small vasodilator response, followed by considerable constriction which was increased during scrotal cooling (see also Figs. 1 and 2).

Respiratory frequency. During the control period the mean respiratory frequency was 35 breaths/min, and this increased to a mean peak value of

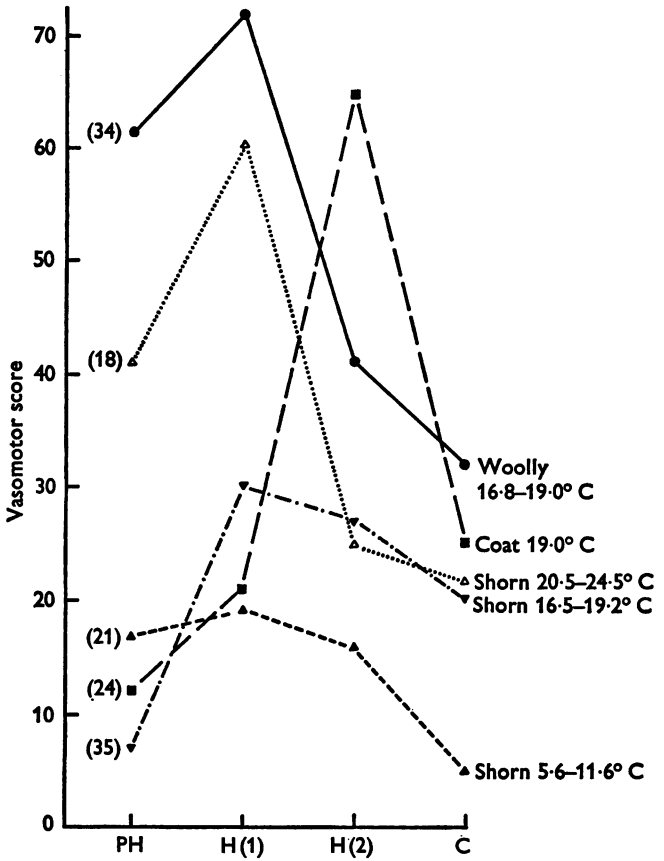

Fig. 3. Mean changes in vasomotor activity is represented by the 'vasomotor score' (see text for full explanation). Number of extremities examined is shown in parentheses. PH: Pre-heat; H (1), H (2): heating the scrotum of woolly and shorn animals with water or the body of shorn animals by covering with a coat, arbitrarily divided into periods (1) and (2); C: cooling the scrotum or the body by removing the coat.

192 breaths/min usually within 5 min of scrotal heating. The frequency often declined slightly as heating continued, so that the mean value was 165 breaths/min (range 127–210 breaths/min). Cooling resulted in an immediate fall in frequency, to a mean of 28 breaths/min within about 5 min.

In six of the eight experiments the peak respiratory frequency occurred within 5–20 min of the start of heating and then remained more or less steady although there was a continuous fall in T_{re}. In the two remaining experiments heating was continued for more than 100 min, and respiratory frequency reached its maximum within a few minutes but then fell steadily to about half the peak value by the end of heating.

Oxygen consumption. The mean control value for oxygen consumption was 268 ml./min (range 240–317 ml./min). Immediately following the start of heating the oxygen consumption showed a transient, highly variable increase, presumably because the animal became excited. Thereafter, an approximately steady rate of 281 ml./min (range 233–318 ml./min) was attained. During cooling the mean value was 271 ml./min (range 232–317 ml./min). Thus, neither heating nor cooling had a significant effect ($P > 0{\cdot}05$).

Respiratory quotient. This did not vary significantly during control, heating or cooling periods, respective values being 0·88, 0·88 and 0·85.

Effects of heating the scrotum of the shorn animal

Figs. 4 and 5 illustrate the results obtained.

Rectal temperature. Scrotal heating resulted in a fall in T_{re} at about the same rate as in the woolly animal, averaging 0·7° C (range 0·2–1·3° C) in 50 min. However, in three experiments this fall was a continuation of a fall throughout the control period. In the remaining nine experiments T_{re} was initially steady, but fell immediately upon scrotal heating in three experiments, and after 10–32 min had elapsed in six experiments. In eight of the fifteen experiments heating was continued for more than 100 min, and in these T_{re} fell to a new steady level (Figs. 4 and 5). In these experiments the mean total fall in T_{re} was 1·3° C.

On cooling the scrotum, T_{re} increased in all but one experiment, by a mean of 0·25° C (range 0·2–0·7° C) in 25 min.

Carotid and meatus temperatures. The pattern of change was similar to that of T_{re}: T_{me} fell by a mean of 0·8° C in 50 min heating (five experiments), and by a total of 1·7° C when a new steady level was attained. T_{ar} fell by 0·6 and 1·3° C in 50 min heating (two experiments), or 1·8 and 2·2° C total fall.

With cooling, T_{me} and T_{ar} increased by a mean of 0·3 and 0·5° C respectively.

Skin temperatures. Mid-side and upper leg temperatures were measured in fourteen experiments (five rams). Neither scrotal heating nor cooling had any significant effect (Figs. 4 and 5), any changes being within 0·5–1·0° C.

Ear temperatures were measured in twelve experiments involving all

animals. Lower leg temperatures were measured in all fifteen experiments, but not always all four limbs. As with the woolly animal responses were apparently erratic with the exception of ram No. 3. This animal exhibited a nicely graded response in different environmental conditions: in 19° C (Fig. 5) and 18·2° C five of the six extremities were dilated during heating, in 11·6° C only two extremities were dilated, and in 8·4° C two extremities dilated but only after 60 min had elapsed. Details of all responses may be

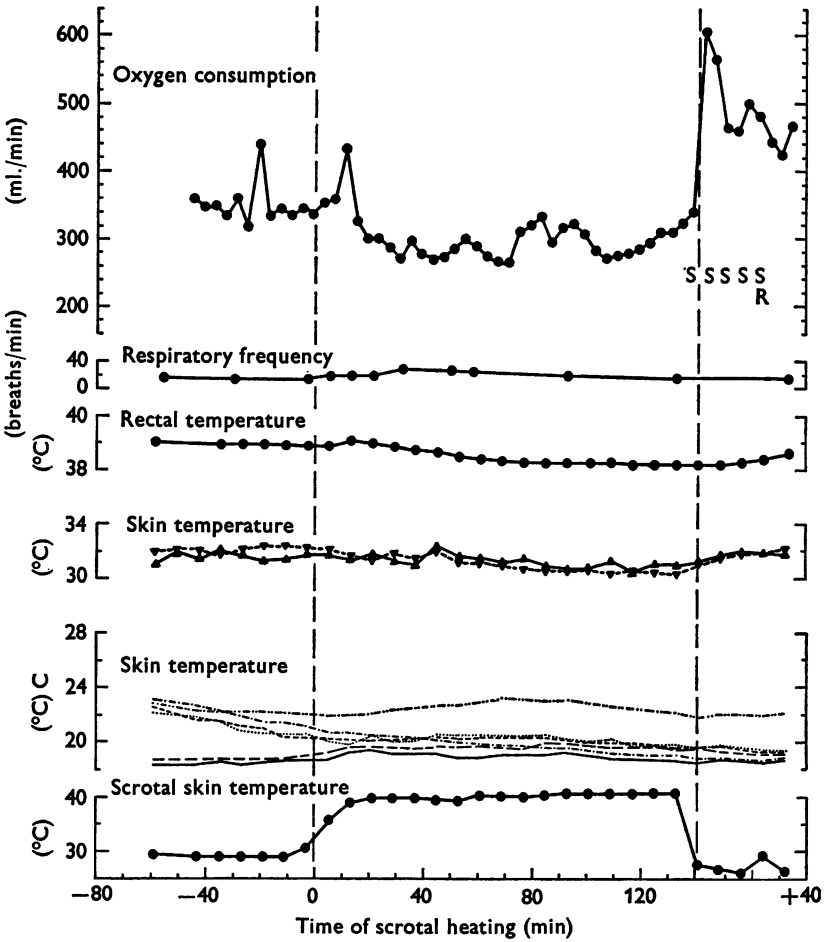

Fig. 4. Effect of heating the scrotum of ram No. 1, on oxygen consumption, respiratory frequency, deep body and skin surface temperatures. Animal shorn, ambient temperature 17° C. The time is in relation to the start of scrotal heating. R: restless; S: shivering. Skin temperatures: mid-side of body (▲—▲), high thigh (▼ – – ▼), right ear (——), left ear (– – –), right low foreleg (–·–·–), left low foreleg (–· ·–· ·–), right low hind leg (· · · · ·), left low hind leg (------).

examined in Table 2. The mean responses are illustrated in Fig. 3. In environments of 5·6–11·6° C there was general constriction during the control period, no response to scrotal heating, but further constriction on cooling. In environments of 16·5–19·2° C the extremities were constricted during the control period; on heating there was a moderate vasodilator

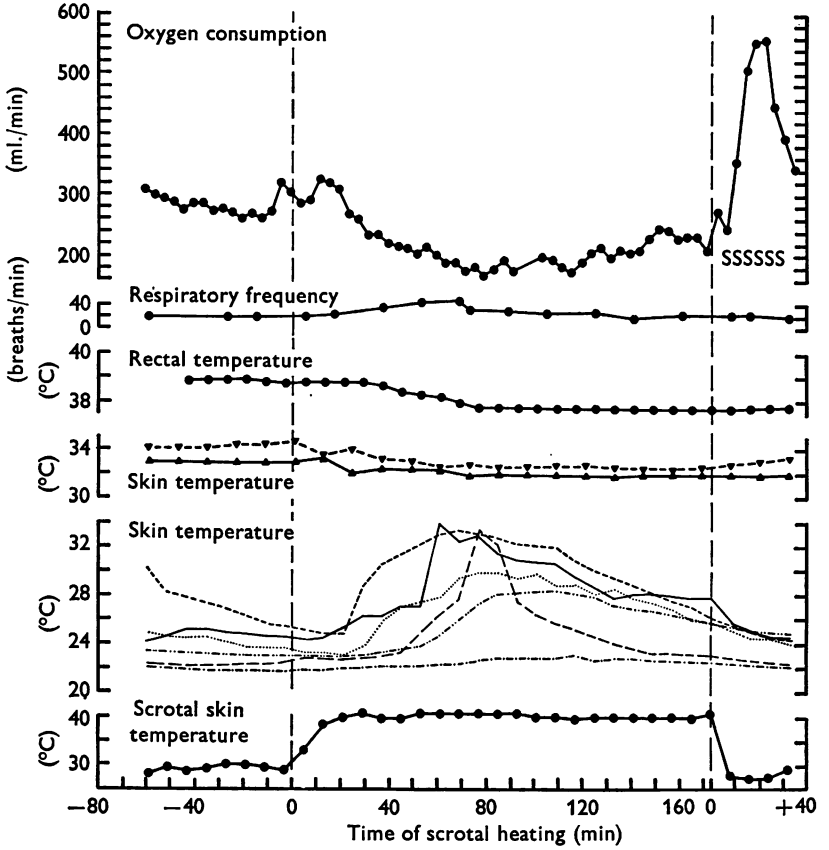

Fig. 5. Effect of heating the scrotum of ram No. 3, on oxygen consumption, respiratory frequency, deep body and skin surface temperatures. Animal shorn, ambient temperature 20° C. The time is in relation to the start of scrotal heating. S: shivering. Skin temperatures: mid-side of body (▲—▲), high thigh (▼ – – ▼), right ear (——), left ear (– – –), right low foreleg (–·–·–), left low foreleg (–·· –·· –), right low hind leg (·····), left low hind leg (-----).

response (somewhat larger than when the rams were woolly) which was slightly reduced by cooling. In environments of 20·5 and 24·5° C the extremities were initially in an intermediate state; with heating there was at first a moderate dilation followed by considerable constriction which persisted during cooling.

Respiratory frequency. During the control period the mean respiratory frequency was 19 breaths/min. In twelve experiments heating the scrotum had little or no effect on respiratory frequency (mean 22 breaths/min), while in three experiments carried out at environmental temperatures of 20·5 and 24·5° C, panting was elicited (mean 99 breaths/min, peak in one experiment 170 breaths/min). Cooling resulted in an immediate fall in frequency, to approximately pre-heating values (mean 21 breaths/min) within about 5 min.

Oxygen consumption. In all animals in all experiments except one (at an ambient temperature of 24·5° C), the oxygen consumption of the shorn animal during the control period was higher than when the animal was woolly. The mean values for the fifteen experiments on shorn animals and the eight experiments on woolly animals were 348 and 268 ml./min respectively ($P < 0{\cdot}01$). At environmental temperatures of 20·5° C and above there was no visible shivering, but below this temperature shivering was usually observed.

On heating the scrotum there was typically a period of about $\frac{1}{2}$ hr during which oxygen consumption was variable at a level approximately equal to or greater than the control; this was presumably due to excitement after the beginning of heating. Thereafter, oxygen consumption declined to 82% of the control level (284 ml./min compared with 348 ml./min, $P < 0{\cdot}001$). The degree of reduction in oxygen consumption caused by scrotal heating was significantly related to ambient temperature as shown in Fig. 6. In the experiments wherein T_{re} assumed a new steady level, the reduction in oxygen consumption was followed by an increase (e.g. Figs. 4 and 5). In half of these experiments the increased level of oxygen consumption exceeded the control value. If shivering occurred before heating, it was reduced or absent when the scrotum was heated. In three of the experiments in which heating was continued for more than 2 hr shivering again became visible towards the end of the heating period when oxygen consumption was increasing.

On cooling the scrotum there was at first a large rise in oxygen consumption usually accompanied by vigorous shivering. Within a few minutes there was a fall of oxygen consumption, but the value remained above that in the preliminary period during the short time over which it was observed.

Respiratory quotient. In the control period this was 0·86, in the period of low oxygen consumption during scrotal heating 0·90, and during cooling 0·84. Thus, the lower the oxygen consumption, the higher was the respiratory quotient.

Effects of heating the scrotum of a shorn animal wearing a sheep skin coat

The changes in rectal, meatus and skin temperatures, respiratory frequency, and oxygen consumption were similar to those recorded with the woolly animals. The vasomotor changes are included in Fig. 3 and Table 2 with those of the woolly animals.

Vasomotor effects of covering the shorn animal with a sheep skin coat

The object of these experiments was to determine whether the ears and lower legs would dilate in a regular manner when the body of the animal,

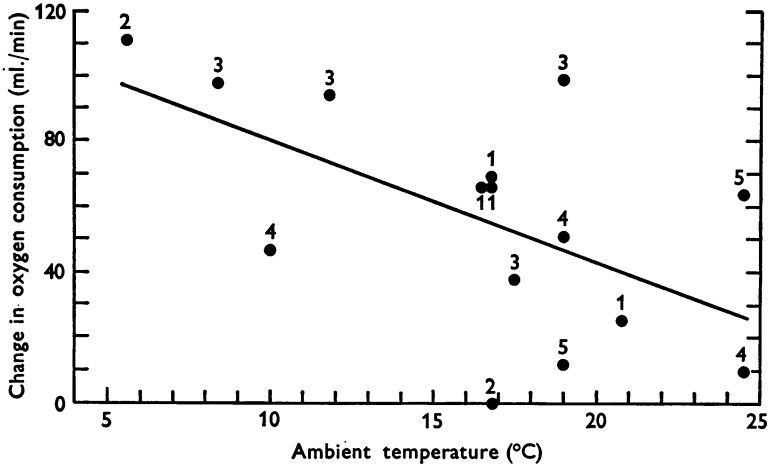

Fig. 6. The decrease in oxygen consumption during scrotal heating of the shorn ram exposed to various ambient temperatures. The individual animals are indicated by their number. The regression line is $y = 117{\cdot}36 - 3{\cdot}68x$; $r = -0{\cdot}572$, $P < 0{\cdot}05$.

as opposed to the scrotum, was heated. Four experiments were conducted on two rams at an ambient temperature of 19° C. The animals used, rams Nos. 1 and 4, had shown little vascular response in the earlier scrotal heating experiments. The shorn animal stood quietly in the climatic room during a control period of at least 2 hr during which time skin temperatures and respiratory frequency were monitored. The ram was then heated by covering the body with a sheep skin carrying a 3·5 cm deep fleece, which was removed after about 2 hr.

Results are illustrated in Fig. 7. Placing the coat caused body skin temperature to increase on average by 5° C within 5–30 min. This resulted in a well organized vasodilator response, details of which may be examined in Table 2. The mean response illustrated in Fig. 3 shows a much greater but slower vasodilation than occurred with scrotal heating in a comparable environment. Removal of the coat caused constriction.

Following application of the coat, rectal temperature did not change significantly in ram No. 4, but increased by 0·3 and 0·4° C in the two experiments on ram No. 1. Respiratory frequency remained at 10–15 breaths/min throughout the entire procedure.

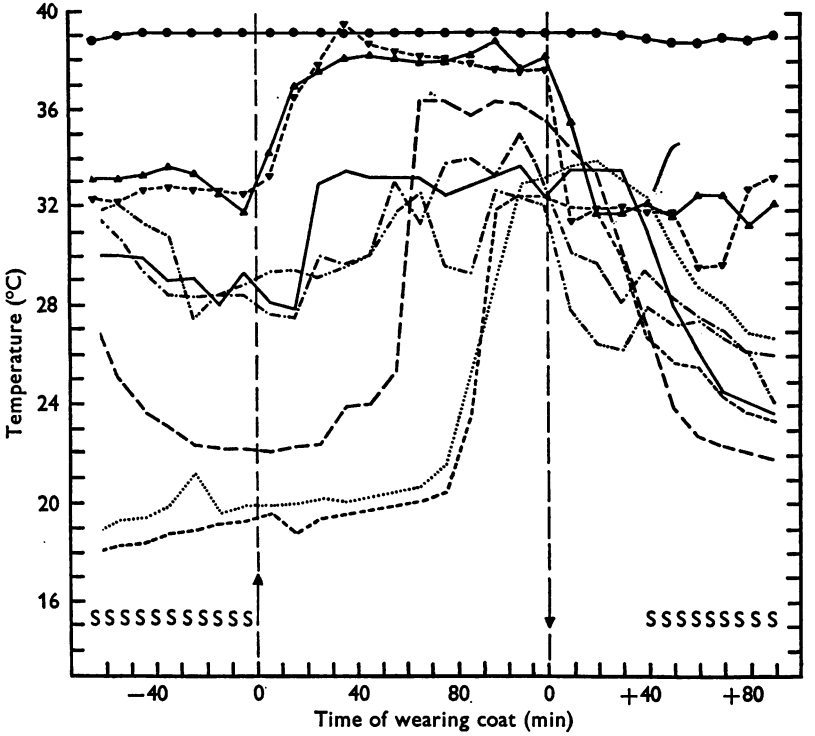

Fig. 7. Effects on rectal and skin surface temperatures of warming the body of ram No. 4, by covering the shorn body with a sheep skin coat in an environment of 19° C dry bulb temperature. The time is in relation to placement of coat. ↑ coat put on, ↓ coat taken off the animal. Respiratory frequency remained at 13–15 breaths/min. S: shivering. Temperatures: rectum (●—●), high midside of body (▲—▲), low mid-side of body (▼--▼), right ear (— —), left ear (– – –), right low foreleg (–·–·–), left low foreleg (–··–··–), right low hind leg (·····), left low hind leg (-----).

DISCUSSION

Effects of scrotal heating on body temperature and panting

The results obtained by Waites (1961, 1962) have been confirmed, in that heating the scrotum of the ram was followed by a marked fall in deep body temperature. This was accompanied by vigorous panting in the woolly animal, which was usually absent in the shorn animal.

As a further index of the temperature of thermoreceptors within the

body, external auditory meatus and carotid artery temperatures were measured in some of our experiments, and exhibited changes parallel to those of the rectum. However, in view of possible heat exchange in the carotid rete between central arterial blood supplying the head and venous blood draining the ears and nasal mucosa (Baker & Hayward, 1968*a*, *b*) hypothalamic temperature need not have been the same as any of the temperatures measured. Depending upon whether there was vasoconstriction or vasodilation in the ears, the brain blood temperature may have been raised or lowered, and vigorous panting occurring in many experiments would have cooled the brain blood. Nevertheless, the temperature of central arterial blood probably provided the best index of the 'average' temperature stimulus acting on receptors within the body.

The very potent effect of thermoreceptors of the scrotum on panting in the woolly animal, even with central body temperature lowered by up to 2° C, is striking. This is much more effective than heating an equal area of skin on the flank (Waites, 1962), and relative to the maximal response possible, the effect of scrotal heating is similar to that of local heating of the hypothalamus of the dog (Hammel *et al.* 1960) and ox (Findlay & Ingram, 1961) or local heating of the spinal cord of the dog (Jessen, 1967) and ox (Hales & Jessen, 1969). One notable difference, however, is that the respiratory response to heating the hypothalamus or spinal cord usually declines markedly within a few minutes, whereas with scrotal heating the response persists for a considerable time (Figs. 1 and 2).

Effects of scrotal heating on oxygen consumption and vasomotor function

The ambient temperatures to which the woolly animals were exposed would have been within the thermoneutral zone (Blaxter, Graham & Wainman, 1959; Armstrong, Blaxter, Clapperton, Graham & Wainman, 1960) and their oxygen consumption would therefore have been at the minimum possible level, corresponding to the basal heat production plus the heat increment of the ration. The fall in body temperature on heating the scrotum could not be expected to be achieved by a fall in oxygen consumption. Contrariwise, a rise in oxygen consumption to meet the energy cost of panting might be expected. However, although respiratory frequency increased eight times, which would at least have doubled respiratory minute volume (Hales & Webster, 1967), there was no significant rise in oxygen consumption. This provides further evidence of the low energy cost of panting in ruminants, as shown by other studies, on the ox (Whittow & Findlay, 1968; Hales & Findlay, 1968) and on the sheep (Hales & Brown, 1971). For the dog the cost seems to be higher (Spaich, Usinger & Albers, 1968, which also refers to earlier papers of Albers and co-workers).

In the shorn animals at environmental temperatures, below 20° C oxygen consumption during the control period was well above the minimum possible because the environments were cooler than the thermoneutral zone for shorn animals. When the scrotum was heated oxygen consumption was considerably reduced after a short delay, reaching the minimal possible value when the environmental temperature exceeded 11·6° C. The cooler the environment, the greater was the reduction in oxygen consumption (Fig. 6). In the three experiments at environmental temperatures of 20·5 and 24·5° C oxygen consumption was less elevated in the control period. In consequence, its reduction during scrotal heating was less and mild or vigorous panting accompanied it from the start.

When scrotal skin temperature was returned to its initial level, and particularly when it was cooled to below this level, there was a large initial rise in oxygen consumption accompanied by shivering. This was followed within 10–15 min by a decline, suggesting rapid habituation to the change in temperature. The animal was not obviously excited at the start of cooling as it was at the start of heating.

These changes in oxygen consumption could explain Waites' (1962) observation of a decline in body temperature during scrotal heating of shorn rams that did not pant. However, the question arises whether vasomotor changes also play a part. The variable and disorganized vasomotor responses to heating suggest that these changes were not important, except perhaps for ram No. 3 which gave well-organized dilations of its extremities on scrotal heating. On the other hand two of the shorn rams, which showed little or no vasodilation of the extremities in response to scrotal heating, could, nevertheless, give well organized dilations when they were warmed with coats. One must ask why there should be this anomaly in their heat regulation. One possible reason is that during scrotal heating non-thermal stimuli may have been causing vasomotor changes, for example, the rather unphysiological procedure of heating the scrotum in a bath of water. Calves in a thermoneutral or mildly warm environment show similar anomalous vasomotor changes of unknown cause (Beakley & Findlay, 1955; Whittow, 1962). The smell of hay and paradoxical sleep can cause vasoconstriction in the nasal mucosa or ear of sheep (Baker & Hayward, 1968*a*, *b*). Feeding can cause peripheral vasodilation in shorn sheep (Webster & Johnson, 1964). Well organized vasomotor responses, as in our coated rams, have been observed in adult sheep and new-born lambs exposed to different environmental conditions (Blaxter, Graham, Wainman & Armstrong, 1959; Alexander, 1961). In contrast to scrotal heating of the sheep, hypothalamic heating in the cat and ox (Ström, 1950; Findlay & Ingram, 1961; Ingram & Whittow, 1962) and spinal cord

heating in the dog and ox (Jessen, Meurer & Simon, 1967; Hales & Jessen, 1971) both elicit definite dilation of the extremities.

Waites & Voglmayr (1963) showed that sweat discharges on the scrotum were still present in shorn animals in which the scrotum was heated at an environmental temperature of 19–21° C. If these discharges also take place over the body, cutaneous evaporative heat loss could also contribute to the fall in body temperature when the scrotum is heated. However, Waites & Voglmayr (1963) noted discharges of sweat on the body simultaneous with those on the scrotum in only two of nine woolly rams. Nevertheless, Hofmeyr, Guidry & Waltz (1969) have found that if evaporative cooling of the scrotum of rams in a hot environment (35 or 40° C) is blocked by covering with a plastic bag, there is a slight increase in evaporation of moisture from the body and in respiratory frequency.

Responses to prolonged scrotal heating

When scrotal heating of shorn animals was continued for more than 100 min the fall in central temperature ceased and a new steady level of deep body temperature was attained (in the two experiments on woolly animals heated for more than 100 min, central temperature was falling, but only very slowly towards the end of heating). In the shorn animals oxygen consumption which had declined while the body temperature was falling, then rose, and the body temperature was thereby maintained. In two experiments in which ears and lower legs were dilated when body temperature was declining, they constricted when it became stable. One can express these findings in terms of the 'set-point' temperature' hypothesis (Hammel, Jackson, Stolwijk, Hardy & Strømme, 1963; Hammel, 1968; Cremer & Bligh, 1969). The effect of heating the scrotum in the circumstances of our experiments would be to reduce the 'set-point' temperature' for the Merino rams by 0·9–1·1° C and for the cross-bred ram by 1·9–2·2° C. This would cause a 'load-error' between the 'set-point' temperature and the actual deep body temperature, evoking the cooling mechanisms of the animal which would then reduce the 'load-error' by reducing central temperature. Bligh & Cottle (1969) have shown that the intraventricular injection of 5-hydroxytryptamine (5-HT) also activates body cooling mechanisms of sheep in both hot and cold environments. The responses we observed were as if heating the scrotum caused an increase in the amount of 5-HT impinging on the hypothalamus, in addition to that present during the control period; this would activate body cooling mechanisms. The central temperature would then fall and cause a reduction in the 5-HT being evoked by the effects of central temperature. This would continue until a new equilibrium of central body temperature and also of the 5-HT present was attained. The anamolous vasomotor changes would have to be achieved by other means.

In nature, the effect of a heat stimulus to the scrotum is not restricted to changing the 'set-point' temperature. Moisture loss used in cooling the scrotum can be as high as 208 g m^{-2} hr^{-1} (Waites & Voglmayr, 1962). This cooling provides negative feed-back from the thermal sensors of the scrotum to the brain. These sensors, therefore, act as local thermostats regulating the temperature of the scrotum and testicles. The sweat would not be wasted in cooling the rest of the body because heat exchange in the pampiniform plexus isolates thermally the scrotum and testicles from the rest of the body (Harrison & Weiner, 1949; Waites & Moule, 1961). Fowler (1967) has reported an average sweating rate of up to 10 g per scrotum per hour (or 160 g m^{-2} hr^{-1}) in eight Merino rams when the scrotal temperature was raised to 39° C by confining the scrotum in a heated chamber. If this sweating rate is considered, together with testicular heat production (measured at 32–35° C and corrected to 39° C assuming a Q_{10} of 2, Setchell & Waites, 1964), it may be calculated that there is sufficient sweat to dissipate more than five times the heat produced.

Inhibition of panting

Bligh (1961*a*, 1963) showed that if a shorn sheep is housed in an environment of approximately 20° C, panting does not begin for 40–50 min on subsequent exposure to a hot environment. Waites (1962) noted the absence of panting during scrotal heating of shorn rams. We found that before scrotal heating, shorn rams in environments of 19° C and below had an oxygen consumption higher than that in a thermoneutral environment (i.e. when they were woolly). Therefore, body temperature could be regulated by changing metabolic rate, and it is not surprising that scrotal heating did not immediately cause panting. However, in many instances the minimal oxygen consumption was reached some time before the central body temperature ceased to fall. It is difficult to understand why panting did not begin at this point and yet had been elicited at the start of scrotal heating in the three experiments at 20·5 and 24·5° C ambient temperature with oxygen consumption close to but above the minimal level; one possible explanation is that deep body and skin temperature were higher in these latter experiments and panting was inhibited in the former experiments because both central and skin temperature had been lowered by the time panting might have become necessary. The anomaly could also be explained in terms of a 'central block' as proposed by Bligh (1963). As in Bligh's experiments the block could be removed by covering the body with a coat. Other inhibitory effects on panting have been observed that are not related to peripheral cold stimuli. Discharge of sweat on the body of the sheep (Bligh, 1961*b*) or on the unclipped scrotum (Waites & Voglmayr, 1963) is accompanied by a marked temporary depression of

panting; yet this sweat, being absorbed by the wool by an exothermic reaction, does not cool but momentarily heats the skin. Pecking of grain by the domestic fowl abolishes established panting for several minutes in a hot environment (Hutchinson & Taylor, 1962).

There are contrasting effects of scrotal, hypothalamic and spinal cord heating on panting, sweating and vasomotor activity in different environments. Thus, raising the temperature of any one of the three sites will elicit panting, vasodilation and sweating in a thermoneutral or warm environment (Fusco *et al.* 1961; Ingram & Whittow, 1962; Ingram *et al.* 1963; Jessen *et al.* 1967; Jessen, 1967; Hales & Jessen, 1969). However, in a cool environment panting and vasodilation do not follow hypothalamic or scrotal heating (Fusco *et al.* 1961; Ingram & Whittow, 1962; Waites, 1962) whereas they are elicited by spinal cord heating even in environments of 0 and 3° C (Jessen *et al.* 1967; Hales & Jessen, 1971). Finally in a relatively cool environment sweating may be stimulated by scrotal heating (Waites & Voglmayr, 1963), but not by hypothalamic (Ingram *et al.* 1963), or by spinal cord heating (Hales & Jessen, 1971).

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Note added in proof

While this paper was in the press, Feldberg, Myers & Veale (*J. Physiol.* (1970), **207**, 403–416) suggested that a possible mechanism for regulating 'set-point' temperature could involve the level of calcium or its permeability in the hypothalamus; they referred particularly to the action of pyrogens in raising 'set-point' temperature. This is an analogue of our suggestion of the role of 5-HT in reducing the 'set-point' temperature of rams (p. 371).

REFERENCES

ALEXANDER, G. (1961). Temperature regulation in the newborn lamb. III. Effect of environmental temperature on metabolic rate, body temperatures, and respiratory quotient. *Aust. J. agric. Res.* **12**, 1152–1174.

ALTMAN, P. L., GIBSON, J. F. & WANG, C. C. (1958). *Handbook of Respiration.* Philadelphia and London: W. B. Saunders Co.

ARMSTRONG, D. G., BLAXTER, K. L., CLAPPERTON, J. L., GRAHAM, N. McC. & WAINMAN, F. W. (1960). Heat production and heat emission of two breeds of sheep. *J. agric. Sci., Camb.* **55**, 395–401.

BAKER, M. A. & HAYWARD, J. N. (1968*a*). Intracranial heat exchange and regulation of brain temperature in sheep. *Life Sci. Oxford* **7**, 349–357.

BAKER, M. A. & HAYWARD, J. N. (1968*b*). The influence of the nasal mucosa and the carotid rete upon hypothalamic temperature in sheep. *J. Physiol.* **198**, 561–579.

BEAKLEY, W. R. & FINDLAY, J. D. (1955). The effect of environmental temperature and humidity on the ear temperatures of Ayrshire calves. *J. agric. Sci., Camb.* **45**, 373–379.

BENZINGER, T. H. (1969). Heat regulation: Homeostasis of central temperature in man. *Physiol. Rev.* **49**, 671–759.

BLAXTER, K. L., GRAHAM, N. McC. & WAINMAN, F. W. (1959). Environmental temperature, energy metabolism and heat regulation in sheep. III. The metabolism and thermal exchanges of sheep with fleeces. *J. agric. Sci., Camb.* **52**, 41–49.

BLAXTER, K. L., GRAHAM, N. McC., WAINMAN, F. W. & ARMSTRONG, D. G. (1959). Environmental temperature, energy metabolism and heat regulation in sheep. II. The partition of heat losses in closely clipped sheep. *J. agric. Sci., Camb.* **52**, 25–40.

BLIGH, J. (1957). The initiation of thermal polypnoea in the calf. *J. Physiol.* **136**, 413–419.

BLIGH, J. (1959). The receptors concerned in the thermal stimulus to panting in sheep. *J. Physiol.* **146**, 142–151.

BLIGH, J. (1961*a*). The delayed polypnoeic response to heat in the shorn sheep. *J. Physiol.* **159**, 84–85*P*.

BLIGH, J. (1961*b*). The synchronous discharge of apocrine sweat glands of the Welsh Mountain sheep. *Nature, Lond.* **189**, 582–583.

BLIGH, J. (1963). Inhibition of thermal polypnoea in the closely shorn sheep. *J. Physiol.* **168**, 764–781.

BLIGH, J. (1966). The thermosensitivity of the hypothalamus and thermoregulation in mammals. *Biol. Rev.* **41**, 317–367.

BLIGH, J. & COTTLE, W. H. (1969). The influence of ambient temperature on thermoregulatory responses to intraventricularly injected monoamines in sheep, goats and rabbits. *Experientia* **25**, 608–609.

CREMER, J. E. & BLIGH, J. (1969). Body temperature and responses to drugs. *Br. med. Bull.* **25**, 299–306.

FINDLAY, J. D. & INGRAM, D. L. (1961). Brain temperature as a factor in the control of thermal polypnoea in the ox (*Bos taurus*). *J. Physiol.* **155**, 72–85.

FOWBER, D. G. (1967). Studies on the association of skin folds and fertility of Merino rams. Ph.D. Thesis, Univ. N.S.W.

FUSCO, M. M., HARDY, J. D. & HAMMEL, H. T. (1961). Interaction of central and peripheral factors in physiological temperature regulation. *Am. J. Physiol.* **200**, 572–580.

HALES, J. R. S. & BROWN, G. D. (1971). The efficiency of panting. *Am. J. Physiol.* (in the Press).

HALES, J. R. S. & FINDLAY, J. D. (1968). The oxygen cost of thermally-induced and CO_2-induced hyperventilation in the ox. *Resp. Physiol.* **4**, 353–362.

HALES, J. R. S. & JESSEN, C. (1969). Increase of cutaneous moisture loss caused by local heating of the spinal cord in the ox. *J. Physiol.* **204**, 40–42*P*.

HALES, J. R. S. & JESSEN, C. (1971). Thermoregulatory mechanisms evoked by alterations in spinal cord temperature in the ox. *J. Physiol.* (in the Press).

HALES, J. R. S., KAO, F. F., MEI, S. S., WANG, C. & GRETENSTEIN, M. (1970). Panting in heated cross-circulated dogs. *Am. J. Physiol.* **218**, 1389–1393.

HALES, J. R. S. & WEBSTER, M. E. D. (1967). Respiratory function during thermal tachypnoea in sheep. *J. Physiol.* **190**, 241–260.

HAMMEL, H. T. (1968). Regulation of internal body temperature. *A. Rev. Physiol.* **30**, 641–710.

HAMMEL, H. T., HARDY, J. D. & FUSCO, M. M. (1960). Thermoregulatory responses to hypothalamic cooling in unanaesthetized dogs. *Am. J. Physiol.* **198**, 481–486.

HAMMEL, H. T., JACKSON, D. C., STOLWIJK, J. A., HARDY, J. D. & STRØMME, S. B. (1963). Temperature regulation by hypothalamic proportional control with an adjustable set-point. *J. appl. Physiol.* **18**, 1146–1154.

HARRISON, R. G. & WEINER, J. S. (1949). Vascular patterns of the mammalian testis and their functional significance. *J. exp. Biol.* **26**, 304–316.

HOFMEYR, H. S., GUIDRY, A. J. & WALTZ, F. A. (1969). Effects of temperature and wool length on surface and respiratory evaporative losses of sheep. *J. appl. Physiol.* **26**, 517–523.

HUTCHINSON, J. C. D. & TAYLOR, W. W. (1962). Effect of exposure to heat on the motor co-ordination of pecking fowls. *Anim. Behav.* **10**, 62–66.

INGRAM, D. L., MCLEAN, J. A. & WHITTOW, G. C. (1963). The effect of heating the hypothalamus and the skin on the rate of moisture vaporization from the skin of the ox (*Bos taurus*). *J. Physiol.* **169**, 394–403.

INGRAM, D. L. & WHITTOW, G. C. (1962). The effect of heating the hypothalamus on respiration in the ox (*Bos taurus*). *J. Physiol.* **163**, 200–210.

JESSEN, C. (1967). Auslösung von Hecheln durch isolierte Wärmung des Rückenmarks am wachen Hund. *Pflügers Arch. ges. Physiol.* **297**, 53–70.

JESSEN, C., MEURER, K-A. & SIMON, E. (1967). Steigerung der Hautdurchblutung durch isolierte Wärmung des Rückenmarks am wachen Hund. *Pflügers Arch. ges. Physiol.* **297**, 35–52.

SETCHELL, B. P. & WAITES, G. M. H. (1964). Blood flow and the uptake of glucose and oxygen in the testes and epididymis of the ram. *J. Physiol.* **171**, 411–425.

SIHLER, C. (1879). On the so called heat-dyspnoea. *J. Physiol.* **2**, 191–201.

SIMON, E., RAUTENBERG, W., THAUER, R. & IRIKI, M. (1964). Die Auslösung von Kältezittern durch lokale Kühlung im Wirbelkanal. *Pflügers Arch. ges. Physiol.* **281**, 309–331.

SPAICH, P., USINGER, W. & ALBERS, C. (1968). Oxygen cost of panting in anaesthetized dogs. *Resp. Physiol.* **5**, 302–314.

STRÖM, G. (1950). Influence of local thermal stimulation of the hypothalamus of the cat on cutaneous blood flow and respiratory rate. *Acta physiol. scand.* **20**, suppl. 70, 47–76.

WAITES, G. M. H. (1961). Polypnoea evoked by heating the scrotum of the ram. *Nature, Lond.* **190**, 172–173.

WAITES, G. M. H. (1962). The effect of heating the scrotum of the ram on respiration and body temperature. *Q. Jl exp. Physiol.* **47**, 314–323.

WAITES, G. M. H. & MOULE, G. R. (1961). Relation of vascular heat exchange to temperature regulation in the testis of the ram. *J. Reprod. Fert.* **2**, 213–224.

WAITES, G. M. H. & VOGLMAYR, J. K. (1962). Apocrine sweat glands of the scrotum of the ram. *Nature, Lond.* **196**, 965–967.

WAITES, G. M. H. & VOGLMAYR, J. K. (1963). The functional activity and control of the apocrine sweat glands of the scrotum of the ram. *Aust. J. agric. Res.* **14**, 839–851.

WEBSTER, M. E. D. & JOHNSON, K. G. (1964). Distribution of temperature in the skin of the sheep exposed to moderate environments. *Nature, Lond.* **201**, 208.

WHITTOW, G. C. (1962). The significance of the extremities of the ox (*Bos taurus*) in thermoregulation. *J. agric. Sci., Camb.* **58**, 109–120.

WHITTOW, G. C. & FINDLAY, J. D. (1968). Oxygen cost of thermal panting. *Am. J. Physiol.* **214**, 94–99.