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HABITUATION AND ACCLIMATIZATION OF SHEEP TO COLD FOLLOWING EXPOSURES OF VARYING LENGTH AND SEVERITY

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SUMMARY

1. Male and female Scottish Blackface sheep were shorn and exposed for 2 weeks either to a thermoneutral temperature (+30° C), to chronic cold (+8° C) or to +30° C interrupted by daily short cold shocks (−10° C). During and at the end of these conditioning treatments, the sheep also received two acute cold exposures (−20° C, 4 m.p.h. wind for 2–8 hr) 1 week apart. Some of these sheep and a fourth (control) group, were subsequently re-shorn and slowly cooled to +8° C.

2. Resting metabolism and the metabolic response to cooling (both inferred from heart rates) were increased by previous chronic cold treatment. Resistance to body cooling (measured during acute cold exposure) was generally increased by both chronic and acute cold, and non-shivering thermogenesis was probably induced in the female sheep. These effects were defined as acclimatization.

3. In contrast, cold shocks reduced the subsequent metabolic response to cold and encouraged facultative body cooling. This pattern of response (defined as habituation) therefore caused greater thermolability.

4. Habituation and acclimatization were antagonistic. Habituation was removed by acute cold exposure and, conversely, acclimatization was inhibited by short cold shocks.

5. There were sex differences in response but these were confounded by probable differences in insulation and in body condition (males thinner).

6. It was concluded that the induction of different forms of adaptation depended on the length, severity and frequency of cold exposures. Habituation to whole body cold exposure apparently involved central nervous system centres normally receiving peripheral cold stimuli.

INTRODUCTION

Shorn sheep can be acclimatized to cold experimentally by acute or chronic cold or, more effectively, by both types of exposure in sequence (Slee & Sykes, 1967; Sykes & Slee, 1968, 1969*a*, *b*; Slee, 1970*a*, *b*). Acclimatization consistently causes an increase in resistance to body cooling during acute cold exposure and this may be taken as the definitive characteristic. Other characteristics of acclimatization, which persist after cold exposure is discontinued, are increased heart rate and increased peripheral skin temperatures. Cold acclimatization apparently involves elevated peak metabolic capability and a temporary elevation in resting metabolism. Webster, Hicks & Hays (1969) showed that cold acclimatization could also be induced in unshorn sheep either by natural exposure to winter temperatures, or by equivalent cold exposure in climate chambers. They confirmed by direct measurements that acclimatization caused increases in peak metabolic response to cold and in resting metabolic rate. These changes were again associated with increased resistance to body cooling and a lowered critical temperature.

The question arose from this work as to how far different types of cold exposure might produce different immediate physiological responses or different forms of adaptation. Relevant factors might be length, severity or total dosage of cold, and whether exposure was steady or fluctuating.

The purpose of the present experiment, therefore, was to investigate the effects of varying the dosage and mode of administration of cold exposure on acclimatization and on rates of body cooling under cold stress. In particular the immediate response to short, discontinuous cold shocks was examined in the same context as the responses to acute and chronic cold and the subsequent effect of adaptation to cold shocks was compared with that resulting from chronic cold. It was found that quite different physiological changes, in terms of thermolability and in the degree of metabolic response to cooling, accompanied these two forms of adaptation.

METHODS

Fifty-six yearling Scottish Blackface sheep were used for these experiments which took place between January and December 1968. The sheep were housed and individually fed maintenance rations of 600 g/head of high protein pelleted cake, increasing to 700 g towards the end of the experiment.

The intention was to measure (i) resistance to obligatory body cooling during acute cold exposure, (ii) the extent of facultative body cooling during moderate cold exposure, and (iii) metabolic responses to acute and moderate cold. The effect of three previous conditioning temperature treatments upon these functions was investigated.

All temperature treatments were carried out in climatic rooms with ambient temperature controlled to within $\pm 1°$ C. Humidity was not controlled but varied from 95–100% r.h. at all temperatures below 0° C D.B. and up to r.h. 40% at

+ 30° C D.B. The sheep were shorn to a standard fleece length of 5 mm before treatment commenced and on the day before each test cold exposure.

Experimental procedure. After shearing the sheep were divided into three groups for conditioning treatment (Table 1). Half of the sheep in each group were males and half were females. In group 1 the sheep were kept at + 30° C (thermoneutral treatment) for 2 weeks; in group 2 the sheep were at + 8° C (chronic cold treatment) for 2 weeks; in group 3 the sheep received ten daily short cold shocks after each of which they reverted to a room temperature of + 30° C (cold shock treatment). Each group also received two acute cold test exposures 1 week apart during its treatment period. Some sheep from groups 2 and 3 were later re-treated and together with untreated controls received a further test exposure which involved slow cooling from + 30 to + 8° C (see Table 1). It was expected that the three conditioning treatments would induce different forms of temperature adaptation, the physiological effects of which could be examined during the test exposures. In addition, however, the first acute cold test exposures themselves produced adaptations which were evident at the second series of acute exposures.

Cold exposures. For the chronic cold conditioning of treatment 2 the sheep remained at + 8° C for 2 weeks except during the two acute exposures and for 16 hr before each of these exposures when the room temperature was raised to + 30° C. Three types of short cold exposure were used: short cold shocks (a conditioning treatment), acute cold test exposures, and slow cooling test exposures. During acute cold exposures ambient temperature fell slowly (1° C/5 min) from + 30° C (thermoneutral) to 0° C. Then a 4 mph wind was introduced and the temperature was lowered in controlled steps to − 20° C. Individual exposures were continued until rectal temperatures fell to 36° C, the time for which varied from 2 to 8 hr. For the short cold shocks room temperature was lowered rapidly from + 30 to − 10° C; 0° C was passed in about 30 min and − 10° C reached within 75 min of commencement. The sheep spent one hour below 0° C and about 15 min at − 10° C before the temperature was re-set for + 30° C which was usually reached in 30 min. A 4 m.p.h. wind was imposed during cooling. Short cold shocks were administered daily in series of four or six. Between cold shocks the ambient temperature remained at + 30° C. For slow-cooling exposures room temperature was lowered at a rate of 1° C/5 min to + 8° C where it remained for 3 hr. In some experiments the temperature was then slowly reduced to + 2° C. No wind was used.

The sheep received their cold exposures in groups of four or eight, but they were restrained individually in separate metabolism crates in a standing position. Wind, when applied, was provided by small electric fans attached to each crate opposite the midside of the sheep.

Measurements. Rectal temperature was measured by 32 S.W.G. copper-constantan thermocouples inserted 13 cm and connected to a Honeywell–Brown potentiometric chart recorder. Skin temperatures were obtained from 36 S.W.G. thermocouples attached to the skin beneath small patches of adhesive tape.

Heart rates were obtained by electrocardiograph equipment using perforated tin plate electrodes attached to the chest wall and midback, and counted from an audible relay confirmed by oscilloscope display.

Shivering intensity was estimated from the mean electrical activity of the pectoral muscles obtained from the same electrodes and from similar electrodes on the thigh over the biceps femoris. Peak to peak voltage changes were integrated and summed over periods of 30 sec.

Heart rate and shivering data were used to make inferences about changes in metabolic rate. It was assumed that heart rate could be used as a guide to metabolic rate in some circumstances (Graham, 1960; Webster, 1967) but not in others

(Brockway & McEwan, 1969). Sykes & Slee (1968, 1969*b*) and Slee (1971) concluded that information about changes in metabolic rate may be inferred from changes in heart rate provided that (i) the changes are fairly large, (ii) comparative differences rather than absolute values are sought, and (iii) comparisons are made between the average responses of groups of trained sheep in controlled environments. In the present experiment these conditions were satisfied.

Statistics. The significance of treatment group differences between sheep at the same ambient temperature was calculated by *t* tests. Analysis of variance was used to test for persistent differences during periods of changing ambient temperature.

RESULTS

Rectal temperature during conditioning treatments

Average daily rectal temperature during the first week of treatment 1 was 39·4° C. The chronic cold treatment of group 2 produced lower rectal temperatures (average 39·0° C) compared with group 1 ($P < 0{\cdot}001$). Conversely, treatment 3 rectal temperatures, measured at +30° C, outside the periods of cold shock exposure, were slightly higher than those of treatment 1, averaging 39·8° C. Males had lower temperatures than females during the chronic cold of treatment 2 ($P < 0{\cdot}001$).

Rectal temperature during short cold shocks

During each of the daily cold shocks of conditioning treatment 3, rectal temperatures fell 1–2° C from about 39·8° C, usually reaching minimum values about 30 min after the lowest room temperature of −10° C had been reached. By then room temperatures had returned to +30° C.

As shown in Fig. 1, rectal temperatures fell lower on each successive cold shock from a mean minimum of 38·8° C on day 1 to 37·9° C on day 6. However, the first acute cold exposure, interposed on day 7 after the 6th shock, interrupted this progression. Thus during the seventh shock on day 8 (following the acute exposure), rectal temperatures fell about 1° C less than in the sixth shock ($P < 0{\cdot}001$), so returning to the pattern shown at the beginning. During the remaining shocks on days 9–11 the sequence of progressively lower rectal temperatures was re-established. Analysis of variance indicated a highly significant ($P < 0{\cdot}001$) over-all effect of day of exposure upon the minimum rectal temperature reached.

There was considerable individual variation in these responses but the general tendency for rectal temperatures to decline more during the later cold shocks suggested that, as the treatment progressed, the sheep were making smaller metabolic (heat production) responses to cold or smaller vasomotor (heat conservation) adjustments.

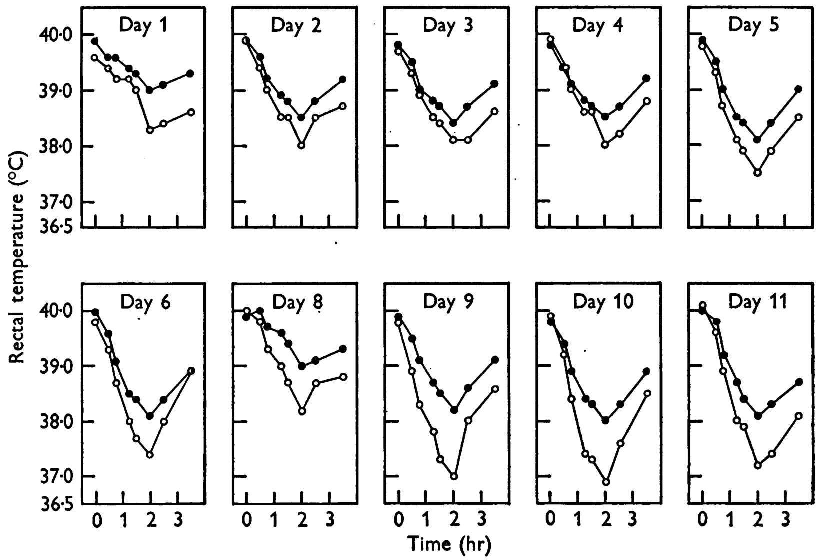

Fig. 1. Changes in mean rectal temperature for sixteen treatment 3 sheep (all shorn) during successive daily cold shocks. An acute cold exposure replaced the cold shock on day 7. Filled circles represent the average of all the sheep (sexes combined); open circles represent the average of the most hypothermic male and female. During each cold shock the minimum ambient temperature of $-10°$ C was reached after $1\frac{1}{2}$ hr and the temperature was returned to $+30°$ C between the second and third hours.

Acute cold exposures

Rectal temperature changes. Acute test exposures comprised two phases; first the $2\frac{1}{2}$ hr period of moderate cooling from $+30$ to $0°$ C, then the acute phase involving wind and a further fall in ambient temperature from 0 to $-20°$ C. Fig. 2 shows that rectal temperatures declined gradually during the first phase until room temperatures fell to about $+8°$ C. They subsequently rose, most quickly during the early part of the acute phase of exposure, before falling again, this time irreversibly. This temporary rise in rectal temperature accompanying acute cold exposure is a typical response of sheep (Joyce & Blaxter, 1964; Slee, 1966; Slee & Sykes, 1967), and is probably associated with the approach to peak metabolism.

Since the metabolic capability of the sheep was sufficient to cause a rise in rectal temperature at ambient temperatures below $0°$ C it may be assumed that the initial decline in rectal temperature was facultative (i.e. a passive decline in body temperature not resisted by maximum metabolic response). In contrast, the hypothermia which eventually occurred at ambient temperatures below $0°$ C was involuntary (i.e. occurred despite

TABLE 1. Experimental procedure

Treatment group	N	Day 1	Conditioning exposures 1st week	Test exposures Day 7	Conditioning exposures 2nd week	Test exposures Day 15	Test exposures 8 weeks after day 15
1	12	at +30° C	+30° C (thermoneutral)	1st acute cold exposure	+30° C	2nd acute cold exposure	—
2	12	Cooled to +8° C	+8° C (chronic cold)	1st acute cold exposure	+8° C	2nd acute cold exposure	Four sheep from treatment 2 and eight sheep from treatment 3 were conditioned again to these treatment temperatures and with sixteen untreated controls were re-shorn and then slowly cooled to +8° C
3	16	at +30° C	6 daily cold shocks at −10° C then returned to +30° C	1st acute cold exposure	4 cold shocks at −10° C then returned to +30° C	2nd acute cold exposure	

maximum metabolic response) and the actual rate of decline represented the ability of the sheep to resist body cooling (Table 2).

Fig. 2 shows mean changes in rectal temperature during acute cold exposures following either 1 week (in the case of the first acute exposure) or 2 weeks (second exposure) of conditioning temperature treatment. Only the female data are shown, but the male responses were generally similar.

TABLE 2. Mean rate of decline in rectal temperature (° C/100 min) during acute cold exposures

	1st exposure		2nd exposure	
	♂	♀	♂	♀
Treatment	Mean S.E.	Mean S.E.	Mean S.E.	Mean S.E.
1	2·16 ± 0·27	1·29 ± 0·13	1·60 ± 0·19	1·02 ± 0·10
2	2·16 ± 0·29	1·22 ± 0·08	4·21 ± 2·03	0·84 ± 0·06
3	2·32 ± 0·43	1·65 ± 0·15	2·08 ± 0·41	1·42 ± 0·08
Over-all means	2·23 ± 0·21	1·42 ± 0·08	2·54 ± 0·61	1·13 ± 0·07

The rates of decline in rectal temperature were calculated individually from the time taken for rectal temperatures to fall to 36·0° C from the initial value in the thermoneutral environment.

Treatment 3 sheep (previously cold-shocked) showed the greatest degree of facultative cooling at ambient temperatures down to 0° C. Their rectal temperatures declined for longer and became significantly lower than treatment 1 sheep ($P < 0{\cdot}05$ to $< 0{\cdot}01$ for males; $P < 0{\cdot}01$ to $< 0{\cdot}001$ for females). Below zero the typical rise in rectal temperature occurred in all sheep, but in treatment 3 the increase was less than in the other treatment groups ($P < 0{\cdot}01$ for males; $P < 0{\cdot}001$ for females). The rise also occurred from a lower base in treatment 3, so these sheep entered and progressed through the acute phase of cold exposure with, on average, significantly lower temperatures than the other groups ($P < 0{\cdot}02$ to $P < 0{\cdot}01$ for males; $P < 0{\cdot}01$ to $P < 0{\cdot}001$ for females).

Cold resistance. Resistance to cooling, calculated from the rate of fall of rectal temperature during acute test exposures (Table 2), was significantly less in males than in females at both exposures ($P < 0{\cdot}001$).

Increased cold resistance at second exposure, especially in females ($P < 0{\cdot}001$), can be attributed to acclimatization resulting from previous cold exposure and was consistent with earlier work (Slee & Sykes, 1967; Sykes & Slee, 1969*a*). Previous acute exposure alone (the effects of which appeared in treatment 1 at second exposure) increased resistance to cooling in both sexes. Chronic moderate cold exposure for 1 week (shown in

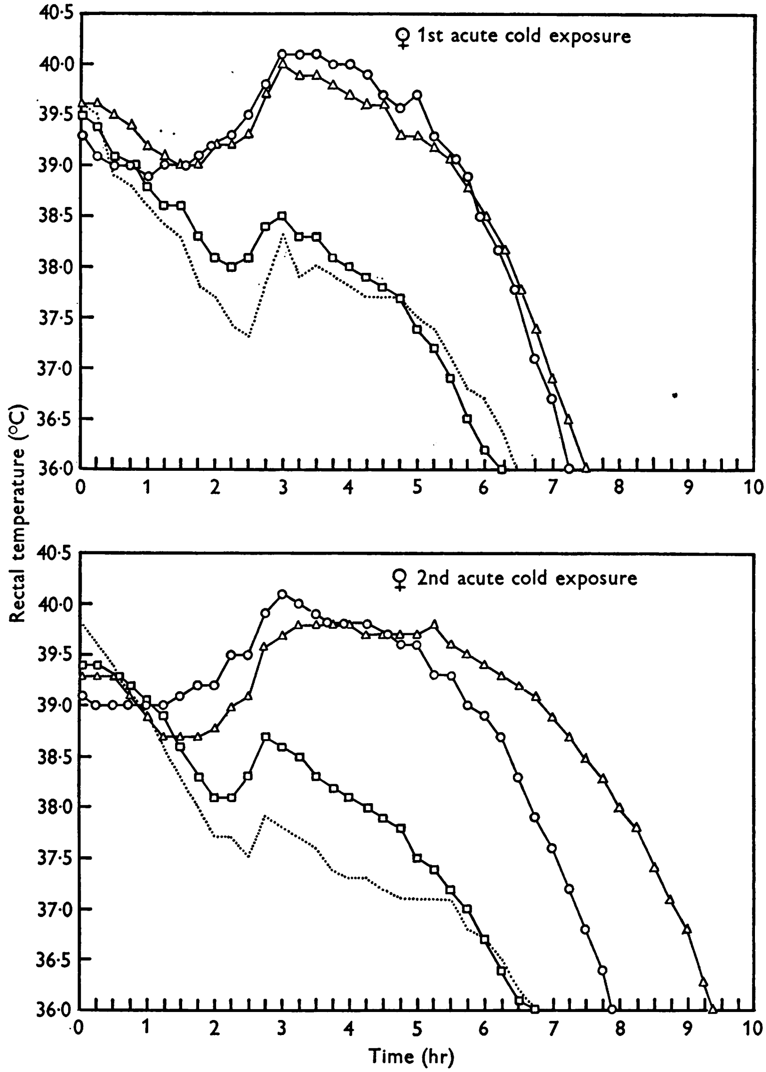

Fig. 2. Mean rectal temperatures during acute cold exposure of eight shorn female sheep from each of the three conditioning treatment groups. Circles represent treatment 1, triangles treatment 2 and squares treatment 3. The individual which had previously shown the lowest rectal temperature during the cold shocks of treatment 3 is indicated separately by dotted lines. Ambient temperature fell from $+30^\circ$ C at the commencement to reach 0° C after $2\frac{1}{2}$ hr, -16° C after 4 hr and -20° C after 7 hr. Wind was introduced at 0° C.

treatment 2, first exposure) did not significantly increase cold resistance. The combined effects of chronic exposure for 2 weeks and acute exposure (seen in treatment 2, second exposure) produced debility in some males, with a low average cold resistance, but induced maximum cold resistance in the females (Table 2).

The short cold shocks of treatment 3 did not increase cold resistance. In females, on the contrary, treatment 3 produced facultative cooling initially (Fig. 2) and also significantly ($P < 0{\cdot}05$) diminished resistance to cooling during the acute phase (Table 2). When continued for a second week the cold shocks significantly ($P < 0{\cdot}01$) reduced the acclimatization expected at second acute exposure as a result of the first acute cold exposure (Table 2, treatments 3 *vs.* 1). Among males the cold shocks also produced facultative cooling initially but had no significant effect on the final resistance to cooling, which was generally low.

Summarizing, previous conditioning by short cold shocks predisposed to facultative lowering of body temperature during the initial moderate cooling phase and to decreased resistance to hypothermia during the subsequent acute phase of cold exposure. Conversely, the first acute cold test exposure induced acclimatization, which was characterized by generally higher rectal temperatures and enhanced resistance to hypothermia at second exposure. Acute cold and short cold shocks were therefore antagonistic in their effects.

Heart rate, shivering and muscle tone. Heart rates were used to indicate average differences between treatment groups in resting metabolic rate at thermoneutrality (+30° C), and to show differences in the extent of metabolic response during cooling.

Heart rates immediately before and during the initial moderate cooling phase of acute cold exposure are shown, for females only, in Fig. 3. The results for males were similar except where indicated below. In both sexes, initial heart rates at +30° C were higher by about 30 beats/min following treatment 2 compared with treatment 1 ($P < 0{\cdot}001$). However, heart rates of treatment 1 sheep began to increase early during cooling and eventually approached those of treatment 2. It was concluded that acclimatization to chronic cold in treatment 2 caused a residual elevation in resting metabolic rate which was still evident in the thermoneutral zone at +30° C. Cooling below about 15° C increased the metabolic rate of treatment 1 sheep to a similar level.

Previous chronic cold exposure (treatment 2), as well as increasing resting metabolism, also apparently increased the metabolic response to cold, as evidenced by high heart rates during later stages of cooling (Fig. 3). The effects of previous acute cold exposure alone (potentially evident at second exposure, treatment 1) did not include increased heart rates, despite the greater cold resistance produced. Previous acute and chronic cold exposure combined (effects evident at treatment 2, second exposure), depressed male heart rates while considerably increasing those of the females. After treatment 2, female heart rates were over-all higher than males ($P < 0{\cdot}001$).

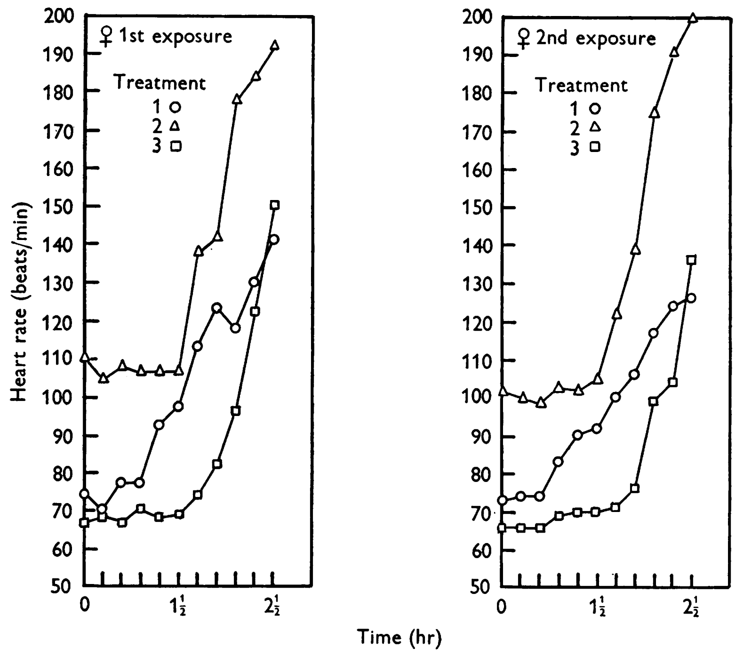

Fig. 3. Mean heart rates of shorn female sheep (eight per treatment group) during the initial moderate cooling phase of acute cold exposure. Ambient temperature fell from + 30° C at a rate of 1° C per 5 min to reach 0° C after 2½ hr.

The short cold shocks of treatment 3 produced little change in heart rates at + 30° C but delayed the point at which heart rates began to increase during subsequent cooling, especially in females. Thus, as ambient temperatures fell below 15° C, heart rates of treatment 3 females became significantly lower ($P < 0{\cdot}001$) than those from treatments 1 and 2. This paralleled the other effect of treatment 3 in lowering female rectal temperatures during cooling.

Electromyograph recordings indicated that, in males, acclimatization due to chronic cold (treatment 2) increased muscle tone at + 30° C ($P < 0{\cdot}05$) and increased shivering intensity during cooling ($P < 0{\cdot}01$); but there was no significant effect in females. The short cold shocks of treatment 3 tended to reduce shivering compared with treatment 1 ($P < 0{\cdot}02$, sexes combined).

Summarizing, chronic cold conditioning treatment apparently increased resting metabolic rate (evident from heart rates at + 30° C) in both sexes and increased subsequent metabolic responses to cooling, especially in females (evident from heart rates at 0° C). Since shivering intensity did not increase in the females, they may have developed an ability for non-shivering thermogenesis. Acute cold exposure alone had little effect on subsequent metabolic rate or shivering responses. Previous cold shocks tended to decrease shivering and cold-induced metabolism, particularly in the females.

Responses to moderate slow cooling

Rectal temperature. The influence of short cold shocks upon facultative body cooling during subsequent cold exposure was investigated further. Preliminary questions were: (i) could this response be induced consistently? (ii) were there repeatable differences between individuals? (iii) how low could rectal temperatures be induced to fall under facultative cooling? and (iv) for how long would rectal temperatures remain subnormal?

Sykes & Slee (1968, 1969*a*) found that closely shorn sheep could withstand ambient temperatures of + 8° C for at least 2 weeks without rectal temperatures deviating from the normal range of 39–40° C. Presumably, therefore, any body cooling induced by room temperatures not lower than + 8° C would be facultative. Four male and four female sheep, which 8 weeks previously had received short cold shocks in treatment 3, were selected for slow cooling tests. Two sheep of each sex had previously shown marked facultative cooling and two had shown a minimum response. These sheep were re-shorn and subjected to 4 daily short cold shocks as for treatment 3. Rectal temperatures again fell lower during each successive cold shock from a mean minimum of 38·9° C on day 1 to 37·8° C on day 4. Assuming that the tendency towards facultative cooling had been sufficiently re-introduced the sheep were shorn again, cooled slowly from + 30 to + 8° C and left for 3 hr. Four cold-acclimatized sheep which had received an acute cold exposure followed by 2 weeks at + 8° C and sixteen control sheep which had received no previous temperature treatment were also shorn and cooled. Thus slow cooling tests were performed on three groups of sheep · (i) untreated controls, (ii) sheep conditioned by short cold shock treatment and (iii) sheep conditioned by chronic and acute cold (i.e. cold-acclimatized).

The resultant changes in rectal temperature are shown in Fig. 4. The acclimatized sheep had the highest rectal temperatures. In contrast, there was considerable body cooling in the sheep which had received cold shocks. Their rectal temperatures fell significantly ($P < 0{\cdot}001$) lower than the controls and lower than the acclimatized sheep ($P < 0{\cdot}02$). Some temperatures remained low for several hours. The fall in temperature was greater in males than in females ($P < 0{\cdot}05$). The rectal temperature of one individual fell to 35·7° C $1\frac{1}{2}$ hr after the room temperature reached +8° C. Even this degree of hypothermia, almost 4° C below normal, was shown to be facultative by a subsequent upward trend when room temperature was further lowered to +2° C (Fig. 4).

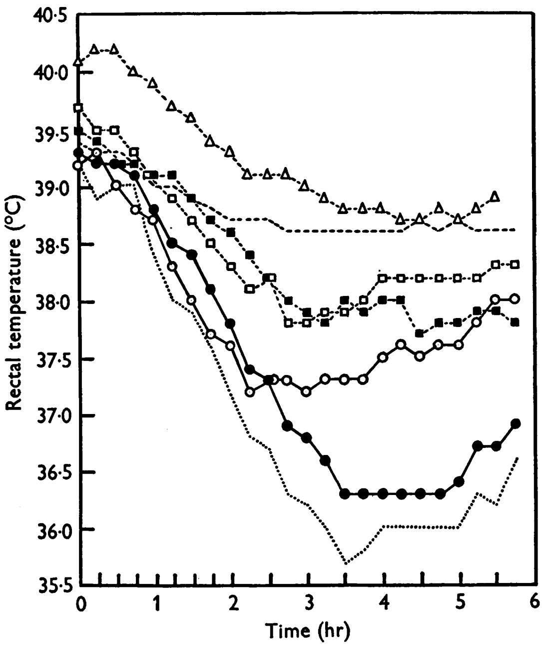

Fig. 4. Mean rectal temperature during slow cooling tests in sixteen previously untreated male and female control sheep (– – –), four cold-acclimatized female sheep from treatment 2 (△) and eight sheep which had received cold shocks (from treatment 3). The cold-shocked sheep (denoted by circles and squares) are subdivided into those which had previously shown maximum (● males, ○ females) or minimum (■ males, □ females) facultative cooling in treatment 3. The individual male whose temperature had previously fallen lowest during treatment 3 is shown separately (· · · ·). All the sheep were shorn. The ambient temperature was lowered from +30 to 8° C in the first 2 hr; then remained at +8° C for 3 hr before being lowered to +2° C in the final hour.

Since these hypothermic sheep had previously shown body cooling during the cold shocks in treatment 3 and during the initial phase of the first and second acute cold exposures, correlations of repeatability could be calculated for the degree of hypothermia shown within individuals on each occasion. Of twenty correlations, all were positive and varied from $+0{\cdot}02$ ($P > 0{\cdot}1$) to $+0{\cdot}89$ ($P < 0{\cdot}001$), indicating variable but positive repeatability. Some individual sheep showed very consistent facultative cooling. On average, the sheep selected previously for maximum cooling response to cold shocks showed lower rectal temperatures during slow cooling than those selected for minimum response ($P < 0{\cdot}001$, sexes combined).

Heart rate. Mean heart rates during slow cooling are shown in Fig. 5. The differences between treatment groups paralleled the differences in rectal temperature (Fig. 4). Cold-acclimatized sheep initially had the highest heart rates, controls came next and the sheep previously conditioned by cold shocks showed the lowest heart rates. Heart rates of the acclimatized sheep remained high during cooling whilst those of the controls rose to a

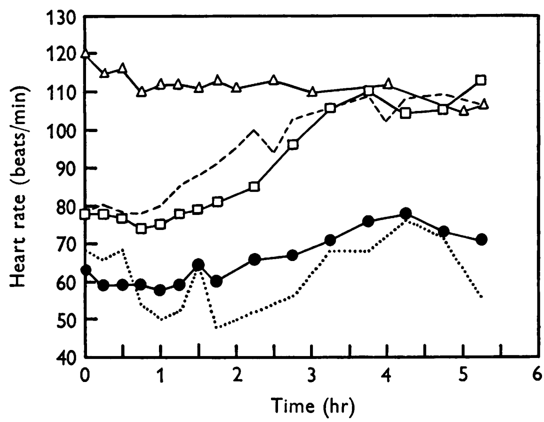

Fig. 5. Mean heart rate during slow cooling tests in sixteen previously untreated control sheep (– – –), four cold-acclimatized sheep from treatment 2 (△) and eight sheep which had received cold shocks in treatment 3 (● males, □ females). The individual male whose temperature had previously fallen lowest during treatment 3 is shown separately (· · · ·). All the sheep were shorn. The ambient temperature was lowered from + 30° C to + 8° C in the first 2 hr; then remained at + 8° C for 3 hr before being lowered to + 2° C.

similar level. The cold shock-treated sheep generally showed later and smaller heart rate responses to cold. Initial treatment group differences at + 30° C were significant ($P < 0{\cdot}001$). Below 12° C, heart rates of the previously cold-shocked sheep became significantly ($P < 0{\cdot}001$) lower than the controls. The individual whose rectal temperature fell to 35·7° C showed periodically very low heart rates between 48 and 50 beats/min. His lowest rectal temperatures tended to coincide with low heart rates, but whenever rectal temperature fell below 36° C heart rate increased until the temperature rose again.

It was provisionally concluded that heart rates during cooling were approximately proportional to metabolic rate and to the level of body temperature maintained. Thus, the cold acclimatized sheep maintained relatively high metabolic rates and high body temperatures at all ambient temperatures. Controls maintained moderate resting metabolic rates which were readily increased in response to cooling in order to maintain body temperature. Sheep which had received cold shock treatment were more variable in response but generally maintained lower metabolic rates and permitted lower body temperatures in the cold.

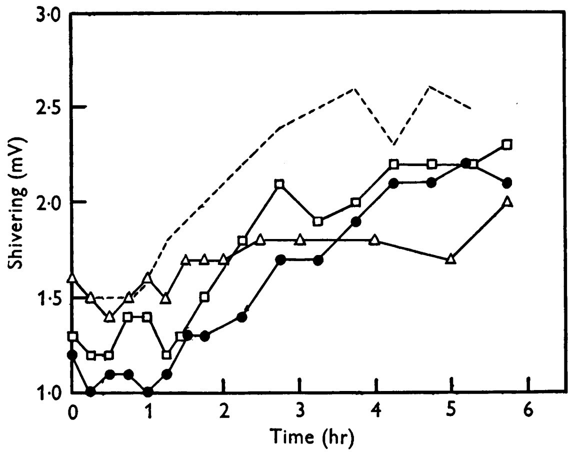

Fig. 6. Mean shivering intensity (electromyograph) during slow cooling tests in sixteen previously untreated control sheep (– – –), four cold-acclimatized sheep from treatment 2 (△) and eight sheep which had received cold shocks in treatment 3 (● males, □ females). Electrical activity at + 30° C is taken to indicate muscle tone. All the sheep were shorn. Ambient temperature was lowered from + 30 to + 8° C in the first 2 hr; then remained at + 8° C for 3 hr before being lowered to + 2° C in the final hour.

Shivering intensity. Mean shivering intensities are shown in Fig. 6. The cold-acclimatized sheep (all females) shivered less than the controls despite their higher metabolic rates (inferred from heart rates). This supports previous evidence from the acute cold exposures that female Scottish Blackface sheep may develop some degree of non-shivering thermogenesis after cold acclimatization. The sheep conditioned by cold shocks shivered less than the controls ($P < 0{\cdot}02$ to $P < 0{\cdot}001$, sexes combined), as expected from their lower heart rates (Fig. 5) and putatively reduced metabolic response to cooling.

To summarize, facultative body cooling during exposure to + 8° C was induced by previous cold shocks. This response was variable between sheep but individual differences tended to be repeatable. Facultative cooling was associated with low shivering intensity and low heart rates in the cold.

Skin temperature

Sykes & Slee (1968, 1969*b*) showed that acclimatization to cold raises skin temperature and delays vasoconstriction on the feet and ears. In the present experiment foot and ear skin temperatures were used to determine the occurrence of vasoconstriction during cooling. The treatment 2 sheep vasoconstricted on average at a lower ambient temperature than treatment 1 sheep (23·3° C *vs.* 27·4° C; $P < 0{\cdot}01$). Vasoconstriction in treatment 3 sheep occurred at 26·6° C, showing no significant difference from treatment 1. This indicated that the chronic cold of treatment 2 had produced an effect consistent with acclimatization, whereas the cold shock treatment had no such effect.

Body skin temperatures were not significantly affected by treatment but there was apparently an effect of sex. Average male midside skin temperatures were higher than in the females, the difference being 2–4° C at ambient temperatures between 15 and 0° C ($P < 0{\cdot}01$). The largest differences occurred during the later cold exposures when the males had lost most weight and appeared thinner than the females. Presumably the males had poorer insulation and therefore higher rates of heat loss.

DISCUSSION

The results may be summarized as follows.

(i) Acute cold exposure increased subsequent resistance to cooling, probably by increasing peak metabolic capability.

(ii) Conditioning to chronic cold generally increased cold resistance and also increased heart rates at thermoneutral temperatures and during mild cooling. These changes implied an elevated resting metabolic rate.

(iii) Unlike acute and chronic cold exposure, conditioning to daily short cold shocks promoted facultative body cooling. The initial responses to cold in terms of heart rate and shivering were reduced by cold shock treatment, suggesting that the normal increase in metabolism in the cold was partially inhibited and that lowered body temperatures resulted accordingly. It was concluded that exposure to acute cold and chronic cold produced acclimatization, as defined here. Cold shocks, in contrast, induced a different form of physiological adaptation.

In so far as the cold shock treatment caused a progressive reduction in the physiological (metabolic) response to a repeated stimulus (daily cold shocks) it seems similar to the phenomenon of habituation. In man, reduced blood pressure and heart rate responses due to habituation following repeated hand immersions in cold water were described by Glaser & Whittow (1957). Glaser & Griffin (1962) showed that rats could habituate to daily whole body immersions in cold water by reducing heart rate during successive days of treatment. On the evidence of Glaser (1966) and Hensel & Hildebrandt (1964) it seems reasonable to interpret the present results in sheep as a form of habituation to whole body cold exposure.

The results of habituation to repeated cold shocks were clearest during subsequent slow cooling to + 8° C when some rectal temperatures fell below 37° C. Hypothermia was facultative since further lowering of room temperature to + 2° C produced increases in rectal temperature. The lowest rectal temperatures were about 1° C below the minimum deep body temperature reported by Bligh, Ingram, Keynes & Robinson (1965) for a sheep (in fleece) exposed outdoors for 1 year to ambient temperatures ranging between − 16 and + 26° C. As Bligh & Harthoorn (1965) have pointed out, the sheep is usually among the most stable of thermoregulating species. However, in several habituated sheep kept at + 8° C in the present experiment, rectal temperatures, having fallen from about 39·5° C, tended to cycle between 36 and 37° C with concomitant decreases or increases in heart rate. The impression was that the set-point for body temperature had been lowered 2–3° C from the normal 39° C and that a coarse system of control was operating. Hardy (1961) and Bligh (1966) have postulated a temperature control system (with regulatory responses proportional to the deviation from set-point) incorporating peripheral and central receptors with both 'broad-band' and fine control. On this hypothesis, the body temperatures of the habituated sheep may have been under 'broad-band' control. Perhaps the hypothalamus was operating at a lowered set point and responding only to relatively large changes in blood temperature, while the drive from peripheral receptors, normally giving early response and fine control, was neutralized by habituation. The fact that vasoconstriction on the extremities occurred at the normal time during cooling of habituated sheep suggests that peripheral receptors were alerted and implies that habituation was imposed at the level of the central receptors. Moreover, since the habituated sheep vasoconstricted normally but showed a reduced metabolic response to cold, it appears that habituation involved those central nervous sites which normally instruct the metabolic responses to cold but not the vasomotor response. Although acclimatization to cold is a predictable and consistent response in sheep, habituation responses seemed variable between individuals, as might be expected if the central nervous system was involved.

In this experiment there were sex differences in response, males showing lower cold resistance than females and less acclimatization. But the ability to develop facultative body cooling with reduced metabolic response to cold seemed greater in males. Both sexes received similar rations. The males grew faster than the females but lost almost three times more weight during cold treatment and so became thinner. This could account for their reduced skin insulation (evidenced by higher body skin temperatures during cooling), and may account for some of the sex differences in response to cold. Nevertheless, the very low metabolic response to mild cooling found in some males after habituation cannot be explained in these terms and may represent an inherent sex difference.

The general conclusion is that the kind of physiological adaptation produced by cold exposure varies according to the mode of administration and the dosage of cold. Acute exposure increases cold resistance. Chronic exposure also increases cold resistance and raises the resting metabolic rate. Exposure to fluctuating cold outdoors causes increased resistance to cooling with a relatively smaller metabolic effort (Webster *et al.* 1969). Repeated short cold shocks do not increase cold resistance but tend to induce habituation involving facultative body cooling and a decreased metabolic response to cold. Habituation is erased by severe, acute cold exposures which produce antagonistic effects involving increased metabolic response to cold and decreased rates of body cooling. These different types of cold adaptation presumably affect heat loss and the efficiency of energy utilization during cold exposure.

It is possible that the form of adaptation resulting from each type of cold exposure depends upon the type of immediate physiological response elicited by that exposure. For example, in this experiment, the cold shocks (which involved a steady decline in ambient temperature from +30 to −10° C with wind throughout) and the acute cold exposures (involving a slow decline from +30 to −20° C with wind suddenly introduced at 0° C) not only produced different forms of adaptation subsequently, but also caused different physiological responses at the time of exposure. Fig. 1 shows that, even on the first day of cold shocks, rectal temperatures fell steadily throughout the period of cold exposure. But during acute cold exposures (Fig. 2), rectal temperatures having first fallen slowly, then rose sharply by about 1° C when the wind was applied.

Presumably this rise resulted from an increased metabolic rate associated with the sudden intensification of cold stress on sheep which were already slightly hypothermic. This type of response was followed subsequently by acclimatization, involving the retention for some time afterwards of elevated metabolism and increased metabolic response to cold. In contrast, the relatively low metabolic response and readily falling rectal temperatures associated with cold shocks were associated with habituation, which involved a progressive consolidation of this type of diminished metabolic response to cold. It seems, therefore, that the type of cold exposure determines the immediate physiological response, and possibly, this affects the form of subsequent adaptation. Such a situation would be analogous to the stimulus–response relationships involved in some types of learning.

The conclusion that the type of cold exposure determines the type of physiological adaptation induced can be related to certain observations in man. Early work produced no clear evidence for human cold acclimatization (Adolph & Molnar, 1946 and Horvath, Freedman & Golden, 1947). Later, two main types of acclimatization were reported. In the first instance, the ability to maintain deep body temperature in the cold was increased (Glaser, 1950; Budd, 1962, 1964). This was not accompanied by changes in shivering and heart rate (Budd, 1964; Budd & Warhaft, 1966) and therefore paralleled the type of acclimatization following acute cold exposure in sheep. Other experiments produced a different type of acclimatization during which rectal temperatures in the cold *decreased* progressively and probably facultatively (Leblanc, 1956; Davis, 1963). Shivering and heat production in response to cold also decreased in some experiments (Davis, 1963; Girling, 1967). These adaptations seem similar to the process termed habituation in the present studies with sheep. The other type of adaptation described here after chronic cold exposure in sheep and also previously in birds (Gelineo, 1934) and rodents (Hart, 1957), involves increased resistance to body cooling with increased resting metabolism and elevated skin temperatures. This syndrome has not yet been demonstrated in man, where the cold exposures have generally been discontinuous or fluctuating. However, in one case at least (Glaser, 1950), where there was continuous cold exposure for 3 days, elevated skin temperatures were reported.

In man, as in sheep, therefore, the type of adaptation may vary with the type of cold exposure. Since experiments with man have differed widely in terms of the length, severity and frequency of cold exposures and the amount of clothing worn, it would seem, by analogy with the sheep, that differences of methodology are sufficient to explain the variations in response (at least within the same racial types).

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