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SUMMIT METABOLISM AND CARDIOVASCULAR FUNCTION IN YOUNG LAMBS DURING HYPEROXIA AND HYPOXIA

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SUMMARY

1. Exposure of lambs at sea level to air containing more than 33% oxygen resulted in a small increase in summit metabolism (maximum oxygen consumption on severe cold exposure) in some lambs, and in an improvement in the ability to maintain summit metabolism in others; there was a concomitant improvement in the maintenance of cardiac output, but no change in the degree of right to left shunting.

2. Hypoxia (12–13% oxygen in inspired air) reduced summit metabolism of new-born lambs by 25%; but there was no effect in lambs 6–11 days old. Hypoxia neither reduced cardiac output, nor affected the degree of right to left shunting.

3. The results suggest that in very young lambs there is some limitation of summit metabolism imposed by the supply of either oxygen or substrate to thermogenic tissues.

INTRODUCTION

In the sheep and other mammals, establishment of normal pulmonary function and circulation of the adult type is not complete until many days after birth (Dawes, review, 1968); and in new-born lambs the arterial oxygen saturation is only about 93% (Cross, Dawes & Mott, 1959; Alexander & Williams, 1970). It seemed likely, therefore, that the maximum metabolic response of lambs to cold (summit metabolism) would be limited by the supply of oxygen to the thermogenic tissues; the results of Thompson & Moore (1968) suggested that this may be so in the new-born rat. A study was therefore made of the effects of exposing lambs to oxygen-enriched air. When this treatment was found to increase summit metabolism, the study was extended to determine whether the results could be explained in terms of oxygen supply to thermogenic tissues, cardiac output, or size of shunts through the foramen ovale or ductus arteriosus; oxygen might be expected to close a still patent ductus (Kovalcik, 1963).

This and an associated study of the effects of hypoxia are presented below. Observations on metabolic and cardiovascular parameters in lambs breathing normal air during the control periods of these experiments are presented in another paper (Alexander & Williams, 1970).

METHODS

Source of data. The data presented below are drawn from two series of experiments (Series 1 and 2) on summit metabolism, in the Merino lambs described previously (Alexander & Williams, 1970). *Series* 1 was the initial study and was concerned primarily with the metabolic effects of increased or decreased oxygen tension in the inspired air. *Series* 2 was an extension of the observations in Series 1.

Apparatus and procedures. The preparation of the animals, the closed circuit apparatus and related procedures for the measurement of *summit oxygen consumption* have already been described together with the data for the initial control periods (Alexander & Williams, 1970). After each control period, the cold chamber, containing the lamb, was opened and warm air was blown in from above to allow rectal temperature to return to normal. Twenty to 30 min later the chamber was closed again, and oxygen concentration in chamber air was allowed to remain unaltered (sham experiments) or was increased or decreased by admitting pure oxygen or pure nitrogen, for 4 or more minutes; these procedures produced chamber oxygen concentrations of 32–54 % and 12–13 % respectively (i.e. approximate oxygen partial pressures of 240–410 and 95 mm Hg). A further 4 min, at least, was allowed for equilibration before measurements of summit metabolism and other parameters were made during two or three consecutive periods ('test' periods) of 10 or 15 min each; in Series 2 there were two periods of 15 min in each experiment. Carbon dioxide expired during equilibration was included in the total collection, so it was not possible to estimate the respiratory quotient accurately. In some experiments in Series 1, the above procedure was reversed and the test period preceded the control period. Oxygen content of air in the chamber during control periods was 20–21 %.

Cardiac output and cardiovascular shunts. Cardiac output and the magnitude of R-L and L-R shunts were estimated by dye dilution techniques, and cardiac output was also estimated from the oxygen consumption by the Fick method; these methods were described in another paper.

Analytical procedures, blood and tissue sampling. Blood oxygen saturation and haemoglobin level were determined as described previously (Alexander & Williams, 1970). The concentration of *glucose* in plasma was determined by the glucose oxidase method of Huggett & Nixon (1957), and the plasma *lactate* concentration was estimated by the enzymatic method of Lundholm, Mohme-Lundholm & Vamos (1963). Blood for these determinations was collected from the posterior vena cava or pulmonary artery during the first half of each $\frac{1}{4}$ hr measurement period in Series 2. The blood was stored at 0° C and the plasma was separated at the completion of the experiment.

Glycogen was estimated in samples of cardiac muscle taken from lambs at the completion of the test period; the lambs were killed by an overdose of pentobarbitone sodium while still exposed to cold, and about 1 g of the tip of the left ventricle was taken into 30 % potassium hydroxide between $1\frac{1}{2}$ and 4 min after death. Digestion, extraction, precipitation and hydrolysis of glycogen were done by modifications of the method of Good, Kramer & Somogyi (1933) and the glucose was estimated as above.

Analysis of results. The mean values of the various parameters were calculated for

each 10 or 15 min period, and these means were then used to calculate the means for the whole of the control period and whole of the test period. Conventional methods were used for statistical examination of these latter means.

RESULTS

Summit metabolism

(i) *Effect of oxygen-enriched air.* In *Series* 1 summit metabolism was elevated by exposure of lambs to 33–46 % oxygen (Fig. 1, Table 1) in nine of ten experiments on lambs less than 3 days old; the mean increase was 0·56 l. O_2/kg.hr (14 % of the mean control value). The effect generally declined with increasing age (twenty-nine experiments on thirteen lambs aged from 3 hr to 22 days). In the twenty-one experiments where control

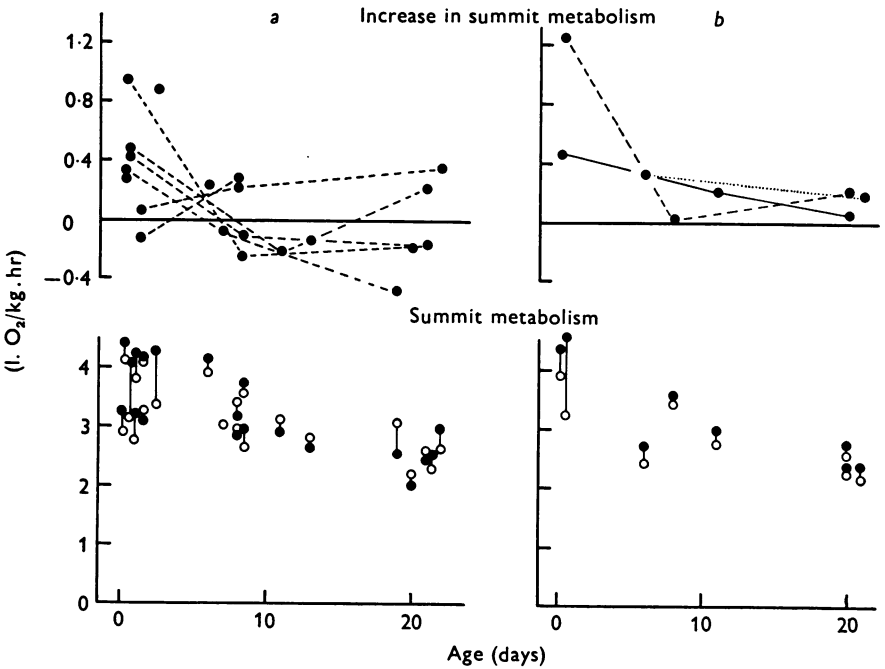

Fig. 1. Changes in summit metabolism due to breathing 32–46 % oxygen in lambs of different ages (Series 1).

a. Experiments where control measurements preceded exposure to oxygen-enriched air; the dashed lines connect data from the same animal.

b. Experiments on three lambs (indicated by dotted, dashed and a continuous line) where control measurements followed exposure to oxygen-enriched air.

The lower graphs show the 'control' (○) and the 'test' values (●) of summit metabolism; the upper graphs show the difference in summit metabolism (test minus control) ascribed to the treatment.

measurements were made before exposure to high oxygen concentrations, the elevation of summit metabolism was significantly greater in lambs less than 3 days old than in the same lambs 6–11 or 13–22 days old ($P < 0.05$, in analysis of variance). There appeared to be a real response to oxygen in only a few lambs older than 3 days (Fig. 1). In the lambs less than 3 days old, there was no evidence that the response to oxygen increased with increasing oxygen concentration within the range of 33–46 %.

TABLE 1. Effect of breathing various concentrations of oxygen on summit metabolism

	Age	% O_2 in test period	No. of lambs	Mean summit metabolism* (l. O_2/kg.hr) Control	Test minus control
Series 1†	3–60 hr	33–46	8	3·44	+0·41
				(0·19)	(0·13)
	6–11 days	32–45	7	3·24	+0·02
				(0·16)	(0·09
	13–22 days	40–45	6	2·61	−0·09)
				(0·13)	(0·11)
	6–60 hr	12–13	5	3·73	−0·84
				(0·13)	(0·12)
	6–11 days	12–13	4	3·05	−0·22
				(0·32)	(0·07)
Series 2	6–24 hr	20–21	8	3·64	−0·49
				(0·10)	(0·05)
	6–48 hr	36–54	11	3·78	+0·01
				(0·09)	(0·12)
	6–30 hr	12–13	5	3·93	−1·10
				(0·18)	(0·32)
	21–28 days	20–21	4	2·51	−0·21
				(0·06)	(0·06)
	24–27 days	36–40	4	2·84	−0·24
				(0·05)	(0·13)

* S.E. of mean in brackets.

† Experiments where the test period preceded the control periods are excluded.

In *Series* 2, summit metabolism was elevated by exposure to oxygen enriched air in only six of eleven lambs less than 2 days old. While this group of lambs maintained a mean summit metabolism during the test period of only 0·01 l. O_2/kg.hr above that in the control period (Table 1), there was a mean fall of 0·49 l. O_2/kg.hr in untreated lambs of a similar age. This difference between the two groups was significant ($P < 0.02$ in t test).

The range of oxygen concentration used in most lambs of Series 2 was too narrow to provide data on the relationship between oxygen concentration and elevation of summit metabolism.

In conformity with Series 1 there was no response to oxygen in lambs 3–4 weeks old (Table 1). The mean difference in summit metabolism between the control and test period in these older lambs was similar to that in the control experiments of Alexander & Williams (1968) (approximately 0·2 l. O_2/kg.hr).

In Series 2, lambs were subjected to considerably more interference in the form of cardiac catheterization, than in Series 1; catheters were known to lie or pass through the heart in thirteen lambs less than 2 days old in Series 2. However, mean summit metabolism during the control period (3·64 l. O_2/kg.hr) was not significantly different ($P \simeq 0{\cdot}07$) from the mean in seven similar lambs (3·94 l. O_2/kg.hr) in which catheters were known not to reach the heart. Only two of six cardiac catheterized lambs responded to oxygen with an increase in summit metabolism, compared with the four of five remaining lambs, but this difference did not approach statistical significance.

In the untreated lambs of Series 2 the intensity of shivering, as judged from the electromyogram, tended to be less during the sham test period than during the initial control period (mean test minus mean control value in arbitrary units was $-0{\cdot}54 \pm 0{\cdot}28$). There was a tendency for oxygen treatment to reduce this decline (the corresponding mean difference was $+0{\cdot}14 \pm 0{\cdot}24$), but the difference between these values was not significant ($0{\cdot}1 > P > 0{\cdot}05$). There was no obvious trend in Series 1.

(ii) *Effect of* 12–13 % *oxygen.* Summit metabolism in Series 1 and 2 was reduced by about 25 % when lambs less than 3 days old were exposed to 12–13 % oxygen (Table 1), but in lambs 6–11 days old (Series 1) the reduction was small and no greater than expected from sham experiments of Alexander & Williams (1968).

Arteriovenous oxygen saturation differences

(i) *Effect of oxygen-enriched air.* Mean arterial saturation in lambs of all age groups (Table 2) exposed to air containing at least 32 % oxygen was elevated by approximately 4 % above that in the control period of exposure to 20–21 % oxygen. Venous saturation and a–v (arteriovenous) differences were also elevated in most lambs; the mean a–v increase in lambs less than 3 days old was 2·5 %.

(ii) *Effect of* 12–13 % *oxygen.* Exposure to low atmospheric oxygen tension (Series 2) reduced arterial oxygen saturation by a mean of 20 %; venous saturation was reduced by a mean of only 9 % and the a–v difference was reduced by 11 % (Table 2).

TABLE 2. Effect of breathing various concentrations of oxygen on oxygen saturation of blood in lambs exposed to extreme cold

	Age	% O_2 in chamber during test period	No. of lambs	% saturation of blood with oxygen*: Arterial: Control	Arterial: Test minus control	Venous: Control	Venous: Test minus control	a-v difference: Control	a-v difference: Test minus control
Series 1†‡	7–30 hr	33–46	4	93·5	+3·5	23·5	+1·0	70·0	+2·5
				(2·0)	(1·0)	(5·1)	(3·5)	(3·5)	(2·5)
	6–11 days	32–45	8	90·8	+5·9	25·2	+4·8	65·6	+1·1
				(3·0)	(1·9)	(3·3)	(2·3)	(2·8)	(2·3)
Series 2	6–48 hr	20–21	8	92·8	+0·8	32·1	+0·1	60·7	+0·7
				(0·9)	(0·8)	(3·5)	(0·4)	(4·1)	(0·7)
		36–54	11	90·6	+5·8	30·9	+3·3	59·7	+2·5
				(0·8)	(0·4)	(2·4)	(1·2)	(2·2)	(1·1)
		12–13	5	91·4	−20·4	29·8	−8·8	61·6	−11·6
				(1·0)	(3·1)	(2·9)	(3·1)	(2·4)	(3·3)
	21–28 days	20–21	4	90·3	+0·5	32·8	−1·5	57·5	+2·0
				(1·6)	(1·2)	(4·6)	(5·0)	(4·4)	(4·0)
		36–40	4	89·5	+5·0	32·3	+1·5	57·2	+3·5
				(1·0)	(1·4)	(7·0)	(2·5)	(6·0)	(2·3)

* S.E. of mean in brackets.

† The only two animals examined in the 13–22 day-old group of Series 1 are omitted.

‡ Experiments where the test period preceded the control period are excluded.

Cardiac output

(i) *Effect of oxygen-enriched air.* In each of eight control lambs, less than 2 days old (Series 2) cardiac output (per unit of body weight), measured by either method, was lower during the sham test than during the control period; the mean difference (test minus control) was -7 ± 1 ml./100 g.min by both methods, i.e. about 10% of control values, and was significantly different from zero ($P < 0{\cdot}01$). In contrast, some lambs given oxygen-enriched air to breathe showed an increase in cardiac output associated with the treatment (six of eleven lambs by the Fick method and four of eleven by the dye method). The mean difference (test minus control) of $2 \pm 2{\cdot}4$ ml./100 g min determined by the Fick method in these lambs was significantly greater than the mean differences of $-7 \pm 1{\cdot}2$ ml./100 g.min in the control animals ($P < 0{\cdot}02$ by t test). Corresponding differences determined by the dye dilution method showed the same trend ($-3 \pm 2{\cdot}0$ and $-7 \pm 1{\cdot}0$ ml./100 g.min) but these values were not significantly different.

Exposure to oxygen-enriched air was without effect on cardiac output in lambs 3–4 weeks old.

(ii) *Effect of* 12–13% *oxygen.* When lambs less than 2 days old were exposed to 12–13% oxygen (Series 2) the mean cardiac output, measured by either method, declined by about the same amount (approximately 6 ml./100 g.min) as in the control group; similarly in lambs 3–4 weeks old.

Shunts through foetal channels

(i) *R-L shunts.* There was no major effect of exposing lambs to more than 36% oxygen or to 12–13% oxygen in air (Table 3). However, the size of the shunt declined in seven of ten lambs when treated with oxygen-enriched air, compared with three of seven lambs in the control group and there was a mean decline in the treated lambs compared with a mean increase in the control lambs (Table 3). The differences were not significant. No shunts were observed in lambs 3–4 weeks old.

(ii) *L-R shunts.* Of the five lambs examined during exposure to oxygen-enriched air, only two showed a L-R shunt, and in these there was no evidence of any change in L-R shunting due to the treatment; the shunts were 29 and 24% of left ventricular output during the control and test period respectively in one lamb, and 7 and 4% in the other. No lamb exposed to oxygen-depleted air was successfully examined.

Heart rate

Heart rate was unaffected by administration of oxygen, or by exposure to oxygen-depleted air.

Blood pressure

Exposure to oxygen-enriched air significantly reduced the fall in blood pressure usually seen in untreated lambs during exposure to summit conditions for two consecutive ½ hr periods (Table 4). There was no significant effect of 12–13 % oxygen on systemic pressure.

TABLE 3. Effect of variation in oxygen content of inspired air upon size of shunt through foramen ovale, in lambs, 6–48 hr old, exposed to extreme cold

% O_2 in chamber during test period	No.* of lambs	Mean size of shunt† (% of blood flow through posterior vena cava)	
		Control	Test minus control
20–21	7	4·0‡	+5·3
		(1·8)	(4·9)
36–54	10	15·5	−4·5
		(4·2)	(3·1)
12–13	4	21·3	−0·3
		(1·8)	(7·2)

* Two lambs given 20–21 % oxygen, and one lamb given 36–54 % oxygen showed no evidence of shunting through the foramen ovale.

† S.E. of mean in brackets.

‡ This comparatively low control value is apparently the result of random selection, and is not due to any difference in the treatment of the animals.

There was no clear effect of increased oxygen percentage on pulmonary arterial pressure (Table 4); pulmonary arterial pressure decreased slightly during the experiments in all of six lambs less than 2 days old, given oxygen, and in three out of four breathing air. However, pulmonary arterial pressure increased in each of two lambs given 12–13 % oxygen, and this difference (one increase in ten lambs compared with two in two lambs) is significant ($P < 0{\cdot}045$).

Effect of breathing oxygen-enriched air on glucose and lactate concentration in plasma and on cardiac glycogen (Series 2)

(i) *Glucose.* As reported previously, plasma glucose levels were high during summit metabolism and often exceeded 200 mg/100 ml. (Alexander, Mills & Scott, 1968). The decrease seen in sham test periods was not affected significantly by exposure to oxygen-enriched or oxygen-depleted air (Table 5).

(ii) *Lactate.* Lactate concentration in plasma was also high during summit metabolism (see also Alexander *et al.* 1968), but relative to the control group the concentration was increased by exposure to oxygen-depleted air and decreased by oxygen-enriched air (Table 5).

TABLE 4. Arterial blood pressure in lambs exposed to variations in oxygen content of inspired air during summit metabolism

Source of data	Age	% O_2 in chamber during test period	Mean systemic blood pressure* (mm Hg)			Mean pulmonary blood pressure* (mm Hg)		
			No. of lambs	Control	Test minus control	No. of lambs	Control	Test minus control
Alexander & Williams (1968)	6 hr–12 days	20–21	6	79 (3·7)	−6 (1·0) †	—	—	—
Series 1	3–6 hr	33–46	6	72 (3·5)	0 (1·9) †	—	—	—
	6–11 days	32–45	6	95 (1·5)	−1 (2·9)	—	—	—
Series 2	6–48 hr	20–21	8	80 (3·9)	−7 (1·5) †	4	31 (4·1)	−2 (2·5)
		36–54	11	76 (3·3)	−3 (1·0) †	6	26 (4·8)	−4 (1·0)
		12–13	5	77 (3·9)	−4 (1·9)	2	31 (7·5)	+9 (6·5)
	21–28	20–21	4	96 (2·5)	−2 (3·1)	4	13 (2·9)	+1 (2·4)
		36–40	3	101 (3·8)	0 (0·9)	2	14 (0·5)	0‡ —

* S.E. of mean in brackets.
† Significance of difference, $P < 0·05$.
‡ One value only.

TABLE 5. Plasma glucose and lactate and cardiac glycogen in lambs exposed to extreme cold and to variations in oxygen content of inspired air (Series 2)

Age	% O_2 in chamber during test period	Mean concentration of metabolites in plasma* (mg/100 ml.)						Cardiac glycogen* (mg/g)		
		Glucose			Lactate					
		No. of lambs	Control	Test minus control	No. of lambs	Control	Test minus control	No. of lambs	Mean	
6–48 hr	20–21	5	231 (15·6)	−30 (8·1)	6	86 (8·8)	−11 (7·1) †	6	16 (3·6)	n.s.
	36–54	4	159 (28·6)	−31 (10·8)	5	93 (6·5)	−25 (3·0) ‡	6	11 (2·6)	
	12–13	4	207 (27·1)	−11 (12·2)	5	86 (9·3)	+15 (4·4)	1	13 —	
21–28 days	20–21	—	—	—	—	—	—	3	15 (1·1)	
	36–40	—	—	—	—	—	—	4	15 (2·1)	

* S.E. of mean in brackets.

† $P = 0{\cdot}04$ (one tailed t test since difference was in direction expected on physiological grounds).

‡ $P < 0{\cdot}01$ in analysis of variance.

n.s. not significant.

(iii) *Cardiac glycogen.* The concentration in heart muscle, collected at the end of experiments on summit metabolism, varied from 3 to 32 mg/g wet wt., but the levels tended to be lowest in the youngest lambs. The mean concentration in lambs exposed to oxygen-enriched air was below the mean in control lambs (Table 5) but the difference was not significant.

DISCUSSION

The initial experiments (Series 1) suggested that summit metabolism was increased by exposure of young lambs to oxygen-enriched air. The result was substantiated in Series 2, but only to the extent that exposure to more than 36% oxygen prevented the decline in summit metabolism that occurred in the sham test periods; however, the extensive catheterization may have reduced the response. The response to oxygen is consistent with the hypothesis that summit metabolism is limited by the supply of oxygen to thermogenic tissues; the increase of only 2·5% in the a–v oxygen saturation difference (Table 2), as opposed to the 4% increase in arterial saturation that accompanied the response, is to be expected since much of the venous blood would drain from tissues without specific thermogenic function. However, interpretation of the result is made difficult by the concomitant relative increase in cardiac output, which, in addition to augmenting oxygen transport, could improve the carriage of metabolites that might limit summit metabolism. For example, the treatment could increase the transport of non-esterified fatty acids from adipose tissue to muscle (Renold, Crofford, Stauffacher & Jeanrenaud, 1965); lipid infusion has been observed to increase summit metabolism in young lambs (Alexander, 1969).

A small increase in the summit or near-summit metabolic response to cold has also been reported in young rats exposed to 50% oxygen (Taylor, 1960); but the response increased with age, in contrast to the effect in lambs. The stimulation by oxygen was thus not due to inefficient pulmonary function either in rats (Taylor, 1960) or in lambs, since arterial oxygen saturation showed no consistent increase with age of lambs (Table 2) while the effect of oxygen decreased with age. In both studies there was an indication that oxygen sustained or increased the intensity of shivering, and indeed the decrease in blood lactate concentration (Table 5) suggests more complete utilization of glycogen by muscle in lambs treated with oxygen than in control lambs, but no data were obtained on muscle glycogen. Alternatively, oxygen may exert a sparing effect on glyco-genolysis by allowing muscle to utilize fat (Masoro, 1966), at an increased rate.

The effect of oxygen on cardiac output is puzzling, particularly as cardiac output was not affected by hypoxia. Oxygen did not appear to

accelerate glycogen utilization by heart muscle (Table 5) which is consistent with the findings of Evans (1934) that the increased cardiac output during exercise is not accompanied by a decrease in cardiac glycogen, at least in the adult rat. An improved maintenance of cardiac output would result from a small reduction in the amount of blood shunted through the ductus arteriosus, since oxygen might be expected to close a still-patent ductus (Kovalcik, 1963), but no reduction was observed. Oxygen might also be expected to increase the output of the left heart by decreasing pulmonary vascular resistance (Dawes, review, 1968) but oxygen had no clear effect on shunting via the foramen ovale, as would then be expected, and there was no evidence that increased oxygen in inspired air reduced pulmonary arterial pressure (Table 4).

Exposure of new-born lambs to 12–13 % oxygen reduced summit metabolism by only 25 %. More marked reductions in the metabolic response to cold during hypoxia have been reported in neonates and adults of other species (Hill, 1959). For example, Hill showed that kittens responded to 10 % oxygen at 26° C, by a 50 % reduction in metabolic rate. Similarly, Heim & Hull (1966) showed that in the new-born rabbit the metabolic response of brown adipose tissue to noradrenaline was almost halved by hypoxia (10 % oxygen). However, shivering, too, may be suppressed by hypoxia since cold-induced thermogenesis is reduced by hypoxia in the adult guinea-pig (Hill, 1959) which lacks brown adipose tissue (Brück & Wünnenberg, 1965). Thus it remains to be determined whether the reduction of summit metabolism due to hypoxia in neonatal lambs is due to a reduction in thermogenesis in brown fat, which supplies about 30 % of summit metabolism, or to a reduction of shivering which supplies about 50 % of summit metabolism (Alexander & Williams, 1968). Certainly in 3 to 4-week-old lambs, which lack brown fat, no reduction of shivering thermogenesis is observed in hypoxia (Table 1).

The depression of thermogenesis by hypoxia was not due to a reduction in cardiac output, but presumably to a decrease in the availability of oxygen to the thermogenic tissues, since the decline in saturation of venous blood due to hypoxia was considerably less than the decline in arterial saturation. The arterial oxygen saturation of about 70 % during hypoxia (Table 2) is considerably higher than the corresponding figure of 50 % indicated by data of Cross *et al.* (1959) for lambs breathing 12–13 % oxygen, but their lambs were anaesthetized and showed abolition of the thermogenic response, in contrast to the conscious lambs in the present experiments.

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