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CARDIOVASCULAR FUNCTION IN YOUNG LAMBS DURING SUMMIT METABOLISM

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SUMMARY

1. Cardiac output in lambs less than 3 days old was 50–100 % higher during summit metabolism than during exposure to thermoneutral conditions.

2. The Fick method, based on oxygen consumption, and the dye dilution method for determination of cardiac output, gave qualitatively similar results, but there were considerable quantitative discrepancies.

3. The thermogenic tissues, muscle and brown fat, extracted some 80 % of the oxygen from the blood perfusing them during summit metabolism.

4. Significant right to left shunts through the foramen ovale were detected in about half of the lambs during the first few days of life; the size and incidence of shunts decreased with advancing age but were apparently independent of environmental conditions.

5. During exposure to extreme cold, left to right shunts through the ductus arteriosus were detected in a high proportion of lambs, < 12 hr old, with detectable foramen ovale shunts. Pressure measurements in pulmonary and systemic arteries in very young lambs indicated that right to left shunting via the ductus was unlikely to occur; none was detected.

6. Summit metabolism was poorly related to oxygen carrying capacity of blood, but significant correlations of summit metabolism with cardiac output (positive) and with oxygen saturation of mixed venous blood (negative) were observed, though not consistently.

7. It is concluded that the great variability of summit metabolism between lambs older than 6 hr is not readily explained in terms of cardiovascular phenomena, but that the quantity and efficiency of thermogenic tissues probably play an important role in limiting summit metabolism.

INTRODUCTION

At birth, sheep (*Ovis aries* L.) have well developed mechanisms for generating heat. In the lamb exposed to cold within minutes of birth, heat production (oxygen consumption) can increase to several times the basal rate, by both shivering and non-shivering mechanisms, but the increase is very variable, ranging from $2\frac{1}{2}$ to 5 times the basal metabolic rate of about 1 l. oxygen/kg.hr (Alexander, 1962*a*; Alexander & Williams, 1968). Reasons for this wide variability in the maximum metabolic response to cold (*summit metabolism*) are unknown and do not appear to be associated with birth weight, pre-natal nutritional history of the ewe or with litter size (Alexander, 1962*a*). Also, there is no information to define the physiological or biochemical processes which are limiting factors in the intense demands that are made upon the lamb during summit metabolism. It seemed reasonable, therefore, to investigate whether the variability in summit metabolism could be explained in terms of cardiovascular function. Considerably more is known about cardiovascular function during intense exercise than during exposure to extreme cold, both of which result in considerable metabolic effort (see review by Horvath & Howell, 1964). Very little is known about the effects of severe cold on the circulation of the new-born animal, although there are some data on cardiac output in the rat (Thompson & Moore, 1968).

This paper presents a study of cardiovascular factors that might be expected to limit summit metabolism in young lambs. The results suggested that other factors play the major limiting role.

METHODS

Source of data. The present paper is confined to the data obtained during the initial control periods of three series of experiments on Merino lambs. The purpose and results of two series are presented elsewhere (Alexander & Williams, 1970).

Animals. Ninety Merino lambs and sixteen Merino × Suffolk × Border Leicester lambs, from well nourished ewes, were used. Many lambs were born indoors and all were brought under cover within 12 hr of birth; none had been exposed to inclement weather before study. The time of birth was known to within approximately 6 hr.

Apparatus and procedures. The closed circuit respiration apparatus and related procedures for the *measurement of summit oxygen consumption* have been described in detail elsewhere (Alexander, 1961*a*; Alexander & Williams, 1968). Wool was clipped from the lambs to facilitate heat loss and a thermocouple was inserted 5 cm into the lamb's rectum. Catheters were inserted as described below, and in most experiments safety-pin electrodes were fastened to the skin for electrocardiographic and electromyographic recording. Each animal, supported in a standing position by a sling of cord netting, was placed in the chamber at an air temperature within the range -5 to $-15°$ C. After connexion of the electrical leads and placement of catheters through airtight glands, the lid was closed and the wind speed in the

chamber was adjusted until rectal temperature began to fall at about 0·03–0·05° C/min. Oxygen consumption (summit metabolism) was then measured during ¼ hr (Series 3) or ½ hr (Series 1 and 2) comprising consecutive periods of 10 min (Series 1) or 15 min (Series 2). Oxygen content of the air in the chamber remained at 20–21 %. A similar procedure was followed for the *measurement of oxygen consumption under thermoneutral conditions*, but the lamb was not clipped, the chamber temperature was 29–30° C and air movement in the chamber was minimal.

Before the chamber was closed in Series 2 and 3, the lamb was heparinized (5 mg/kg I.V.) to facilitate handling of blood for dye dilution studies; heparin was without effect on summit metabolism in control experiments (unpublished observations).

Catheterization. Blood vessels were catheterized for blood sampling, injection of indicator dye and drug infusion. Polyvinyl chloride catheters (Dural Plastics, Dural, N.S.W.) of i.d. 0·8 mm, o.d. 1·27 mm or occasionally of i.d. 1·0 mm, o.d. 1·5 mm were used.

All catheters were inserted under local anaesthesia using diethylamino-dimethyl-acetanilide (Xylotox, Pharmaceutical Manufacturing Co. England). Catheters in the jugular vein and carotid artery were inserted by a modification of the method of Herd & Barger (1964), and were cemented into position with isobutyl-2-cyano-acrylate (Ethicon, tissue adhesive, Ethnor Pty. Ltd). Catheters in the posterior vena cava and aorta or femoral artery were introduced via the recurrent tarsal vein and saphenous artery. Carotid catheters were introduced via a thyroidal artery in some lambs of Series 1. Catheters were jacketed in electrically heated sheaths to prevent freezing.

In Series 1 catheters were placed so that blood was sampled from the posterior end of the posterior vena cava, from the femoral or posterior end of the aorta, as well as from the carotid artery in some animals.

In Series 2 the sampling site of the catheter introduced via the recurrent tarsal vein varied from caudal to the renal inflow to within the heart, but was usually at about the level of the diaphragm and above the hepatic vein. Catheters in the jugular vein were inserted until the orifice lay about 2 cm above the heart, or, in about half of the experiments, the catheter was successfully passed into the pulmonary artery with the orifice usually distal to the ductus arteriosus. Catheters in the carotid artery were usually passed into the left ventricle. Successful placement of arterial catheters was indicated by the blood pressure trace, and in many animals catheter positions were checked at post mortem examination.

In Series 3, catheters were placed in the posterior vena cava, the femoral artery or posterior end of the aorta and in the jugular vein as in Series 2.

Indicator dye dilution curves. Dye dilution curves were obtained during the latter half of each period of measurement of oxygen consumption, and the curves were used for the estimation of cardiac output and the size of shunts through the foramen ovale; procedures similar to that of Stahlman, Merrill & Le Quire (1962) were used.

A known amount (0·5–1 mg) of indocyanine green dye (Cardiogreen, Hynson, Westcott & Dunning Inc. Baltimore, U.S.A.) in 0·20 ml. distilled water was introduced into the jugular, posterior vena cava or carotid catheter, and sufficient saline (0·9 g sodium chloride per 100 ml. water) was injected to bring the dye solution to within 0·3 ml. of the catheter orifice; 1·1 ml. saline was then injected into the catheter over ½–1 sec to deliver the dye into the blood stream. Insignificant traces of dye remained in the catheter after this procedure. Immediately before injection of dye, withdrawal of blood from the femoral artery, through a densitometer cuvette (X-250, The Waters Co., Minn., U.S.A.) was commenced. Withdrawal was effected by a Harvard pump and steady flows of approximately 20 ml./min could usually be achieved, although in a few lambs it was necessary to reduce withdrawal rates to 8, and very occasionally to 4 ml./min, to achieve a steady flow. The 'dye dilution curve'

was traced on a potentiometric recorder, connected to the cuvette through its associated control unit, the output of which was proportional to the concentration of dye in the blood passing through the cuvette.

The 5–10 ml. blood withdrawn during the completion of each curve was immediately returned to the lamb, and the whole process was then repeated, using the same or another injection site. There was no evidence of haemolysis in plasma samples collected after 12 or more injections in any of the lambs. During each ¼ hr period of measurement of oxygen consumption, curves were usually obtained in sets of two or three, using injection into the posterior vena cava and jugular vein and sometimes into the carotid artery. Replicate curves were sometimes obtained for one or more injection sites. The cuvette was flushed through with detergent (Pyroneg, Diversey, Sydney, Australia) and rinsed with saline after each set of injections.

A similar procedure was used for the detection of left to right shunts through the ductus arteriosus; dye was injected into the left ventricle and blood was withdrawn from the pulmonary artery at the rate of 12, or occasionally at 6 ml./min.

At the start of each experiment a blood sample was withdrawn and divided into two parts. The electrical output of the cuvette was then calibrated by the use of a known solution of dye in one subsample of blood. The other subsample was used to zero the instrument for calibration.

Cardiac output was calculated by conventional methods from the area under the dye dilution curve, using a semi-logarithmic plot and straight line extrapolation (for example see Stahlman *et al.* 1962). Right to left shunts were demonstrated by an early appearance time (Fig. 2) and two peaks in the curve. The size of the shunt relative to the total blood flow past the point of exit of the shunt was estimated by assuming that the extrapolated semi-logarithmic disappearance slope for the dye passing through the shunt was parallel to that for the main disappearance phase of the curve, estimated as above; the area under the shunt curve was then expressed as a percentage of the total area (see Stahlman *et al.* 1962).

Left to right shunts were demonstrated by the early appearance of dye, in pulmonary artery blood (Fig. 2) superimposed on the normal appearance curve after injection into the left ventricle. The area under the curve, due to the shunt, was estimated by superimposing the characteristic normal re-appearance curve, freehand, and the area was then expressed as a percentage of the mean area of the curves obtained by femoral sampling during the same experimental period, and after injection of the same amount of dye.

Estimation of cardiac output by the Fick method. The mean cardiac output, during ¼ hr periods of measurement of metabolic rate, was estimated by dividing the rate of oxygen consumption by the product of the oxygen capacity and the difference between the oxygen saturation percentage of arterial and mixed venous blood. Oxygen capacity and oxygen saturation were determined from single blood samples drawn during the first half of the relevant ¼ hr period.

Blood sampling and analytical procedures. Blood was collected anaerobically, via the various catheters during the first half of each period of measurement of oxygen consumption; samples were stored at 4° C until analysed at the end of the experiment. *Arterial and venous oxygen saturations* were determined by the spectrophotometric method of Verel, Saynor & Kesteven (1960), calibrated against the manometric method of Peters & Van Slyke (1932). *Haemoglobin* was measured as oxyhaemoglobin by spectrophotometric absorption at 540 mμ (Dacie, 1956). *Oxygen capacity* (ml./100 ml. blood) was calculated from the haemoglobin percentage by conventional methods.

Analysis of results. Mean values of the various parameters measured during the ½ hr period of Series 1 and 2, and the ¼ hr period of Series 3, were calculated for each animal, and these means were used in conventional statistical analyses. Data from the first ¼ hr period of Series 2 pooled with that from Series 3, were also examined.

RESULTS

Arteriovenous oxygen saturation differences

The mean oxygen saturation in the blood in the descending aorta ranged from 90 to 98 % in the various groups of Series 1, 2 and 3 (Table 1); there was no clear difference between lambs exposed to severe cold and lambs under thermoneutrality (Series 3).

In determination of arteriovenous (a–v) oxygen saturation differences, it was assumed that blood in the right ventricle or pulmonary artery represented mixed venous blood. In lambs from which right heart blood was not obtained, oxygen saturation of mixed venous blood was estimated as the average saturation of blood in the posterior vena cava and anterior vena cava (or jugular vein). These saturations were usually similar and of the same magnitude as in right heart blood from other lambs; in nine experiments of Series 2 and seven experiments of Series 3, mean saturation ($\pm$ S.E. of mean) of posterior vena caval blood during summit metabolism was 7 ± 3 % and 6 ± 2 % lower than saturation of the anterior vena caval sample. Differences tended to be greater when posterior caval blood was drawn below the renal veins; saturations as low as 12 % were sometimes encountered in such samples and the low venous saturations recorded in Series 1 (Table 1) are no doubt reflexions of the sampling site.

In young lambs under thermoneutral conditions (Series 3) the mean saturation of 'mixed venous blood' was 70 %, and the mean a–v oxygen saturation difference was 28 % (Table 1). However, during summit metabolism the mean percentage saturation of mixed venous blood in the lambs less than 3 days old in Series 2 and 3 (Table 1) was 31 and 37 and mean a–v difference was 60 and 58 respectively.

The arterial and venous saturations and a–v differences appeared to be independent of age (Fig. 1, for example).

The mixed venous oxygen saturation was significantly and negatively correlated with summit metabolism in the young lambs in Series 2 and in Series 2 and 3 pooled (Tables 3 and Fig. 1) but not in Series 3. There was no indication of such a relationship in the older lambs of Series 2 (Fig. 1).

Cardiac output

(i) *Comparison of methods.* Although changes in cardiac output followed the same trends (see below) when determined by the Fick method or by dye dilution, numerical agreement between the two methods was not good. In Series 3 when data from the twenty-one experiments on cold exposure and exposure to thermoneutral conditions were pooled, the coefficient of correlation between the values obtained by the two methods was 0·78 ($P < 0{\cdot}001$). However, in Series 2 in which the range of values of cardiac

output was more restricted, due to inclusion of cold conditions only, the coefficient was a mere 0·24 (twenty-three experiments). In lambs less than 12 hr old, the Fick method tended to give higher values than the dye dilution method, whereas in lambs 12–60 hr old the direction of the differences was more random; in Series 2 the mean difference ± S.E. of mean (Fick minus dye) of 12 ± 2·4 ml./100 g.min in the younger group was significantly higher than the mean of − 1·2 ± 3·7 in the 12–60 hr old lambs ($P < 0·01$ in t test). Similar results were obtained from Series 2 and 3, pooled. In lambs

TABLE 1. Oxygen saturation of blood in lambs during exposure to extreme cold and to thermoneutral conditions

	Age	Environmental conditions	No. of lambs	Mean % saturation of blood with oxygen*		
				Arterial	Mixed venous	a–v difference
Series 1	7–30 hr	Summit	4	94	24	70
				(2·0)	(5·1)	(3·5)
	6–11 days	Summit	8	91	25	66
				(3·0)	(3·3)	(2·8)
	21 days	Summit	2	97	27	70
				(2·0)	(7·0)	(8·0)
Series 2	6–48 hr	Summit	24	91	31	60
				(0·5)	(1·6)	(1·7)
	21–28 days	Summit	8	90	33	57
				(0·9)	(3·9)	(3·4)
Series 3	6–54 hr	Summit	10	95	37	58
				(1·4)	(1·3)	(2·1)
	6–54 hr	Thermoneutral	11	98	70	28
				(0·8)	(1·1)	(1·4)

* S.E. of means shown in brackets.

3–4 weeks old (Series 2), values were 10 % higher by the Fick than by the dye method (mean difference 7·0 ± 3·4 ml./g.min), but the difference from zero was not significant.

(ii) *Cardiac output during summit and thermoneutral conditions.* In Series 3, the mean cardiac output per unit of body weight in cold exposed lambs, less than 3 days old, was considerably higher than in lambs exposed to thermoneutral conditions (approximately 70 ml./100 g.min and 40 ml./100 g.min respectively) when determined by either method (Table 2). A decline in cardiac output, per unit of body weight, with increasing age was also indicated by both methods in cold exposed lambs in Series 2 (Table 2); thus total cardiac output did not increase at the same rate as body weight which increased threefold in 4 weeks. Mean cardiac output ± S.E. of mean in twelve lambs with catheters in or passing through the heart and in seven

lambs without cardiac catheters was $75 \pm 2{\cdot}0$ and $74 \pm 3{\cdot}1$ ml./100 g.min respectively, by the Fick method and $63 \pm 2{\cdot}2$ and $68 \pm 5{\cdot}0$ by the dye method (Series 2). Thus there was no obvious effect of cardiac catheterization on cardiac output, although blood pressure fell abruptly when extrasystoles occurred in most lambs with cardiac catheters (Fig. 5).

There was no apparent relation between summit metabolism and cardiac output, measured by either method, in the data from Series 2 (Fig. 1), nor in Series 2 and 3 combined, although in Series 3 the coefficient of correlation was significant when the dye method was used, and approached significance when the Fick method was used (Fig. 1).

TABLE 2. Cardiac output in lambs during exposure to extreme cold and to thermoneutral conditions

	Age	Environmental conditions	No. of lambs	Mean cardiac output* (ml./100 g.min) Fick method	Dye method
Series 2	6–48 hr	Summit	24	73 (1·7)	66† (2·2)
	21–28 days	Summit	8	63 (1·7)	56‡ (1·9)
Series 3	6–54 hr	Summit	10	72 (5·2)	76 (5·4)
	6–54 hr	Thermoneutral	11	48 (4·9)	37 (2·4)

* S.E. of mean in brackets.
† Twenty-three lambs.
‡ Seven lambs.

In Series 2 cardiac output determined by the Fick method was positively correlated with mixed venous oxygen saturation ($r = +0{\cdot}48$, $P < 0{\cdot}05$) as might be expected since the former was calculated from the latter, but there was no correlation when the dye results were used. In Series 2 and 3 singly and combined, cardiac output (Fick and dye) was negatively correlated with oxygen carrying capacity of blood; the better correlations obtained by the Fick method ($r = -0{\cdot}43$ to $-0{\cdot}73$) than by the dye method ($r = -0{\cdot}18$ to $-0{\cdot}45$) may also result from the dependence of the calculation of cardiac output upon oxygen capacity.

Shunts through foetal channels

(i) *Right to left (R–L) shunts.* Shunting through the foramen ovale is indicated when an extra peak appears on the dilution curve, after injection into the posterior vena cava, but not after injection into the jugular vein, right heart or upper pulmonary artery; shunting through the ductus

arteriosus is indicated if extra peaks appear after both posterior vena caval and jugular vein injections.

Dye was injected into the posterior vena cava in thirty-four lambs, less than 4 days old, during exposure to summit conditions (twenty-one lambs from Series 2, eight from Series 3, and five miscellaneous lambs). Evidence of a significant R–L shunt (3–63 %, but usually < 25 % of the blood flow in the posterior vena cava) was obtained in fifteen of the thirty-four; very small shunts (< 3 %) were detected in another nine (examples in Fig. 2).

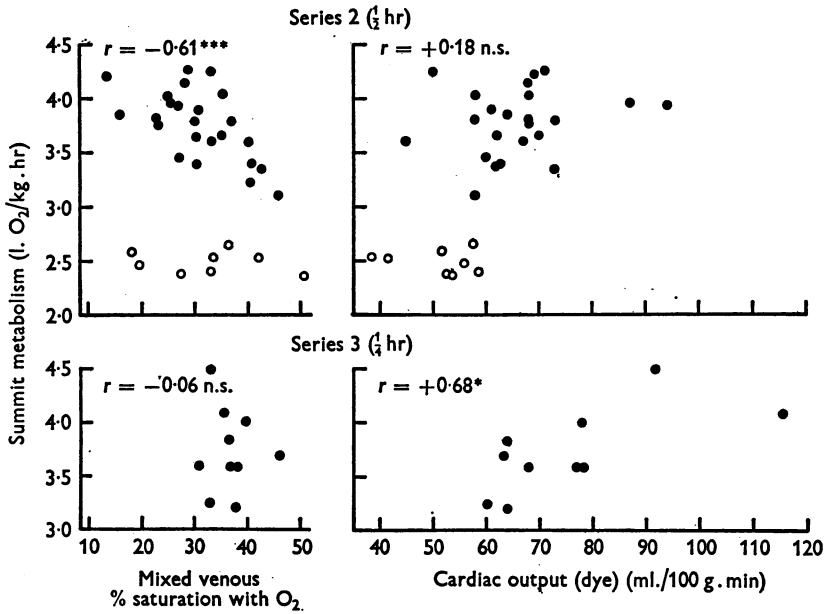

Fig. 1. Relation of summit metabolism (Series 2 and 3) to % oxygen saturation of mixed venous blood and to cardiac output determined by the dye method. ● Lambs < 3 days old; ○ lambs 3–4 weeks old; r is the correlation coefficient. * $P < 0{\cdot}05$; *** $P < 0{\cdot}001$; n.s. not significant.

Shunts observed during the first and second ¼ hr control periods of cold exposure were usually of similar size (twenty-one lambs, Series 2) but there were wide variations in several animals. For example, in one lamb approximately 6 hr old, duplicate estimates of the shunt size during the first ¼ hr were 63 and 30 %, and zero in the second ¼ hr; in another lamb of similar age the respective estimates were < 3 % and 43 %. When the former lamb was excluded, the means (± S.E. of mean) of the shunt size during the first and second ¼ hr periods were 12·1 ± 2·9 and 11·6 ± 2·8 % respectively.

The incidence and magnitude of R–L shunts declined with increasing age (Fig. 3), and shunts were not detected in any of eight lambs examined under summit conditions at 3–4 weeks of age (Series 2).

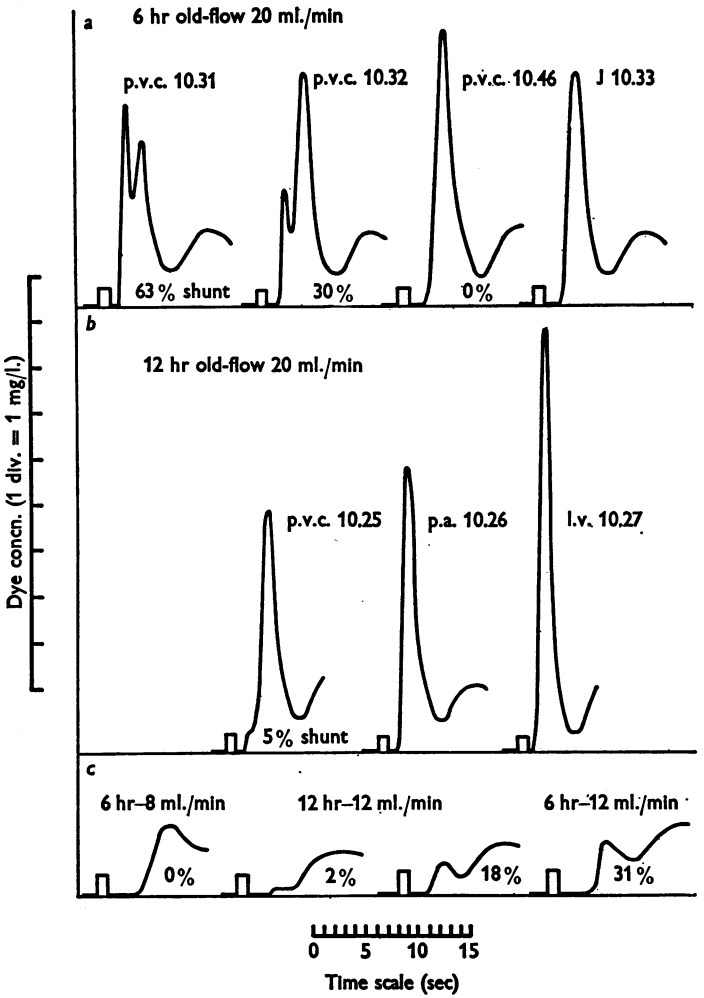

Fig. 2. Sample dye dilution curves. Duration of injection (0·50–0·67 mg dye in 0·20 ml.) is indicated by rectangular deviation of the trace from the base line; the time of injection is shown for lambs that received more than one injection.

(*a*) Curves obtained at withdrawal rates of 20 ml./min from the aorta, after injection into the posterior vena cava (p.v.c.). Lamb 6 hr old. Curves show varying R–L shunt.

(*b*) Curves obtained as in (*a*). Lamb 12 hr old. Curves show small R–L shunt and variable appearance time of dye depending on injection site (p.a. = pulmonary artery; l.v. = left ventricle).

(*c*) Curves obtained by withdrawal at 6–12 ml./min from pulmonary artery distal to ductus arteriosus, after injection into left ventricle. Various degrees of L–R shunting are illustrated. The longer appearance time in (*c*) than in (*a*) and (*b*) is due to lower rates of withdrawal and a longer withdrawal catheter.

R–L shunts were also detected in four of nine lambs examined under thermoneutral conditions (Series 3), but there were too few animals in this group to show whether the size of the shunt depended on the environmental conditions.

Following injection into the jugular, right ventricle or pulmonary artery there was evidence of a R–L shunt in the curves from only one of eighteen lambs injected during summit metabolism, and in none of the nine lambs

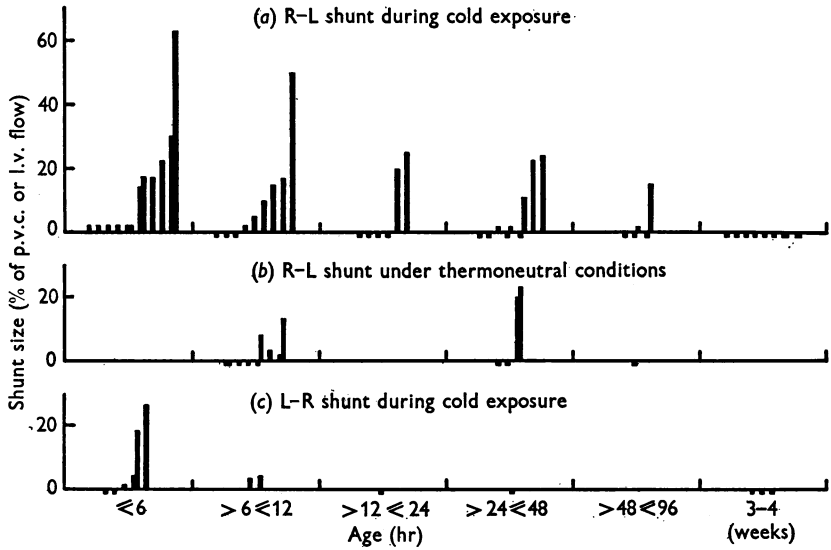

Fig. 3. Shunting through foetal channels in relation to age of lamb. The size of R–L shunts is expressed as a percentage of blood flow in the posterior vena cava; the size of L–R shunts is expressed as a percentage of left ventricular output.

(*a*) R–L shunt during first $\frac{1}{4}$ hr of cold exposure in Series 2 and 3.

(*b*) R–L shunt during first $\frac{1}{4}$ hr of exposure to thermoneutral conditions in Series 3.

(*c*) L–R shunt during first $\frac{1}{4}$ hr of cold exposure in Series 2.

Each column represents a single determination. Duplicate estimates in any one lamb are indicated by conjoined columns; each lamb was examined at a single age. Absence of a shunt is indicated by a short column below the horizontal axis.

similarly treated under thermoneutral conditions. In the one exception the tip of the injection catheter lay close to the ductus arteriosus so that some of the dye might have been injected into that vessel, under pressure.

There was no evidence of significant R–L shunts through the ductus arteriosus in five lambs less than 30 hr old during exposure to severe cold; the oxygen saturation of arterial blood in the aorta, posterior to the ductus arteriosus, was within 2 % of the saturation of blood in the carotid artery.

Although shunting of blood through the foramen ovale occurred in at least half of the lambs less than 4 days old, there was no apparent relationship between the size of the shunt and summit metabolism nor was there any relation between shunt size and percentage saturation of aortic blood ($r = -0{\cdot}07$, 29 values, in both Series 2 and 3).

(ii) *Left to right* (*L–R*) *shunts.* Experimental requirements for a valid examination of lambs for L–R shunting through the ductus arteriosus, were achieved in nine lambs less than 3 days old, and three lambs 3–4 weeks old (all in Series 2); in several other lambs post mortem examination

TABLE 3. Incidence and size of L-R shunts during exposure of lambs to extreme cold (Series 2)

Approx. age (hr)	Size of shunts in consecutive measurements (% of left ventricular output)
6	1, 3
6	4, 18, 5
6	0, 0, 0
6	26, 29, 31
6	0, 0, 0, 0
12	2, 3
12	3, 2, 18, 3
13	0, 0, 0, 0
36	0, 0, 0

indicated that the sampling catheter in the pulmonary artery lay between the heart and the ductus arteriosus. In several older lambs the injection catheter was ejected from the heart, during the experiment, and at post mortem examination was found to lie in the aorta with the tip posterior to the ductus arteriosus. However, in all the older lambs the lumen of the ductus was virtually obliterated.

L–R shunts of up to 31 %, usually less than 10 % of left ventricular output, were detected in Series 2 (Fig. 2), during the initial $\frac{1}{2}$ hr measurement periods in five of the nine young lambs, and in none of the three older lambs. Shunts were not detected in any lamb older than 12 hr (Fig. 3). As with R–L shunts, most of the two to four replicate measurements during the $\frac{1}{2}$ hr were of similar size (Table 3), and there was no indication of any relation between shunt size and summit metabolism. However, percentage oxygen saturation in the distal aorta appeared to be slightly increased by shunting; the mean saturation $\pm$ S.E. of mean in the young lambs not showing a shunt was $89{\cdot}5 \pm 0{\cdot}3$ % compared with $92{\cdot}0 \pm 1{\cdot}1$ % in lambs with a detectable shunt ($P = 0{\cdot}05$, by a single tailed t test since the difference was in the direction expected on physiological grounds).

Haemoglobin concentration

Haemoglobin concentration in the blood of lambs tended to decrease, usually by 0·5–1 g per 100 ml. blood (i.e. by about 5 %) during the course of experiments in Series 2, in which sampling usually entailed a blood loss of 10–15 ml.

Haemoglobin concentration (or oxygen capacity) in lambs less than 3 days old was unrelated to summit metabolism, measured at the same time and was also unrelated to a–v oxygen saturation differences (r = approximately $-0{\cdot}16$ in each set of data).

Heart rate

Heart rate was considerably higher during cold exposure (approximately 300/min) than during exposure to thermoneutral conditions (approximately 200/min). Rates of 300/min were observed in lambs less than 3 days old and in lambs 3–4 weeks old.

There was no apparent relation between heart rate and summit metabolism nor was there any significant relation between heart rate and cardiac output measured either by the Fick method or by dye dilution (r ranged from $+0{\cdot}6$ to $-0{\cdot}3$ in the three sets).

Blood pressure

(i) *Systemic pressure.* In Series 3, mean blood pressure (diastolic pressure plus one third of pulse pressure) in lambs exposed to summit conditions (Table 4) was 8 mm Hg higher than that in lambs under thermoneutral conditions ($P < 0{\cdot}05$ by t test). Blood pressure during summit metabolism increased with age (Table 4) but there was no apparent relationship with age in lambs less than 3 days old (Series 2).

Blood pressure was significantly and positively correlated with summit metabolism in Series 1, but the correlation was significant and negative in Series 3.

(ii) *Pulmonary arterial pressure.* In contrast to systemic pressure, the mean pulmonary arterial pressure clearly declined with increasing age from about 35 mm Hg at about 6 hr of age to about 15 mm Hg before the lambs were 3 weeks old (Fig. 4).

In each of fourteen lambs (twelve in Series 2 and two in Series 3) examined under summit conditions within 3 days of birth, the mean pulmonary pressure was considerably below the mean systemic pressure. However, there were considerable variations in pulmonary arterial pressure associated with respiration (Fig. 5) which increased the excursions of pressure from an average pulse pressure of 29 mm Hg to an average of about 50 mm Hg; and in six of twelve animals, 13 hr old or less, the peak

pulmonary pressure exceeded the diastolic systemic pressure of about 60 mm Hg, during some phase of the cardio-respiratory pressure cycles. Since the systemic catheter was directed towards the heart, and the pulmonary artery catheter away from the heart, the pulmonary artery pressure probably exceeded systemic arterial pressure by a larger amount than

TABLE 4. Systemic arterial blood pressure in lambs during exposure to extreme cold and to thermoneutral conditions

Source of data	Age	Environmental conditions	No. of lambs	Mean blood pressure* (mm Hg)
Alexander & Williams (1968)	6 hr–12 days	Summit	6	79 (3·7)
Series 1	3–60 hr	Summit	6	72 (3·5)
	6–11 days	Summit	6	95 (1·5)
Series 2	6–48 hr	Summit	24	77 (2·1)
	21–28 days	Summit	8	98 (2·0)
Series 3	6–54 hr	Summit	10	81 (2·8)
	6–54 hr	Thermoneutral	11	73 (2·5)

* S.E. of mean in brackets.

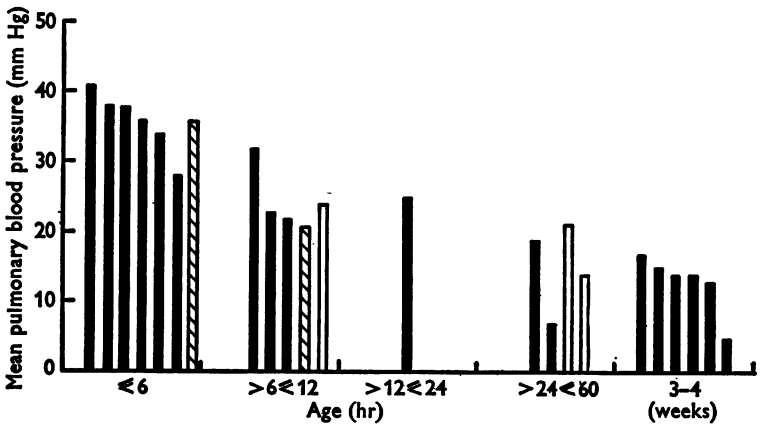

Fig. 4. Mean blood pressure in pulmonary artery of lambs in relation to age. The solid columns represent the mean pressure during the ½ hr period of summit conditions in Series 2. The shaded and open columns represent the mean pressures during the ¼ hr period of exposure to summit and to thermoneutral conditions, respectively, in Series 3. No lamb is represented more than once.

observed, and in more lambs than observed. However, it is unlikely that the pulse waves at either end of the ductus arteriosus would be so out of phase as to result in a R–L pressure gradient.

There was no apparent relation between mean pulmonary arterial pressure and size of R–L shunt.

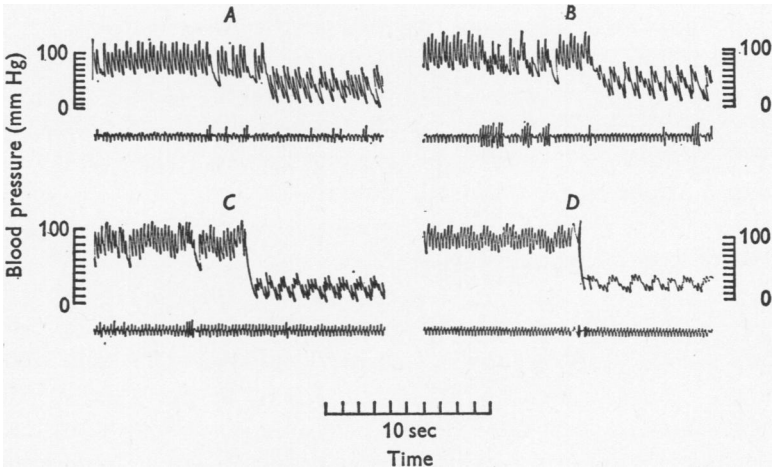

Fig. 5. Blood pressure and electrocardiogram in four lambs during exposure to severe cold. *A* and *B*. Lambs approximately 6 hr old. *C* and *D*. Lambs 3–4 weeks old. Pulmonary pressure (second half of record) was measured within a few seconds of aortic pressure (first part of record), and with the same transducer. The pressure in the pulmonary artery shows marked fluctuations associated with respiration. In the younger lambs peak pulmonary pressure barely equalled systemic diastolic pressure so that even transient R–L shunting via the ductus arteriosus does not appear likely. Pulmonary arterial pressure decreased with advancing age.

Extrasystoles with associated falls in systemic pressure occurred in lambs *A*, *B* and *C* with catheters in the left ventricle, but cardiac output was not significantly affected (see text).

DISCUSSION

In lambs exposed to extreme cold (Series 2 and 3) cardiac output per unit of body weight increased to reach about 70 ml./100 g.min. However, this 50–100 % increase in cardiac output in the cold is considerably less than the fivefold increase in man, from about 5 ml./100 g.min during rest to 25 ml./100 g.min during maximum work load (Hamilton, 1964; Douglas & Becklake, 1968). Janksy & Hart (1968) reported an increase of 46 % in cardiac output of adult rats exposed to 9° C, but at this temperature metabolic rate would be considerably less than summit metabolism as indicated by the data of Depocas, Hart & Héroux (1957). In new-born rats (12 days old) at 20° C cardiac output was only 25 % higher than at 35° C (Thompson

& Moore, 1968), but metabolic rate at 20° C was probably close to summit in rats of this age.

Previous measurements of cardiac output in lambs by Cross, Dawes & Mott (1959) using the Fick method, and by Downing & Rocamora (1968) using the dye method were done with the animals under anaesthesia and in rather ill-defined thermal conditions; the mean values per unit of body weight (33 and 20 ml./100 g.min respectively) were somewhat lower than those recorded in the present experiments by the Fick and dye methods (58 and 37 ml./100 g.min) in resting conscious lambs under strict thermoneutrality (Series 3). The higher values (up to 60 ml./100 g.min) in the data of Cross *et al.* (1959) may have been due to rates of heat loss that were greater than those authors suspected; room temperatures were some 10° C below the critical temperature for lambs (Alexander, 1961*b*), and if the coat was still wet, heat loss would have been higher still (Alexander, 1962*b*).

Heart rate in lambs exposed to extreme cold was about 50 % higher than the rate in lambs under thermoneutral conditions (300 and 200/min respectively), but the effects of cold on stroke volume are not clear. On the basis of the Fick measurements stroke volume was approximately 2·4 ml./kg under both thermoneutral and cold conditions; but on the basis of the dye method, stroke volume increased from 1·8 ml./kg, under thermoneutrality, to 2·5 ml./kg during cold exposure (Series 3). An increase in stroke volume accompanies strenuous exercise, at least in man (see review by Horvath & Howell, 1964).

The poor agreement between the Fick and dye methods of estimating cardiac output (Table 2) may have arisen from several factors. In the presence of a shunt through the foramen ovale, blood in the right ventricle or pulmonary artery would not be truly representative of mixed venous blood; the use of the average saturation of anterior and posterior venous samples as an estimate of mixed venous saturation may introduce errors; oxygen saturation of blood was not continuously monitored during the periods of measurement of oxygen consumption; and the two methods could not be applied simultaneously. Differences may also be partly due to patency of foetal shunts; for example, dye rapidly recirculated (L–R) through the ductus arteriosus would reduce the slope of the disappearance curve, and lead to over-estimation of the area of the curve, and hence under-estimation of the cardiac output, which is in conformity with the results on lambs less than 12 hr old; problems of interpretation of estimations of cardiac output in the presence of foetal shunts have been stressed by Cross *et al.* (1959). A low order repeatability of the dye method (Hanson & Tabakin, 1964) may also have contributed to the discrepancies.

In addition to the considerable increase in cardiac output due to acute

cold exposure in new-born lambs, there was a marked increase in the degree of extraction of oxygen from the blood; the mixed venous oxygen saturation was reduced from about 60 to 30 %. Similar changes have been seen in new-born rats (Thompson & Moore, 1968) and in exercising men (Saltin, Blomqvist, Mitchell, Johnsson, Wildenthal & Chapman, 1968). In the present experiments the venous saturation distal to the renal veins was usually about 20 % and sometimes as low as 12 %, so that the thermogenic tissues (muscle and brown adipose tissue) from which this blood largely drains, were able to extract some 80 % of oxygen from the blood flowing through them. A similar high extraction of oxygen from brown adipose tissue was reported by Heim & Hull (1966) when new-born rabbits were stimulated by infusion of noradrenaline.

Oxygen saturation of arterial blood was not obviously affected by exposure of neonatal lambs to extreme cold; the low mean figure of about 93 % is in conformity with figures of Cross *et al.* (1959). Surprisingly, there was no marked depression in arterial saturation due to shunting via the foramen ovale; however, shunting and arterial saturation were not examined simultaneously. Arterial saturation was slightly elevated by L–R shunting via the ductus arteriosus; but it is doubtful whether this would represent a physiological advantage in lambs more than a few hours old (Dawes, Mott & Widdicombe, 1955).

Shunts through foetal channels were detected in about half of the lambs examined under summit conditions; and the combination of a L–R shunt through the ductus arteriosus and R–L shunt through the foramen ovale, considered by Cross *et al.* (1959) to be improbable, was encountered in five of the six lambs less than 12 hr old in which the relevant examinations were made.

Results from the few animals examined under thermoneutral conditions suggested that there was no major effect of cold exposure on the degree of shunting via the foramen ovale. The measurements of foramen shunting made by Stahlman *et al.* (1962) in young lambs, apparently near thermal neutrality, were slightly higher than those in the present cold exposed lambs; available data are therefore not consistent with an increase due to cold exposure. Data were not obtained in L–R shunting through the ductus arteriosus under thermoneutral conditions, but L–R shunting appeared to be unimportant in lambs older than 6 hr (Fig. 3).

Right to left shunts through the ductus arteriosus were not detected in the present studies, nor in those of Stahlman *et al.* (1962), although peak pulmonary pressure transiently exceeded diastolic systemic pressure during part of the cardio-respiratory cycle in some of the youngest lambs (Fig. 5). The high initial pulmonary artery pressure and the rapid decline with advancing age (Fig. 4) was also seen in the sheep by Polosa, Dagianti,

Guiliano & Condorelli (1957), in dog and goat by Rudolph, Auld, Golinko & Paul (1961), in the calf by Reeves & Leathers (1964) and in man by Emmanouilides, Moss, Duffie & Adams (1964); presumably these observations were made under conditions approximating thermoneutrality.

The use of correlation coefficients, to indicate factors that restrict the metabolic response to cold, is of somewhat limited value, for the magnitude of the correlation coefficient depends largely on the range of values encountered amongst the factors under examination. Examples are illustrated in Fig. 1. Nor do correlations distinguish cause from either primary or secondary effect. However, oxygen-carrying capacity of blood, heart rate and size of R–L shunt appeared to be of little or no importance, while cardiac output, blood pressure, and mixed venous saturation assumed importance under some circumstances; errors in the methods of measuring cardiac output, particularly in the presence of shunts, may have masked the full degree of dependence of summit metabolism upon this parameter. Interpretation of the correlations with blood pressure is difficult, since there were both significant positive and negative correlations with summit metabolism.

The significant negative correlation between summit metabolism and mixed venous saturation focuses attention on the extraction of oxygen from the circulation by the thermogenic tissues. The relationship could arise from differences between animals in the proportion of muscle and brown fat in the body, or from differences in the efficiency with which these tissues extract oxygen from the blood, but data are not available on these points. The low oxygen content of the venous blood draining the thermogenic tissues suggest that summit metabolism in lambs may be limited to some extent by the supply of oxygen to the thermogenic tissues; if this is so a correlation between summit metabolism and oxygen-carrying capacity of blood might be expected, but the absence of correlation is at least partly explained by the tendency for lambs with low oxygen capacity to have high cardiac output. Summit metabolism may, on the other hand, be limited by cardiac output, and the circulation of metabolites other than oxygen, but the correlations suggest that this dependence is not very strong. In 12-day-old rats (Thompson & Moore, 1968) both cardiac output and supply of oxygen to heat producing tissues appear to limit the metabolic response to cold, but elucidation of the relative importance of oxygen supply and cardiac output, at least in lambs, must await more specific experimentation. However, it appears that the major source of individual variations in summit metabolism does not lie in the circulation; these variations seem more likely to reside in the amount of thermogenic tissue possessed by the individual or in the capacity per unit weight of this tissue to produce heat. It must be emphasized, however, that the present results

apply to lambs mostly older than 6 hr; the limiting factors in younger lambs may well include circulatory phenomena.

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