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REGIONAL DISTRIBUTION OF CARDIAC OUTPUT IN YOUNG LAMBS: EFFECT OF COLD EXPOSURE AND TREATMENT WITH CATECHOLAMINES

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SUMMARY

1. Lambs less than 3 days old, exposed to thermoneutral or intensely cold conditions in a respiration chamber, were infused with adrenaline or noradrenaline, 1 or 10 μg/kg.min; and the effects on oxygen consumption, cardiac output and its distribution to skin, skeletal and cardiac muscle, liver, spleen, kidney, gut, brown adipose tissue and brain were determined. Cardiac output was estimated by the Fick and dye dilution methods and the distribution of cardiac output by Sapirstein's method of fractional distribution of indicators.

2. Under thermoneutral conditions, metabolic rate was stimulated by both doses of noradrenaline and by the low, but not the high dose of adrenaline. Under cold conditions, the low dose of catecholamines had little effect on the already elevated metabolic rate, but the high doses depressed the metabolic response to cold.

3. The low dose of adrenaline increased cardiac output under thermoneutral conditions whereas the high dose decreased cardiac output; the effects of noradrenaline were less marked, in contrast to reported effects in new-born rabbits. The low doses of catecholamine given under cold conditions had little effect on the already elevated cardiac output, but the high doses, particularly of adrenaline, decreased cardiac output.

4. Blood flow through the skin of the extremities was markedly reduced by cold exposure, while flow through the peri-renal fat was doubled, flow through the skeletal muscle was quadrupled and flow through the cardiac muscle was trebled. These increases, particularly in skeletal muscle, were due to increased cardiac output and to vasodilation, as indicated by the reduced ratio of blood pressure to blood flow. Results are contrasted with published reports that blood flow through brown fat in new-born rabbits was greatly increased by cold, but muscle flow was scarcely altered.

5. In almost all organs examined the high doses of adrenaline infused in either environment markedly reduced blood flow, presumably by generalized vasoconstriction. Changes due to noradrenaline were small under thermoneutral conditions and flow through brown fat was increased by only 60 % during infusion of 10 μg/kg.min. Much greater increases have been reported in new-born rabbits. Under cold conditions the high doses of noradrenaline tended to decrease flow in most organs including brown fat and muscle.

6. The results provide likely explanations for published reports that adrenaline failed to stimulate non-shivering thermogenesis or suppressed the mobilization of metabolites and the metabolic response to cold.

INTRODUCTION

The metabolic response to cold in new-born eutherian mammals is due to stimulation of metabolism in brown adipose tissue (non-shivering thermogenesis) and in muscle (shivering thermogenesis). In the new-born of some species such as the rabbit, non-shivering thermogenesis predominates (Hull, 1965), in others such as the guinea-pig and sheep, both shivering and non-shivering contribute significantly (Brück & Wünnenberg, 1965; Alexander & Williams, 1968), while there are others, such as the pig and ox which appear to lack brown adipose tissue and to rely entirely on shivering thermogenesis (LeBlanc & Mount, 1968; Jenkinson, Noble & Thompson, 1968).

Studies on the redistribution of cardiac output that occurs on stimulation of thermogenesis by cold exposure of the new-born have indicated that there is considerable diversion of blood to the brown adipose tissue, but these studies have been restricted to the rabbit (Heim & Hull, 1966; Járai, 1969). This paper presents data on the new-born sheep, a species which relies more heavily on shivering than on non-shivering thermogenesis (Alexander & Williams, 1968). Experiments on the effects of physiological and supra-physiological doses of catecholamines on regional blood flow, particularly through thermogenic tissues, are also included since there is conflicting evidence about the effects of catecholamines on thermogenesis in young animals. For example, thermogenesis appears to be reduced by infusion of catecholamines in kittens (Scopes & Tizard, 1963) and in lambs (Alexander, 1969) exposed to cold. On the other hand in the absence of cold, non-shivering thermogenesis may be stimulated by catecholamines in guinea-pigs (Dawes & Mestyán, 1963) and lambs (Alexander, 1969).

METHODS

Some of the methods such as the preparation of the lambs, the measurement of oxygen consumption, and the estimation of cardiac output have already been described in detail (Alexander & Williams, 1970). Summaries only are presented below.

Experimental design. New-born lambs were exposed to thermoneutral conditions under which metabolic rate was minimal (resting metabolism), or to conditions of extreme cold under which metabolic rate was maximal (summit metabolism). Oxygen consumption of each lamb was measured during an initial *control period*, and then during a *test period* during which 1 or 10 μg/kg.min of adrenaline or noradrenaline, was infused. Control lambs received no hormones. During these periods, cardiac output was determined by a dye dilution technique and by the Fick method based on oxygen consumption. While the animals were still undergoing the respective thermal and drug treatments the regional distribution of cardiac output was examined, using the distribution of radioactive indicators (Sapirstein, 1958). The fractional distribution of radioactive rubidium (^{86}Rb) provided estimates of blood flow through most organs of interest; however, Rb is largely excluded from the brain by the blood–brain barrier (Sapirstein, 1958) so tritiated water (TOH) was used to provide an estimate of brain blood flow (Chatwin, Linzell & Setchell, 1969). The distribution of each indicator was examined after two different intervals between injection of indicator and killing of the lamb (see below). Experiments were carried out over 2 successive years (1968 and 1969). Thus the variables in the series of experiments were two environmental treatments, five infusion treatments (one saline control, two noradrenaline doses and two adrenaline doses), two times of killing and 2 years; forty lambs were used and each lamb received a different combination of treatments.

Animals. Merino lambs were used; all were born indoors and the time of birth was known to within 8 hr. The lambs were allowed to remain with their mothers and to suck at will, until they were about to be used. Ages ranged from 6 hr to 3 days and body weights from 2·8 to 5·4 kg. Treatments were randomly allocated.

Preparation of lambs. A thermocouple was inserted 5 cm into the rectum and safety-pin electrodes were attached to the skin for electromyographic and electrocardiographic recording. Catheters of polyvinylchloride (dimensions 1·0 mm i.d., 1·5 mm o.d.) were inserted into a femoral artery via a saphenous artery, into the right ventricle or pulmonary artery via an external jugular vein, into the posterior vena cava via the recurrent tarsal vein and directly into the other external jugular vein. Heparin (5 mg/kg) was injected intravenously to facilitate withdrawal of blood for dye dilution curves. The animals were supported in a standing position by a sling of cord netting and, to facilitate heat loss, wool was partially clipped from lambs just prior to exposure to cold. The arterial catheter was connected to a strain-gauge transducer for the measurement of blood pressure.

Measurement of oxygen consumption. Oxygen consumption was measured in a closed-circuit respiration chamber. Resting metabolism was determined with the chamber temperature at 29–30° C and minimal air movement; summit metabolism was determined at chamber temperatures within the range of -5 to -15° C, and air movement was adjusted, within the range of wind speeds 0–30 km/hr, to induce a rate of fall in rectal temperature of 0·03–0·05° C per min. Oxygen consumption of each lamb was measured, first, over a *control period* of 15–20 min, when saline alone was infused into the jugular vein at approximately 0·1 ml./min, and secondly during a *test period* of similar length when the experimental infusions were given.

Drug infusion. Adrenaline and noradrenaline (1 or 10 μg/kg.min) were infused as the bitartrate salts (British Drug Houses), freshly dissolved in physiological saline

(0·9 g NaCl/100 ml. water) containing 0·03% ascorbic acid as preservative. A priming dose equivalent to the amount administered during 3 min of infusion was given initially. Physiological saline was infused into the control animals during the test period. The lower catecholamine doses approximate the maximal output by adrenal glands of new-born lambs (Comline & Silver, 1961).

Estimation of cardiac output. Cardiac output was estimated from the concentration of dye in blood from the femoral artery after injection of 0·5–1 mg indocyanine green into the right heart or posterior vena cava; the arterial blood was withdrawn, at about 18 ml./min through a densitometer cuvette (Waters Corp. type XC250A) for dye detection. Two or three estimates were made during the first and last 5 min of each control or test period and the averages obtained during each period were used in the analyses of results. Cardiac output was also estimated by the Fick method from the oxygen consumption and the percentage saturation and haemoglobin content of arterial blood and mixed venous blood from the right heart; the blood was sampled mid-way through each control and experimental period and the percentage saturation was estimated by the method of Verel, Saynor & Kesteven (1960); haemoglobin was estimated by the method of Dacie (1956).

Estimation of relative blood flow [^{86}Rb]Cl (100 μCi in 0·5–1·0 ml. physiological saline) was injected rapidly, through the cannula in the posterior vena cava, within several minutes of the completion of the measurement of oxygen consumption, but with the experimental thermal and hormone treatments continuing. The indicator was washed in rapidly with 5 ml. saline. Fifteen or 30 sec later 100 μCi TOH in 0·5 ml. saline was given, similarly, and the lamb was killed after a further 15 or 30 sec, by rapid I.V. injection of 40 ml. saturated KCl solution. Thus half of the lambs were killed 60 sec after ^{86}Rb injection and 30 sec after TOH injection, and the other half 30 sec after ^{86}Rb and 15 sec after TOH injection. The lamb was removed from the chamber and dissection was commenced within 5 min of ^{86}Rb injection. The kidneys, perirenal adipose tissue, the liver, heart and spleen, were rapidly removed. Skin on the ear, the lower leg (over the metatarsus) and the midside was closely clipped and samples of approximately 0·5–1 g were taken. The gut was removed and separated into stomach, small intestine and large intestine, and the ends were tied to avoid loss of contents. Muscle was removed from the hind leg; in 1968 a mass of muscle was taken from both legs without regard to identity, but in 1969 the sample was composed of semitendinosus, semimembranosus, gastrocnemius, vastus lateralis, gluteus medius and longissimus dorsi, all on the side contralateral to the femoral arterial cannula. Finally, the brain was removed and stored in an air-tight jar at −15° C.

The larger organs were placed in preweighed plastic bags immediately after removal, and the skin samples were placed in preweighed counting tubes (Packard Instrument Co., Illinois); containers plus contents were weighed. The larger organs were minced, care being taken to prevent contamination from one specimen to another, and duplicate samples of approximately 2 g of well mixed mince were weighed into Packard counting tubes. Since some of the radioactivity passes into the gut lumen, gut contents were removed and weighed separately and then mixed with the minced gut wall prior to sampling for counting; gut flow was calculated on the basis that all the radioactivity was in the wall. These samples together with a suitably diluted aliquot of the original [^{86}Rb]Cl solution were counted for 10 or 20 min with a single channel of a Spectrometer (Packard Auto-gamma model 5212). The 'relative blood flow' through the various tissues was computed as:

$$\frac{\text{Blood flow per g tissue}}{\text{Cardiac output per g body weight}} = \frac{\text{Counts per unit time per g of tissue}}{\text{Counts per unit time in } ^{86}\text{Rb injected, per g of body weight}}.$$

Absolute organ blood flow was then calculated by multiplying this ratio with the mean of the estimates of cardiac output obtained during the test period. Total blood flow through the liver was estimated as the sum of hepatic arterial and portal flows; portal flow was estimated as the sum of the individual flows through stomach, intestine and spleen (each uncorrected for organ weight), divided by the weight of the liver.

For the estimation of brain blood flow, brains were allowed to thaw and approximately 1 ml. water was collected as described by Chatwin *et al.* (1969). TOH standards and aliquots of 0·1 ml. distillate were counted in a liquid scintillation counter (Packard, Tricarb, model 3375) after dilution with 5 ml. of a scintillation mixture. Relative blood flow through the brain was calculated by the same method as blood flow through the other organs.

Relative and absolute organ blood flow and changes (test minus control period) in metabolic rate, cardiac output, blood pressure and heart rate were examined by conventional analyses of variance.

Sources of variation and the associated degrees of freedom, in the analyses of organ blood flow are shown in Table 4. In most other analyses effects of years and killing time were ignored, so that in each group there were four lambs under similar thermal and infusion treatment.

Resistance to blood flow. The mean blood pressure during the test period in each group of four lambs was divided by the mean absolute blood flow in each organ, to provide a measure of the resistance to flow and an indication of whether the treatment caused vasodilation or vasoconstriction.

RESULTS

Metabolic rate

Resting metabolism. Resting oxygen consumption was consistently increased by infusion of 1 μg/kg.min of adrenaline whereas there was a corresponding decrease in each lamb that received saline only (Table 1, $P < 0{\cdot}05$). There was also a small but less consistent increase in lambs treated with 1 μg/kg.min of noradrenaline. In contrast, 10 μg/kg.min of adrenaline tended to decrease metabolic rate, while the same infusion rate of noradrenaline clearly increased metabolic rate ($P < 0{\cdot}01$).

Mean oxygen consumption ($\pm$ S.E. of mean), during the control period in all twenty lambs under thermoneutral conditions was $1{\cdot}30 \pm 0{\cdot}05$ l.O_2/kg.hr which is about 0·3 l. O_2/kg.hr higher than is usually expected in new-born lambs (e.g. Alexander & Williams, 1968). This is readily explained by the absence of any preliminary period for the lambs to settle down, which also explains the reduction in metabolic rate in control lambs during the sham test period (Table 1).

Summit metabolism. Infusion of 1 μg/kg.min of either catecholamine, produced little effect on summit metabolism, although the mean decline tended to be less in these two groups than in the control lambs (Table 1). However, infusion of 10 μg/kg.min of catecholamine, particularly of adrenaline, reduced summit metabolism substantially. Mean summit meta-

bolism during the control period in the twenty lambs was 3·71 ± 0·09 l. O_2/kg.hr, and there was a mean decline of 0·13 l. O_2/kg.hr in the four lambs that received saline only. All these results are consistent with previous data (Alexander, 1969).

TABLE 1. Change in metabolic rate due to treatment with catecholamines under thermoneutral and summit conditions

Treatment	Change in oxygen consumption (l. O_2/kg.hr)†‡§	
	Thermoneutral conditions	Summit conditions
Saline only	−0·19	−0·13
Adrenaline 1 μg/kg.min	+0·49*	−0·09
Adrenaline 10 μg/kg.min	−0·43	−1·36*
Noradrenaline 1 μg/kg.min	+0·28	+0·03
Noradrenaline 10 μg/kg.min	+0·79*	−0·67
5% L.S.D.‖	0·65	1·03
1% L.S.D.‖	0·91	1·42

† The change in each animal was expressed as the difference between the average value obtained during the control period and the average value obtained during the test period.

‡ Mean oxygen consumption (± S.E. of mean) during the control periods was 1·30 ± 0·05 l. O_2/kg.hr under thermoneutral conditions and 3·71 ± 0·09 l. O_2/kg.hr under summit conditions.

§ The values shown each represent the mean for four animals.

‖ Difference between means which must be exceeded if difference to achieve significance at 1 or 5% level of probability (*, $P < 0·05$; **, $0·05 > P > 0·01$). Significances shown refer only to differences from 'saline only' group.

Cardiac output

Comparison of methods. Under resting conditions, mean cardiac output (per unit of body weight) during the control period was significantly higher ($P < 0·01$) when determined by the Fick method than by the dye method (Table 2), but the discrepancy was not so great under summit conditions. Possible reasons for this methodological discrepancy are discussed elsewhere (Alexander & Williams, 1970). Estimates by the two methods were positively correlated (coefficients of correlation for thermoneutral and summit conditions and for the pooled data were 0·82, 0·48 and 0·74); variability between animals tended to be greater in the Fick estimates than in the dye estimates. Results of analysis of treatment effects were somewhat dependent on the method for estimating cardiac output; and the results presented (Table 2) are therefore based on estimates by each method as well as on the mean of the two estimates.

Thermoneutral conditions. Infusions of 1 μg/kg.min of adrenaline tended

TABLE 2. Change in cardiac output due to treatment with catecholamines under thermoneutral and summit conditions

Treatment	Mean change in cardiac output (ml./100 g.min)†§¶					
	Thermoneutral conditions			Summit conditions		
	Fick	Dye	Mean	Fick	Dye	Mean
Saline only	−5·2	+0·6	−2·3	−3·4	−0·3	−1·9
Adrenaline 1 μg/kg.min	+9·8	+17·1*	+13·5*	+0·1	+2·3	+1·2
Adrenaline 10 μg/kg.min	−27·2	−15·8*	−21·5*	−29·5***	−20·9*	−25·2***
Noradrenaline 1 μg/kg.min	−5·2	+6·3	+0·5	+1·3	−1·8	−0·2
Noradrenaline 10 μg/kg.min	−4·0	+17·9**	+7·0	−13·7	−6·9	−10·3
5% L.S.D.¶	23·2	12·1	15·6	11·5	15·3	12·0
1% L.S.D.¶	32·2	16·8	21·6	15·8	21·1	16·6
(Mean cardiac control ± S.E. of mean during control period)	(44 ± 3)	(35 ± 3)	—	(66 ± 4)	(68 ± 4)	—

*** $P < 0·001$

¶ Cardiac output is expressed in terms of ml. blood/100 g. body wt. min.
For key to other symbols see footnote to Table 1.

to increase cardiac output (Table 2), whereas the higher dose clearly decreased cardiac output. The low dose of noradrenaline was without consistent effect while the high dose increased cardiac output, but only as determined by the dye method.

Summit conditions. Cardiac output was clearly increased by cold exposure (Table 2), as indicated above, and the magnitude of the increase was consistent with that in earlier results (Alexander & Williams, 1970). Infusions of either catecholamine, 1 μg/kg.min, were without consistent effects, but adrenaline, 10 μg/kg.min, markedly depressed cardiac output (Table 2); noradrenaline, 10 μg/kg.min, also tended to depress cardiac output but less consistently than adrenaline.

Blood pressure, heart rate and the electromyogram

Thermoneutral conditions. Mean blood pressure (diastolic plus one third of pulse pressure) increased in all lambs during infusion of catecholamines and the increase was clearly greater at the higher than at the lower doses (Table 3). Changes in heart rate were less consistent (Table 3) but the higher doses of both catecholamines tended to increase heart rate.

Summit conditions. Blood pressure and heart rate were increased by exposure to cold conditions, as previously reported (Alexander & Williams, 1970). There was little or no effect of catecholamines at the low infusion rates upon blood pressure, but pressures were clearly increased by 10 μg/kg.min of either catecholamine. Heart rates fell in all cold exposed animals treated with catecholamines and the fall was greater after 10 than after 1 μg/kg.min (Table 3).

Shivering as indicated by the electromyogram was considerably reduced in several lambs treated with 10 μg/kg.min of catecholamines, but this trend was not statistically significant.

Regional blood flow

Analysis of variance showed that estimates of relative blood flow were virtually independent of killing time, and independent of the year of the experiment, except for skeletal muscle, leg-skin and stomach, but these differences were small in comparison with the effects of environment and catecholamine treatment. The differences no doubt arise from slightly different sampling procedures in the 2 years, particularly for muscle, and these sources of variation are not considered further. The absence of significant differences between the animals killed at the two times indicates that there were no marked departures from Sapirstein's criterion that the indicator content of each tissue examined is constant at the time of killing; the use of the technique under these conditions therefore appears valid (Sapirstein, 1958; Chatwin *et al.* 1969).

TABLE 3. Change in blood pressure and heart rate due to treatment with catecholamines under thermoneutral and summit conditions

	Mean blood pressure§ (mm Hg)				Mean heart rate§ (beats/min)			
	Thermoneutral conditions		Summit conditions		Thermoneutral conditions		Summit conditions	
Treatment	During control period	Change†	During control period	Change†	During control period	Change†	During control period	Change†
Saline only	79·0	−1·5	87·5	−1·0	206	+5	287	−4
Adrenaline 1 μg/kg.min	74·8	+23·7**	90·0	+0·5	200	+13	294	−43*
Adrenaline 10 μg/kg.min	73·5	+55·8**	93·5	+49·8**	174	+71	294	−110**
Noradrenaline 1 μg/kg.min	80·8	+23·0**	93·3	+2·0	197	−40	304	−24
Noradrenaline 10 μg/kg.min	77·0	+48·8**	89·5	+36·0**	194	+60	306	−87**
5% L.S.D.‖	—	11·9	—	10·1	—	70	—	31
1% L.S.D.‖	—	16·5	—	14·0	—	97	—	43

For key to symbols see footnotes to Table 1.

The estimates of absolute flood flow depend to some extent on the method used for estimating cardiac output since absolute flow was calculated from relative flow multiplied by cardiac output, but in general the direction of change in absolute flow due to treatment was independent of the method. The absolute flows in Table 4 are therefore based on the average of the cardiac output as estimated by the Fick and dye methods. Changes in relative blood flow did not always reflect changes in absolute blood flow since environmental and catecholamine treatment altered cardiac output; changes in relative blood flow are, in general, mentioned below only where their direction appears to differ from that of changes in absolute flow.

Effect of environment. In almost all organs there was clear evidence that relative blood flow was dependent upon the environmental conditions. In the liver (hepatic artery flow), kidneys, spleen and gut and in the three skin samples relative blood flow was lower during cold exposure than under thermoneutral conditions, while in the skeletal muscle, and possibly the cardiac muscle, the difference was in the reverse direction, but there was no consistent trend in the peri-renal adipose tissue or brain. However, the absolute flow in the liver, kidneys, spleen, gut and brain did not change greatly upon cold exposure, while absolute flow through the peri-renal adipose tissue was doubled (Table 4), and there was a threefold increase in flow through cardiac muscle and a fourfold increase in skeletal muscle.

Effect of catecholamines. Treatment with catecholamines, particularly with adrenaline, 10 μg/kg.min, clearly affected the distribution of cardiac output under thermoneutral and cold conditions (Table 4).

In the skin, effects were somewhat dependent on the sampling site, but in general 10 though not 1 μg adrenaline/kg.min, given under thermoneutral conditions markedly reduced flow, while 1 but not 10 μg noradrenaline/kg.min, increased flow. Under cold conditions the same trends occurred in midside skin, but the ear flow was somewhat increased by all treatments, particularly by noradrenaline, 10 μg/kg.min.

In the skeletal muscle, blood flow was reduced by 10 μg/kg.min of either catecholamine under summit conditions. Under thermoneutral conditions the high dose of adrenaline also tended to reduce flow, but the low dose tended to increase flow.

Kidney blood flow was generally reduced by the catecholamines, particularly under cold conditions. The greatest reduction was produced by adrenaline, 10 μg/kg.min; and as with muscle flow the same dose of noradrenaline produced marked effects only under cold conditions.

Although relative blood flow through liver (hepatic artery) was increased by infusion of adrenaline, 10 μg/kg.min, absolute flow (hepatic, portal and

TABLE 4. Blood flow through various tissues and organs of lambs treated with catecholamines under thermoneutral and summit conditions

Mean blood flow [ml./100 g tissue.min]§

	Cardiac output	Skin (ear)	Skin (leg)	Skin (mid-side)	Skeletal muscle	Kidney	Liver			Spleen	Cardiac muscle	Peri-renal adipose tissue	Stomach	Small intes-tine	Large intest-tine	Brain
							Hepatic	Portal	Total							
Thermoneutral conditions																
Saline only	36	27	14	25	21	323	62	230	292	75	158	71	61	257	102	83
Adrenaline 1 μg/kg.min	51	42	20	26	38	352	74	293	367	101*	282*	95	95	234	158	92
Adrenaline 10 μg/kg.min	23	5*	3	8*	11	103**	52	128	180	40**	258	57	38	103	71	86
Noradrenaline 1 μg/kg.min	38	46*	30*	32	20	289	56	211	267	75	186	81	59	267	134	82
Noradrenaline 10 μg/kg.min	47	24	23	26	23	305	85	253	338	86	407**	114	74	260	100	138*
Summit conditions																
Saline only	72	8	3	19	92	348	94	346	440	72	480	145	95	340	129	134
Adrenaline 1 μg/kg.min	64	10	3	16	81	268	83	283	366	59	361	105	66	238	104	123
Adrenaline 10 μg/kg.min	40**	11	4	8	37**	132**	72	198	270	41*	366	66**	69	171	74	124
Noradrenaline 1 μg/kg.min	67	19	7	26	90	293	75	239	314	57	387	118	62	222	120	126
Noradrenaline 10 μg/kg.min.	58	30*	4	14	55**	180**	95	247	342	75	442	119	78	211	101	170
5% L.S.D.‖	16	19	14	14	19	106	35	140	165	23	123	48	34	204	67	47
1% L.S.D.‖	21	26	19	20	26	144	48	190	224	31	167	65	46	277	91	64
Analysis of variance																
Source of variation	Degrees of freedom															
Treatment (1)	4	***	**	*	**	***	***	N.S.	N.S.	N.S.	***	*	*	N.S.	N.S.	N.S.	*
Environment (2)	1	***	**	***	*	***	N.S.	*	N.S.	N.S.	**	***	*	N.S.	N.S.	N.S.	***
1×2	4	N.S.	*	N.S.	N.S.	**	N.S.	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.
Killing time (3)	1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.
1×3	4	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
2×3	1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Years	1	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Error	23																
Total	39																

For key to symbols see footnote to Tables 1 and 2.

total) tended to decrease, particularly after the high dose of adrenaline, but in general changes in liver flow were small.

In heart muscle (ventricles) high doses of catecholamines produced an increase in relative blood flow; and all treatments, particularly 10 μg/kg.min of noradrenaline, tended to increase absolute flow under thermoneutral conditions while under cold conditions all treatments tended to decrease flow.

In both environments, relative blood flow in brain was increased by the high doses of catecholamines, but absolute flow was increased only by 10 μg/kg.min of noradrenaline.

Changes in blood flow in the remaining organs examined (spleen, adipose tissue, stomach, small and large intestine) were small; but as with muscle, kidney, liver and midside skin, flows were usually smallest after 10 μg/kg.min of adrenaline than after the other treatments, both under thermoneutral and summit conditions. Under thermoneutral conditions absolute blood flow through the peri-renal adipose tissue was increased by 10 μg/kg.min noradrenaline, though not significantly.

Resistance to blood flow

On exposure to cold there was a marked increase in calculated resistance to flow, and hence vasoconstriction, in the skin, particularly on the ear and leg, while in the skeletal and cardiac muscle and adipose tissue, and possibly in the liver, stomach and brain there was a decrease in resistance indicating vasodilation (Table 5). The net effect of cold exposure was to produce a marked decrease in over-all peripheral resistance (Table 5).

Infusion of 10 μg/kg.min adrenaline in either environment clearly produced a generalized increase in resistance and hence vasoconstriction, while 10 μg/kg.min of noradrenaline given in cold conditions produced similar though less marked effects in all organs except the ear which was apparently dilated (Table 5). The lower doses of catecholamines also appeared to produce a smaller degree of generalized vasoconstriction under cold conditions but there was some evidence that noradrenaline produced vasodilation of the ear and leg skin.

DISCUSSION

The results show that the redistribution of cardiac output due to cold exposure in new-born lambs is qualitatively similar to that in other animals such as the new-born rabbit (Járai, 1969) and adult laboratory rat (Jansky & Hart, 1968).

The redistribution is of clear advantage for survival. The reduced flow to the skin favours heat conservation, and the increased flow to skeletal

TABLE 5. Resistance to blood flow (blood pressure ÷ blood flow) through various organs and tissues of lambs treated with catecholamines under thermoneutral and summit conditions

	Mean flow resistance‡ [mm Hg ÷ ml/100 g.min]															
							Liver									
Treatment	Whole body	Skin (ear)	Skin (leg)	Skin (mid-side)	Skeletal muscle	Kidneys	Hepatic	Portal	Total	Spleen	Cardiac muscle	Peri-renal adipose tissue	Stomach	Small intes-tine	Large intes-tine	Brain
Thermoneutral conditions																
Saline only	2·1	2·9	5·7	3·1	3·7	0·2	1·3	0·3	0·3	1·0	0·5	1·1	1·3	0·3	0·8	0·9
Adrenaline 1 μg/kg.min	2·0	2·3	4·8	3·8	2·6	0·3	1·3	0·3	0·3	1·0	0·4	1·0	1·0	0·4	0·6	1·1
Adrenaline 10 μg/kg.min	5·8	24·6	50·1	16·0	11·8	1·3	2·5	1·0	0·7	3·3	0·5	2·3	3·4	1·3	1·8	1·5
Noradrenaline 1 μg/kg.min	2·8	2·3	3·5	3·3	5·3	0·4	1·9	0·5	0·4	1·4	0·6	1·3	1·8	0·4	0·8	1·3
Noradrenaline 10 μg/kg.min	2·7	5·3	5·6	4·8	5·4	0·4	1·5	0·5	0·4	1·5	0·3	1·1	1·7	0·5	1·3	0·9
Summit conditions																
Saline only	1·2	11·0	25·3	4·6	1·0	0·3	0·9	0·3	0·2	1·2	0·2	0·6	0·9	0·3	0·7	0·7
Adrenaline 1 μg/kg.min	1·4	8·9	35·2	5·8	1·1	0·3	1·1	0·3	0·3	1·5	0·3	0·9	1·4	0·4	0·9	0·7
Adrenaline 10 μg/kg.min	3·6	13·3	38·5	19·1	3·9	1·1	2·0	0·7	0·5	3·5	0·4	2·2	2·1	0·8	1·9	1·2
Noradrenaline 1 μg/kg.min	1·4	5·1	14·3	3·7	1·1	0·3	1·3	0·4	0·3	1·7	0·3	0·8	1·5	0·4	0·8	0·8
Noradrenaline 10 μg/kg.min	2·2	4·2	30·7	8·7	2·3	0·7	1·3	0·5	0·4	1·7	0·3	1·1	1·6	0·6	1·3	0·7

‡ Calculated from means in Tables 3 and 4 and used as crude indication of vasoconstriction or vasodilatation. These data were not examined statistically.

muscle, brown fat and heart favour increased metabolism. The relatively rapid flow through the small intestine (also seen by Járai (1969) in new-born rabbits) was well maintained during cold exposure, and is presumably associated with the need for rapid digestion in a small rapidly growing but cold-susceptible animal.

The twofold increase (from 70 to 145 ml./100 g.min) in blood flow through the peri-renal adipose tissue (brown fat) in lambs exposed to cold was considerably less than the three- to fourfold, increase (from 90 to 306 ml./100 g.min) found by direct collection of blood from the cervical brown fat of new-born rabbits (Heim & Hull, 1966) and the fivefold increase (from 36 to 180 ml./100 g.min) in the same tissues as found by Járai (1969) using radioactive microspheres.

Blood flow and changes in blood flow in brown fat of new-born lambs exposed to cold were of the same magnitude as determined in the brown fat of adult rats by Jansky & Hart (1968) and Kuroshima, Konno & Itoh (1967) using Sapirstein's method. However, comparisons between species are difficult to interpret without an indication of the degree of elevation in metabolic rate produced by the cold exposure. Certainly in lambs the flow changes observed are likely to have been maximal since changes in metabolic rate were maximal.

While the flow changes in the brown fat of cold-exposed lambs were small, the changes in muscle were large (from 21 to 91 ml./100 g.min) in comparison with those in new-born rabbits (from 12 to 17 ml./100 g.min bone included – Járai, 1969) and in adult rats (Jansky & Hart, 1968). Thus in the lamb which relies largely on shivering for its thermogenic response to cold (Alexander & Williams, 1968) the circulatory adjustments on cold exposure appear to favour muscle, while in the new-born rabbit which relies principally on non-shivering thermogenesis, the adjustments favour brown adipose tissue.

In addition to the effects of cold exposure there were marked effects of catecholamine infusions, particularly of 10 μg/kg.min of adrenaline. Under thermoneutral conditions, metabolic rate tended to increase after each treatment except infusion of 10 μg/kg.min of adrenaline. The increases are consistent with previous work (Alexander & Williams, 1968) and are probably due to stimulation of brown fat metabolism (Hull, review, 1966). In conformity with earlier trends (Alexander, 1969) the responses appear to be dose dependent, those due to noradrenaline being higher at 10 than at 1 μg/kg.min, but those due to adrenaline showing the reverse trend. The tendency for 10 μg/kg.min of adrenaline, given under thermoneutral conditions, to depress both metabolic rate and cardiac output was unexpected and may have been partly due to suppression of voluntary muscular activity, through vasoconstriction, rather than to a reduction in

true basal metabolism or resting cardiac output. However, it can be calculated from Tables 2 and 3 that this treatment markedly reduced cardiac stroke volume (from about 10–3 ml.); and since blood pressure rose the treatment appears to have produced a most unusual haemodynamic situation. The changes in stroke volume produced by the other treatments were small and equivocal.

The increase in cardiac output after 1 μg/kg.min of adrenaline under thermoneutral conditions, but not after the same dose of noradrenaline is consistent with findings in adult rats (Takács, 1965), but the small changes after either dose of noradrenaline (Table 2) contrast with the substantial increase from 27 to 41 ml./100 g.min in new-born rabbits stimulated with 2 μg/kg.min of noradrenaline (Heim & Hull, 1966).

Changes in blood flow due to catecholamine infusion under thermoneutral conditions varied widely according to the amine, the dose, and the organ examined. The marked and consistent reduction in cardiac output and in organ blood flow due to 10 μg/kg.min adrenaline clearly resulted from generalized vasoconstriction in all organs but the heart (Table 5), while the tendency for one tenth of this dose to increase flow in skeletal muscle was apparently due to vasodilatation. This dose dependent action of adrenaline in muscle is well known in other species and is explained in terms of a greater sensitivity of β-sympathetic receptors, that produce vasodilatation, than of α-receptors that produce vasoconstriction (Celander, 1954).

Effects of noradrenaline, given at either infusion rate under thermoneutral conditions, were generally less marked, although 10 μg/kg.min appeared to produce some constriction in all organs except the heart, adipose tissue and brain. This dose also appeared to have a direct effect on heart action, since cardiac output (at least as determined by the dye method) increased in the face of a general increase in resistance to flow (Tables 2 and 5); a similar result has been reported in rats (Evonuk & Hannon, 1963). Infusion of 1 but not of 10 μg/kg.min noradrenaline increased flow and dilated the extremities, while 10 but not 1 μg/kg.min caused dilation and a great increase in flow in cardiac muscle. Both doses of noradrenaline produced vasoconstriction in skeletal muscle. Noradrenaline increased flow through the brown fat only by 60% and apparently does not cause vasodilatation there, contrasting markedly with the finding of Heim & Hull (1966) of a fourfold increase (from 87 to 360 ml./100 g.min) in new-born rabbits infused with 2 μg/kg.min noradrenaline; substantial increases have also been reported to occur in adult rats infused with 2 μg/min (Kuroshima *et al.* 1967; Evonuk & Hannon, 1963).

Under summit conditions where metabolic rate, cardiac output and its distribution were already considerably altered by cold exposure, the

effects of treatment were generally different from those under thermoneutral conditions. Low doses of catecholamines did not affect metabolic rate or cardiac output, but high doses depressed both parameters; the effects on metabolic rate are consistent with previous data (Alexander, 1969). The depression of metabolic rate and cardiac output was clearly associated with generalized vasoconstriction and a decrease in flow through the thermogenic tissues (muscle and brown fat). Changes in all these parameters were less marked after noradrenaline than after adrenaline treatment, when flows through fat and muscle were more than halved. The lower doses of catecholamines also tended to produce generalized vasoconstriction and flow reduction, but to a very much smaller extent than 10 μg/kg.min. There was, however, some evidence of vasodilation in the extremities due to both infusion rates of noradrenaline.

Some of the catecholamine effects that depend on environmental conditions may be due to a background of adrenal medullary secretion under cold conditions. For example, skeletal muscle in which flow was apparently increased by adrenaline at 1 μg/kg.min in warm conditions, may, under cold conditions, already have responded maximally to endogenous adrenaline. However, the massive increase in flow that occurs in some organs on cold exposure does not seem explicable by endogenous adrenaline or noradrenaline secretion, since the effects of catecholamines under thermoneutral conditions were relatively small. It would be desirable, nevertheless, to examine the effects of physiological mixtures of catecholamines and also to test the effects of related compounds; in new-born lambs the maximum catecholamine output of the adrenal glands is about 1 μg/kg.min of a mixture of noradrenaline and adrenaline in the ratio of 1:2 (Comline & Silver, 1961).

Whatever the explanations of the observed effects, it is clear that catecholamine responses may be considerably modified by thermal conditions.

The results provide likely explanations of some of the previously observed effects of catecholamines on the metabolism of new-born animals. For example, adrenaline is consistently as effective as noradrenaline in stimulating metabolic rate in the new-born guinea-pig (Dawes & Mestyán, 1963), but only sometimes in the lamb (Alexander, 1969), and barely at all in the kitten (Moore & Underwood, 1960; Scopes & Tizard, 1963). It now seems likely that these differences are due, at least in part, to differences in sensitivity to the vasoconstrictive effects of adrenaline on thermogenic tissue, the guinea-pig presumably being least sensitive, the kitten most sensitive and the lamb variably sensitive in the dose range used (1–10 μg/kg.min). Adrenaline also suppresses the metabolic response of new-born rabbits and kittens to cold (Dawes & Mestyán, 1963; Scopes & Tizard, 1963) and it seems likely that this is due to vasoconstriction in brown fat,

which may also explain the rapid decline in the concentration of free-fatty acids in the plasma of a proportion of lambs following a single I.V. injection of adrenaline 25 μg/kg (Alexander & Mills, 1968).

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