J. Physiol. (1977), **265**, *pp.* 743–762
With 8 text-figures
Printed in Great Britain

THE DEVELOPMENT OF SOME METABOLIC RESPONSES TO HYPOXIA IN THE FOETAL SHEEP

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(*Received 14 July 1976*)

SUMMARY

1. Foetal and maternal plasma metabolite and catecholamine concentrations have been measured in chronically catheterized sheep, 95–145 days pregnant.

2. With increasing gestational age there was rise in foetal plasma lactate, free fatty acid and ketone body concentration and in maternal plasma in free fatty acid and ketone body concentration. With the exception of α-amino nitrogen none of the plasma metabolites showed any correlation with foetal blood gas or pH values; α-amino N was inversely related to foetal blood pH.

3. Hypoxia in the foetuses was induced by causing the ewe to breathe 9 % O_2 with 3 % CO_2 in N_2. This had a small effect on plasma metabolites in the ewe, mainly producing an increase in free fatty acid and ketone body concentration.

4. In the foetus hypoxia was associated with a large rise in plasma lactate and a small rise in α-amino N, the magnitudes of which did not change over the gestational range studied. Consistent and large increases in foetal plasma glucose, free fatty acid and ketone body concentration in response to hypoxia were seen only between 130 and 145 days.

5. In foetuses of 130–145 days the magnitude of the hypoxia-induced rise in plasma glucose and free fatty acid concentration was proportional to the plasma catecholamine concentration.

6. The concentration of acetate in foetal plasma was lower than and proportional to that in the maternal plasma. Neither concentration changed significantly during hypoxia.

7. The results are discussed in relation to the ability of the foetal sheep independently to control the concentration of its plasma metabolites and to mobilize its carbon stores at times of need. They indicate that in the sheep plasma catecholamines are important regulators of plasma glucose and free fatty acid concentrations late in foetal life.

INTRODUCTION

During foetal life large changes occur in tissue and plasma composition and particularly in the concentration of energy stores such as glycogen (Dawes & Shelley, 1968) and triglyceride (Widdowson, 1950; Roux & Yoshioka, 1970; Shelley & Thalme, 1970; Edson, Hudson & Hull, 1975; Jones, 1976). These stores are essential for early neonatal survival. The extent to which they are mobilized in foetal life and contribute to maintaining an adequate supply of metabolic substrates for the foetal tissues at times of need is not clear. Insulin does in part regulate foetal blood glucose utilization and foetal pituitary hormones are important for glycogen deposition and lipid mobilization (Shelley, 1973; Bassett, Madill, Nicol & Thorburn, 1973; Jost & Picon, 1970; Jones, 1976). In addition, adrenaline will stimulate glycogen break-down and fatty acid release from foetal tissues (Bassett & Jones, 1976; Jones, 1976). This suggests that the foetus should be capable of mobilizing and affecting deposition of its energy stores at times of increased demand, as is shown in the anoxic exteriorized foetus (Dawes, Mott & Shelley, 1959).

In the present experiments the stimulus of hypoxaemia has been used to study the development of the ability of the foetus to change the plasma concentration of a number of metabolic substrates as an indication of the development of a degree of metabolic autonomy in the foetal sheep.

METHODS

Animal handling. Forty-four sheep of various breeds, 95–145 days pregnant were used. Vascular catheters were implanted into each ewe and one of its foetuses as previously described (Dawes, Fox, Leduc, Liggins & Richards, 1972). A period of at least 4 days was allowed for the sheep to recover from the effects of surgery before the experiments were performed. Six of the pregnant sheep (109, 118, 123, 127, 135, 140 days) were used as controls in which air was administered for 60 min at 40 l./min into a polyethylene bag placed over the ewe's head. The remaining sheep were subjected to a 60 min period of hypoxia in which 9% O_2 and 3% CO_2 in N_2 instead of air was passed into the polyethylene bag. In all but two of these sheep only one period of hypoxia was given; in the other two the 60 min period of hypoxia was repeated once every 24 hr for 8 days. Thirty-five of the ewes had twins, the remainder with single foetuses showed responses that were not significantly different and these have been included. Since most of the pregnant sheep studied were subsequently used for other experiments complete information on the further course of pregnancy is not available. The two ewes subjected to repeated episodes of hypoxia did not deliver prematurely and gave birth to live healthy lambs of normal weight.

Blood sampling. Carotid artery blood samples (maternal 7 ml., foetal 4 ml.) were taken 120 and 10 min before, 10, 25 and 60 min during, and 10 and 60 min after the period of hypoxia, except for the two sheep subjected to repeated hypoxia when samples were collected 60 min before and at the end of 60 min of hypoxia.

Also in foetuses younger than 105 days 4 ml. blood samples were collected only at 60 min before, 25 and 60 min during, and 60 min after the period of hypoxia. At the same time samples were taken into glass syringes for immediate determination of pH, PO_2 and PCO_2 using a Model 27 Acid-Base Analyzer (Radiometer, Copenhagen).

The carotid arterial blood for metabolite and hormone determinations was collected into plastic syringes containing 15–20 u. heparin fluoride (Boots Pure Drug Co. Nottingham) transferred immediately to polystyrene tubes and placed on ice. Plasma was removed after centrifugation at 1500 *g* and 2° C for 20 min then stored at −2° C.

Hormone and metabolite assays

Catecholamines. Adrenaline and noradrenaline was assayed within 24 hr of sample collection by the fluorimetric method of Haggendahl (1962) as described by Jones & Robinson (1975).

Free fatty acids. Plasma free fatty acids were extracted by the method of Dole (1956) then determined by the colorimetric method of Duncombe (1963). Palmitate was used as a standard; its recovery in the Dole extraction was 92·3 ± 4·7 (6)% no significant phospholipid extraction occurred.

Glucose. Plasma for glucose determination was first deproteinized with 0·4 M-$HClO_4$ and the supernatant neutralized with 2 M-$KHCO_3$. After the removal by centrifugation of $KClO_4$ that precipitated after standing on ice for 30 min, glucose was assayed in the supernatant using glucose oxidase (Huggett & Nixon, 1957).

Lactate. Plasma was deproteinized as described for the glucose determination then lactate concentration was determined using lactic dehydrogenase (Gutmann & Wahlefeldin, 1974).

α-Amino N. Plasma was deproteinized as described for the glucose determination then the total α-amino N concentration was determined colorimetrically with trinitrobenzene sulphonic acid (Mokrasch, 1967). Glycine was used as the standard.

Acetate. Plasma was deproteinized as described for the glucose determination and the acetate concentration was determined using acetyl-CoA synthetase as described by Jones & Ashton (1976).

Ketone bodies. Fresh plasma was deproteinized as above then ketones determined with β-hydroxybutyrate dehydrogenase as described by Williamson, Mellanby & Krebs (1962).

Expression of results

Where appropriate the results are given as means ± S.D., with the number of observations in parentheses. Statistical significance was calculated using the Student's *t* test.

RESULTS

Blood gas and pH values

In the thirty-eight pregnant sheep and their foetuses the mean carotid blood P_{a,O_2} and pH values were 96·3 ± 8·1 and 7·44 ± 0·12 and 24·2 ± 4·1 mmHg and 7·35 ± 0·18 respectively. The changes during hypoxia were similar to those previously reported (Boddy, Dawes, Fisher, Pinter & Robinson, 1974) with values after 60 min of the administration of 9% O_2 with 3% CO_2 of 46·8 ± 4·9 mmHg and 7·43 ± 0·1 and 16·9 ± 3·8 mmHg

and 7·27 ± 0·14 for ewe and foetus respectively. The blood gas and pH changes observed in the various age groups were not significantly different (Table 1). The mean maternal and foetal carotid blood P_{a,CO_2} were 28·9 ± 3·1 and 42·4 ± 6·7 mmHg respectively; they did not change significantly during hypoxaemia.

TABLE 1. The changes in the carotid P_{a,O_2} and pH values and in the plasma catecholamine concentrations of foetal sheep during hypoxia

Gestational ages (days)	P_{a,O_2} (mmHg)	pH	Adrenaline (ng/ml.)	Noradrenaline (ng/ml.)
95–118	7·9 ± 2·8 (6)	0·06 ± 0·037 (6)	—	—
120–129	7·1 ± 2·4 (8)	0·04 ± 0·026 (8)	3·8 ± 4·0 (5)	8·0 ± 9·2 (5)
130–139	7·5 ± 1·9 (12)	0·059 ± 0·032 (12)	5·2 ± 5·1 (6)	8·3 ± 12 (6)
140–145	6·9 ± 3·4 (9)	0·068 ± 0·043 (9)	4·8 ± 3·6 (4)	10 ± 7·3 (4)

The values are the means ± S.D. (number of animals in parentheses) of the difference between the control value and the value after 60 min of administration of 9% O_2 plus 3% CO_2 in N_2 to the ewes.

In the six sheep administered air instead of the gas mixture the control values for P_{a,O_2}, pH and P_{a,CO_2} were 23·7 ± 2·1 mmHg and 98·7 ± 6·2 mmHg, 7·32 ± 0·11 and 7·42 ± 0·1 and 44·7 ± 8·4 mmHg and 29·2 ± 1·4 mmHg for the foetuses and ewes respectively. After 60 min of air administration they were 23·1 ± 3·1 mmHg and 97·3 ± 4·8 mmHg, 7·3 ± 0·13 and 7·45 ± 0·14 and 42·7 ± 4·2 mmHg and 28·4 ± 3·3 mmHg respectively.

Plasma catecholamines

In the control period plasma catecholamines were not consistently detectable with concentrations of normally < 50 pg/ml. for the ewes and < 100 pg/ml. for the foetuses. During hypoxia, as previously demonstrated (Jones & Robinson, 1975), both adrenaline and noradrenaline concentrations rose substantially; noradrenaline attained the highest concentration (Table 1). Between 120 and 145 days there were no significant changes in the extent of the catecholamine rise or in the adrenaline/noradrenaline ratio (Table 1), although there was substantial variation in the size of the responses.

Plasma glucose

Between 95 days and term there were no significant changes in foetal or maternal plasma glucose concentrations, the mean values were 0·99 ± 0·06 (30) and 3·72 ± 0·12 (30) μmole/ml. respectively. The small fluctuations in the normal plasma glucose concentration were not related to any natural variations in blood gas or pH values. During hypoxia foetal plasma glucose rose by an amount dependent on the stage of

pregnancy (Fig. 1). Before 120 days there was a gradual rise following a similar rise in the concentration in maternal plasma (Fig. 1*D*). At 120–129 days there was a similar small rise in the foetus but little change in the ewe (Fig. 1*C*), while at 130–140 days and particularly between 140 days and term there were large and progressive rises in foetal plasma

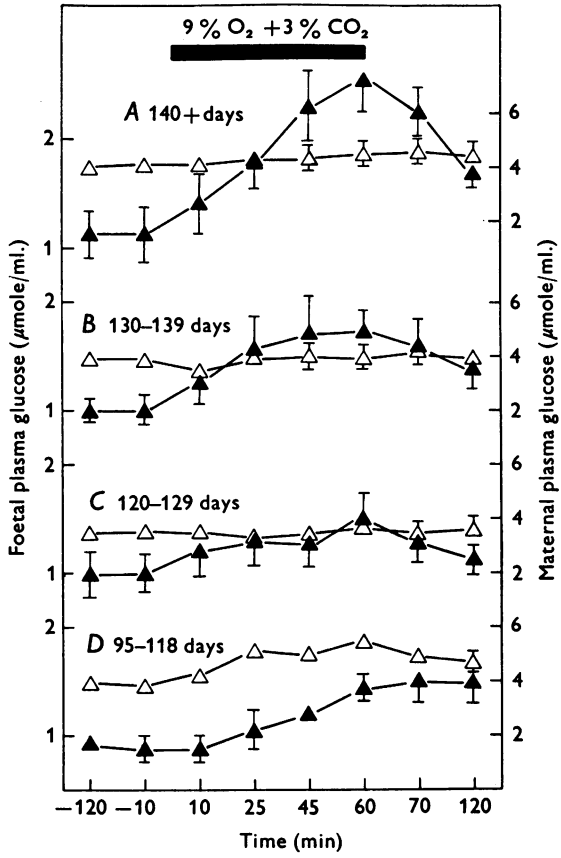

Fig. 1. Maternal and foetal carotid plasma glucose concentrations before, during and after the administration of 9% O_2 and 3% CO_2 in N_2 to the pregnant ewes. The stage of pregnancy was: *A*, 140+ days (n = 8); *B*, 130–139 days (n = 11); *C*, 120–129 days (n = 6); *D*, 95–118 days (n = 5). Foetal (▲); maternal (△); vertical bars = S.D. of means.

glucose concentration (Fig. 1*A* and *B*). This increased response between 120 days and term was also seen in two pregnant sheep given a 60 min period of hypoxia once every 24 hr (Table 2). Between 130 days and term the increase in foetal plasma glucose concentration was not related to the small variations in the fall in P_{a,O_2} or pH. It was linearly correlated

with the rise in plasma catecholamines (Fig. 2). Such a relationship could not be detected in maternal plasma since during hypoxia measurable rises in maternal plasma catecholamines were infrequently observed (Jones & Robinson, 1975).

During the administration of air to six sheep 109–140 days pregnant no consistent changes in maternal or foetal plasma glucose were observed.

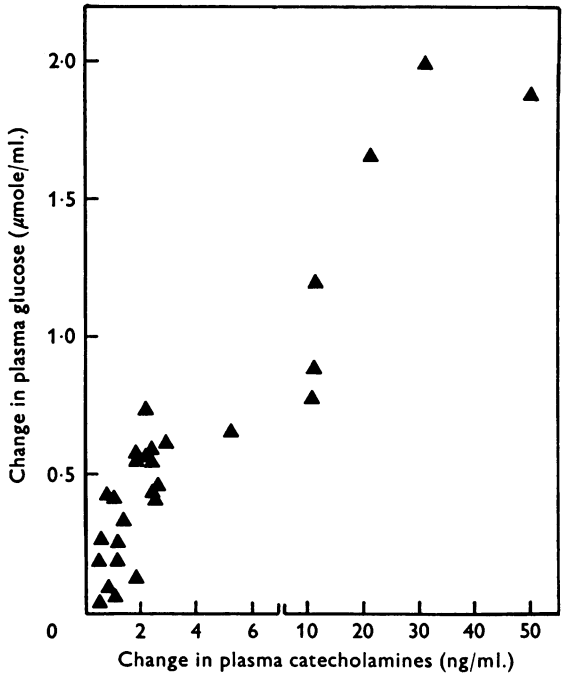

Fig. 2. The relationship between the change in plasma glucose and the rise in plasma catecholamine concentration in foetal sheep during hypoxia. Values for plasma glucose and plasma adrenaline plus noradrenaline were taken from foetuses of 130–145 days at 25, 45 and 60 min of hypoxia. Correlation coefficient, $r = 0{\cdot}90$ ($P < 0{\cdot}001$).

Plasma lactate

The lactate concentration in foetal plasma at 95–118 days and at 120–129 days was significantly lower ($P < 0{\cdot}05$ and $< 0{\cdot}01$ respectively) than at 140 days to term (Fig. 3*A*). No significant changes were observed in the ewes. The control values were not related to blood gas, pH or plasma glucose values in either the ewes or the foetuses. During hypoxia there was a large and progressive rise in the lactate concentration of foetal plasma (Fig. 4) that was similar at the four age-ranges studied. It was still very high 60 min after the period of hypoxia. At 5–8 hr after hypoxia

the mean value was 3·4 ± 1·1 (5) μmole/ml. and 24 hr after hypoxia it was within the normal range. The rise in plasma lactate was not related to the small variations in the extent of the fall in blood P_{a,O_2} or pH, nor was it related to the rise in plasma catecholamine concentration. The plasma lactate concentration of the ewes rose to a small extent during hypoxia and

TABLE 2. The changes in the concentration of glucose and free fatty acids in the plasma of foetal and pregnant sheep during hypoxia*

	Glucose (μmole/ml.)				Free fatty acids (μmole/ml.)			
	− 60 min†		+ 60 min†		− 60 min		+ 60 min	
Gestational age (days)	M	F	M	F	M	F	M	F
Ewe 14								
129	3·46	0·96	3·87	1·34	0·29	0·031	1·26	0·052
130	3·51	1·14	4·12	1·39	0·27	0·040	1·53	0·073
131	3·17	0·99	3·36	1·57	0·42	0·024	1·17	0·054
132	3·25	0·85	3·49	1·84	0·31	0·052	1·64	0·089
133	3·54	1·07	4·23	1·62	0·38	0·046	1·32	0·096
134	3·82	1·19	3·97	1·97	0·4	0·061	1·73	0·14
135	3·36	1·15	3·92	2·31	0·35	0·053	1·25	0·171
136	3·54	1·27	3·78	2·04	0·42	0·058	1·38	0·224
Ewe 100								
127	3·64	0·83	4·04	1·25	0·46	0·017	1·16	0·036
128	3·29	0·87	3·47	1·43	0·15	0·028	1·32	0·042
129	3·87	0·93	4·19	1·39	0·34	0·031	1·47	0·051
130	3·92	0·84	4·67	1·52	0·29	0·025	1·26	0·032
131	3·67	1·06	4·12	1·48	0·35	0·047	1·73	0·067
132	3·72	1·13	3·96	1·87	0·37	0·036	1·51	0·089
133	3·65	0·95	3·84	1·93	0·39	0·043	1·35	0·125
134	3·52	1·02	3·73	2·10	0·44	0·052	1·48	0·18

* Each ewe was given 9% O_2 + 3% CO_2 in N_2 to breathe for 60 min once every 24 hr for 8 days.

† Samples were taken 60 min before and at the end of the 60 min period of hypoxia.

M, maternal; F, foetal.

had returned to normal values within 60 min. In the foetuses made hypoxic for 60 min every 24 hr there was no significant change in the plasma lactate response between 127 and 136 days, nor was there a progressive rise in the control plasma lactate concentration.

There were no significant changes in maternal or foetal plasma lactate concentrations during the administration of air to four ewes 118–138 days pregnant.

Plasma free fatty acids

Before 130 days the plasma free fatty acid concentration in foetal sheep was very low (< 0·02 μmole/ml.); it rose significantly ($P < 0·001$)

between 95 days and term (Fig. 3 *B*). The concentration in maternal plasma was substantially higher and also rose significantly ($P < 0{\cdot}05$) between 95 days and term. During hypoxia the free fatty acid concentration in maternal plasma rose by about 200% at each age range studied (Fig. 5). In contrast the concentration in foetal plasma rose consistently only

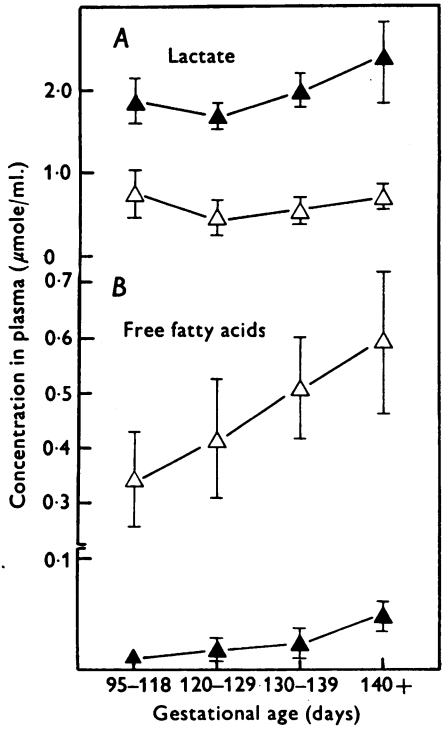

Fig. 3

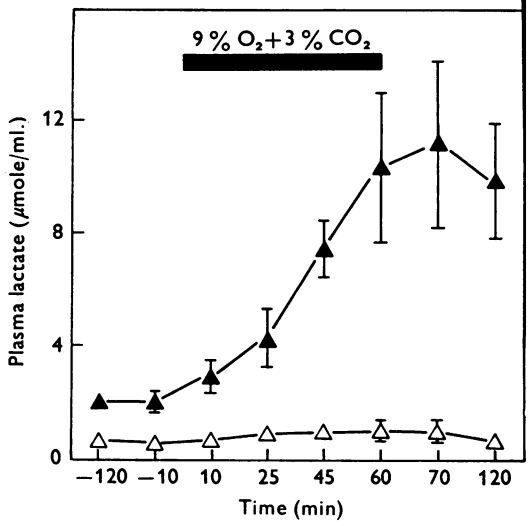

Fig. 4

Fig. 3. The changes in foetal and maternal carotid plasma lactate and free fatty acid concentrations during the latter third of pregnancy. *A*, lactate, *B*, free fatty acids. Foetal (▲); maternal (△); vertical bars = S.D. of mean.

Fig. 4. The foetal and maternal carotid plasma lactate concentration before, during and after the administration of 9% O_2 and 3% CO_2 in N_2 to pregnant ewes. The sheep were 130–140 days pregnant ($n = 11$). Foetal, (▲); maternal (△); vertical bars = S.D. of mean.

after 130 days and attained relatively high values only after 140 days (Fig. 5*A* and *B*). Between 130 days and term the rise in foetal plasma free fatty acid concentration was unrelated to the small variations in the extent of the P_{a,O_2} or pH fall nor was it consistently related to the rise in plasma glucose. It was linearly related to the rise in the catecholamine

concentration in foetal plasma that occurred during hypoxia (Fig. 6). The change in the response of the foetal plasma free fatty acid concentration to hypoxia was also observed in the two pregnant sheep subjected to repeated episodes of hypoxia between 127 and 136 days (Table 2).

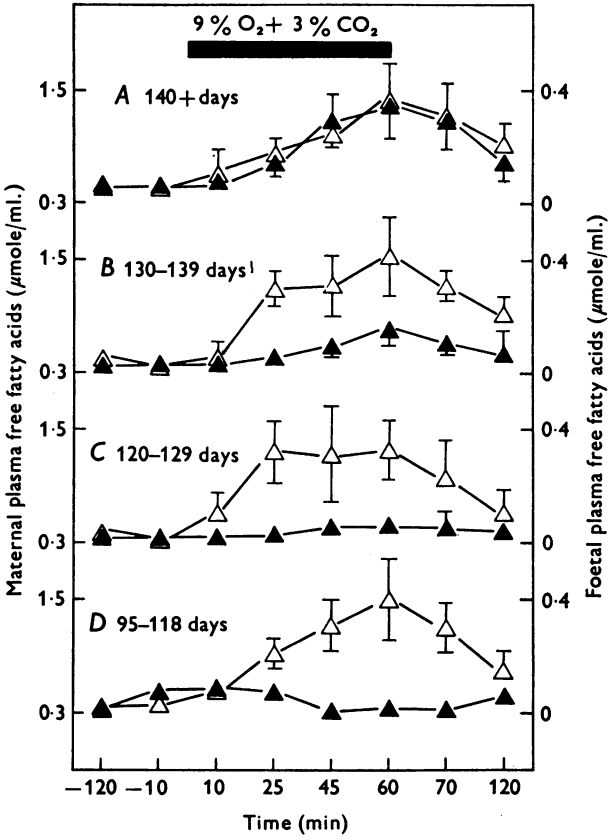

Fig. 5. The foetal and maternal carotid plasma free fatty acid concentration before, during and after the administration of 9 % O_2 and 3 % CO_2 in N_2 to pregnant ewes. *A*, 140 + days (n = 7); *B*, 130–139 days (n = 9); *C*, 120–129 days (n = 5); *D*, 95–118 days (n = 5). Foetal, (▲); maternal, (△); vertical bars = S.D. of mean.

No consistent changes were observed in maternal or foetal plasma free fatty acid concentrations during the administration of air to four ewes 118–138 days pregnant.

Plasma α-amino nitrogen

Between 95 days and term the normal plasma α-amino N concentration of the foetuses and the ewes did not vary significantly; the mean values

were 7·45 ± 1·32 (29) and 3·6 ± 0·41 (29) μmole/ml. respectively. The concentration in foetal plasma was inversely related to the blood pH (Fig. 7). During hypoxia there was about a 25% rise in the α-amino N concentration in foetal plasma but no change in maternal plasma. The foetal plasma concentration remained elevated for at least 1 hr after hypoxia.

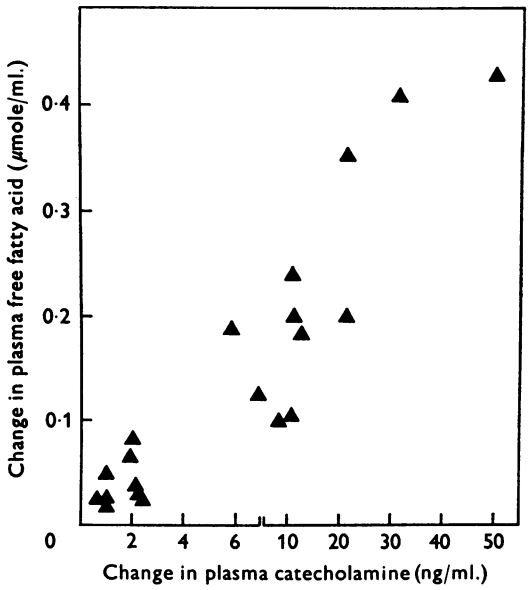

Fig. 6. Relationship between the change in plasma free fatty acid and the rise in plasma catecholamine concentration in the foetal sheep during hypoxia. Values for plasma free fatty acid and plasma adrenaline plus noradrenaline were taken from foetuses 130–145 days at 45 and 60 min of hypoxia. Correlation coefficient $r = 0{\cdot}92$ ($P < 0{\cdot}001$).

The magnitude of the rise did not change between 95 days and term. It did not correlate with any of the other measurements made and was not observed in pregnant sheep breathing air instead of 9% O_2.

Plasma ketone bodies

Between 95 days and term there was a small but significant rise in the concentration of β-hydroxybutyrate in maternal plasma. A similar increase was observed in foetal plasma although the concentration was much lower (Table 3). The concentration of acetoacetate in maternal plasma also rose during pregnancy so that the β-hydroxybutyrate/acetoacetate ratio did not change (6·42 ± 1·3 (14)). No acetoacetate (i.e. < 0·01 μmole/ml.) was detected in foetal plasma at any time during the control period. Hypoxia caused a rise in both the β-hydroxybutyrate and acetoacetate

concentration in maternal plasma (Fig. 8*A*) and a rise in the β-hydroxybutyrate/acetoacetate ratio (8·63 ± 1·51 (14), $P < 0·001$). The response did not change during the latter third of pregnancy (Table 1). Before 130 days there were small changes only in the β-hydroxybutyrate concentra-

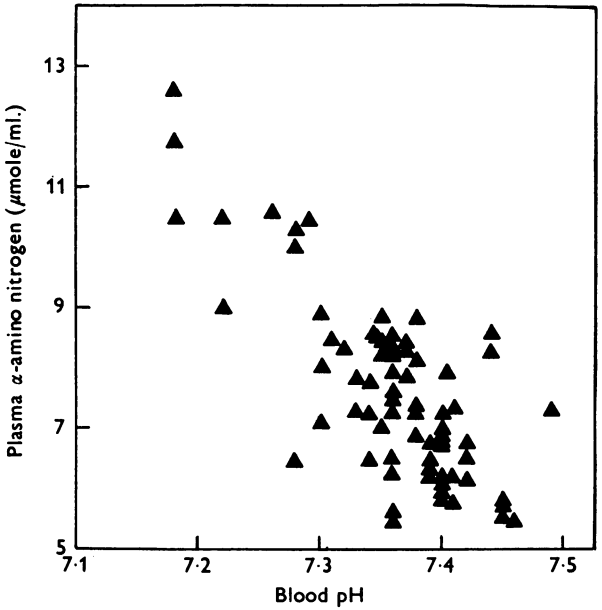

Fig. 7. The relationship between carotid arterial blood pH and plasma α-amino N concentration in foetal sheep. Gestational age range was 95–145 days. Correlation coefficient, $r = 0·73$ ($P < 0·001$).

tion of foetal plasma during hypoxia and no acetoacetate was detected (Table 3). Between 130 days and term hypoxia caused a threefold rise in β-hydroxybutyrate and acetoacetate was detected in foetal plasma (Fig. 8*B*). There was a linear relationship between the hypoxia-induced rise in free fatty acids and in ketone bodies in those foetuses between 130 days and term in which both metabolites were assayed (the plot of foetal carotid against maternal carotid concentration gave a regression line of $y = 1·42x - 0·03$; $r = 0·88$, $P < 0·001$; $n = 17$).

Plasma acetate

The mean concentration of acetate in the plasma of eleven adult and foetal sheep between day 110 and day 143 of pregnancy was 0·73 ± 0·24 and 0·45 ± 0·17 μmole/ml. respectively. There were no consistent changes either during the latter third of pregnancy or before, during or after the period of hypoxia. In those pregnant sheep maternal and foetal plasma

TABLE 3. The changes in foetal and maternal carotid plasma ketone body concentrations during the latter third of pregnancy

	Control (μmole/ml.)		Hypoxia (μmole/ml.)	
	Acetoacetate	β-hydroxybutyrate	Acetoacetate	β-hydroxybutyrate
Foetus (days)				
95–118	UD	0·017 ± 0·009 (3)	UD	0·025 ± 0·017 (3)
120–129	UD	0·039 ± 0·025 (4)	UD	0·074 ± 0·037 (4)
130–139	UD	0·046 ± 0·029 (5)	0·043 ± 0·026 (5)	0·16 ± 0·048 (5)
140–145	UD	0·071 ± 0·022 (5)	0·073 ± 0·035 (5)	0·18 ± 0·071 (5)
Ewe (days)				
95–118	0·07 ± 0·025 (3)	0·39 ± 0·15 (3)	0·14 ± 0·053 (3)	1·03 ± 0·36 (3)
120–129	0·086 ± 0·03 (3)	0·6 ± 0·29 (3)	0·13 ± 0·056 (3)	1·27 ± 0·25 (3)
130–139	0·096 ± 0·027 (4)	0·69 ± 0·18 (4)	0·13 ± 0·046 (4)	1·29 ± 0·43 (4)
140–145	0·11 ± 0·039 (4)	0·78 ± 0·22 (4)	0·16 ± 0·05 (4)	1·37 ± 0·4 (4)

The values are the means ± S.D. (number of animals in parentheses) of observations in the control period or at the end of 60 min of administration of 9% O_2 plus 3% CO_2 in N_2 to the ewes.

UD, undetectable.

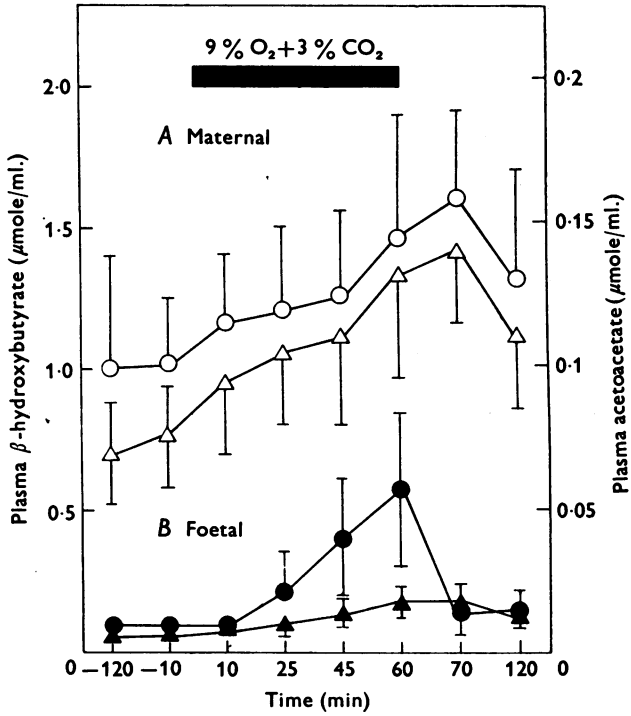

Fig. 8. Foetal and maternal carotid plasma ketone body concentrations before, during and after the administration of 9% O_2 and 3% CO_2 in N_2 to pregnant ewes. The sheep were 130–145 days pregnant (n = 10). *A*, maternal, *B*, foetal; β-hydroxybutyrate (△, ▲); acetoacetate (○, ●); vertical bars = ± S.D. of mean.

acetate concentrations were positively correlated (the plot of foetal carotid against maternal carotid concentration gave a regression line of $y = 0{\cdot}67x - 0{\cdot}041$; $r = 0{\cdot}92$; $P < 0{\cdot}001$).

DISCUSSION

Plasma metabolites during development

It has been proposed that the major respiratory fuel of the foetal sheep is glucose (Alexander, Britton & Nixon, 1966*a*) but more recent evidence indicates that other substrates, notably amino acids, are also important (Battaglia & Meschia, 1973). Lipids, short-chain fatty acids and ketone bodies are of minor significance (Alexander, Britton & Nixon, 1967; Alexander, Britton, Cohen & Nixon, 1969; James, Meschia & Battaglia, 1971).

The absence of a change in foetal or maternal plasma glucose concentration, over the age range studied, confirm the observations of Shelley (1960) with anaesthetized ewes and the serial observations of Comline & Silver (1970) on unanaesthetized sheep. The high foetal plasma lactate concentration compared with that of the ewe is probably a consequence of the relative impermeability of the sheep placenta to lactate (Britton, Huggett & Nixon, 1967), and much of the lactate in the foetal plasma probably arises from the foetal tissues. Recent evidence indicates that the placenta may also produce lactate at relatively high rates but the quantitative significance of this is not yet established (Burd, Jones, Simmons, Makowski, Meschia & Battaglia, 1975; Jones & Rurak, 1976).

The progressive increase in the plasma free fatty acid concentration of the ewe during the latter third of pregnancy is consistent with the higher values in pregnant compared with non-pregnant ewes and the rise in free fatty acid concentration several days before parturition (Reid, 1968; Noble, Steele & Moore, 1971; Comline & Silver, 1972). A rise in maternal plasma free fatty acid concentration during the latter part of pregnancy has been observed in several species (Jones, 1976). The very low foetal plasma free fatty acid concentration in sheep has been reported previously (Van Duyne, Parker, Havel & Holm, 1960; James *et al.* 1971; Comline & Silver, 1972). This is consistent with the small quantity of lipid in the foetal sheep (Body & Shorland, 1964; Body, Shorland & Gass, 1966). The progressive increase in basal free fatty acid concentration in the foetal sheep parallels the rise in maternal plasma. It is unlikely that the foetal increase is of maternal origin because of the poor placental permeability of free fatty acids in the sheep (Van Duyne *et al.* 1960) and the different nature of fatty acids in maternal and unsuckled new-born plasma (Leat, 1966).

The changes in maternal and particularly foetal plasma ketone body concentrations during pregnancy follow the rise in plasma free fatty acid concentrations. The foetal results are the opposite of those of Reid (1962), who described acetoacetate as the major ketone body with little β-hydroxybutyrate in the foetal plasma, but similar to those of Alexander *et al.* (1969). The low foetal concentration is probably related to both the low foetal plasma free fatty acid concentration and the poor ability of the foetal liver to produce significant quantities of ketone bodies from fatty acids until several days before term (Alexander, Andrews, Britton & Nixon, 1973). The rates of ketone body synthesis immediately after birth are relatively high (Hird & Weidemann, 1964). Ketone bodies are rapidly oxidized by the foetal sheep (Alexander *et al.* 1966*b*).

The plasma acetate concentrations observed in the ewes were similar to values reported for the non-pregnant (Annison, 1964) and the pregnant sheep (Char & Creasy, 1976), and the foetal values were similar to those of exteriorized foetal sheep (Pugh & Scarisbrick, 1955; Alexander *et al.* 1967; Char & Creasy, 1976). Acetate is transported across the sheep placenta and rapidly metabolized by the foetus (Pugh & Scarisbrick, 1955; Alexander *et al.* 1967; Char & Creasy, 1976).

The responses to hypoxia

Moderate hypoxia causes little change in the cardiac output of the foetal heart (Cohn, Sacks, Heymann & Rudolph, 1974) but large changes occur in the venous returns to both sides of the heart and the distribution of the cardiac output to the foetal tissues (Assali, Holm & Sehgal, 1962; Campbell, Dawes, Fishman & Hyman, 1967; Dawes, Lewis, Milligan, Roche & Talner, 1968; Dawes, Duncan, Lewis, Merlet, Owen-Thomas & Reeves, 1969). Blood flow to the placenta, adrenals, heart and brain increases and that to the carcass, lungs, kidneys and spleen declines (Cohn *et al.* 1974). Thus any changes in metabolite concentrations in the foetal circulation during hypoxia may be caused not only by an increase in the break-down of glycogen, triglyceride or protein but also by changes in the tissue uptake of metabolites because of changes in organ blood flow. Such a mechanism, however, would not explain the development of the glucose, free fatty acid or ketone body responses to hypoxia between 120 days to term since the cardiovascular responses are already well developed by this time (Shinebourne, Vapaarouri, Williams, Heymann & Rudolph, 1972; Cohn *et al.* 1974).

Some of the metabolic effects of hypoxia in foetal sheep have been investigated previously (Acheson, Dawes & Mott, 1957; Britton, Nixon & Wright, 1967; Mann, 1970; Alexander, Forsling, Martin, Nixon, Ratcliffe, Redstone & Tunbridge, 1972). However these studies used anaesthetized

ewes with exteriorized foetuses. Under such conditions the foetal plasma catecholamine concentration is about 25 ng/ml. (Jones & Rurak, 1976) which is considerably higher than the value of < 100 pg/ml. at rest and higher than the value of 1–5 ng/ml. during moderate hypoxia (Jones & Robinson, 1975) for the foetal sheep *in utero*. Thus the metabolic response of the exteriorized foetus or that of the anaesthetized ewe must be interpreted with caution.

Besides transport across the placenta the blood glucose concentrations in foetal sheep is in part controlled by foetal insulin secretion (Shelley, 1973; Battaglia & Meschia, 1973; Bassett *et al.* 1973; Bassett & Madill, 1974). During hypoxia plasma insulin concentration falls to a small extent and may be partly responsible for the rise in foetal plasma glucose concentration. In late foetal life many of the foetal tissues accumulate large quantities of glycogen which disappears after birth (Dawes & Shelley, 1968). The only tissues capable of mobilizing glycogen to extracellular glucose are the liver and the kidney (Scrutton & Utter, 1968), as indicated by the presence of glucose 6-phosphatase (Ballard & Oliver, 1965) and the parallel rise in blood glucose and fall in hepatic glycogen during asphyxia (Dawes *et al.* 1959). The placenta has no glucose 6-phosphatase activity (Lea & Walker, 1962) and its glycogen is unlikely to contribute directly to foetal blood glucose. The foetal blood glucose concentration is increased by the infusion of physiological doses of adrenaline or noradrenaline (Comline & Silver, 1972). In addition adrenaline increases the production of glucose from the foetal guinea-pig liver (Bassett & Jones, 1976). Thus it is possible that a significant proportion of the rise in plasma glucose during hypoxia is produced through a stimulation of hepatic glycogenolysis induced either by plasma adrenaline or increased splanchnic stimulation (Edwards & Silver, 1972). The close correlation between the rise in plasma glucose and the plasma catecholamine concentration suggests an important role for plasma catecholamines. The explanation for the increasing glucose response to hypoxia between 120 days and term is not clear, as foetal hepatic glycogen concentration is high by 120 days and then changes only slightly (Shelley, 1960). If plasma adrenaline was the main stimulus for glycogen break-down, then the observed increase between 120 and 140 days in the adrenaline response of the foetal adrenal to severe hypoxia (Comline & Silver, 1961) may provide an explanation although this was not confirmed by plasma catecholamine measurements (Jones & Robinson, 1975).

In the present studies the absence of a change in the lactate response to hypoxia over the gestational age-range investigated indicates that the magnitude of the lactate rise does not simply correlate with the changes in tissue glycogen (Dawes & Shelley, 1968). Though, if the liver and heart

were the major sites of lactate production from glycogen, the rise in hepatic glycogen could compensate for the fall in cardiac glycogen (Dawes & Shelley, 1968). After hypoxia the foetal plasma lactate concentration declines very slowly despite a rapid return to normal foetal blood gas values. This is consistent with the nature of lactate dehydrogenase of foetal sheep heart and liver which is largely the LDH-1 isoenzyme, as is 30–40 % of the enzyme in skeletal muscle (Hinks & Masters, 1964). This particular isoenzyme is inhibited by high lactate and pyruvate concentrations and has a lower K_m for both substrates than does LDH-5 the major isoenzyme found in adult sheep muscle and in the liver of the non-ruminant (Wilkinson, 1970). Thus it is unlikely that the liver, the heart or to a lesser extent the skeletal muscle are able to increase their rates of lactate utilization in response to the rise in plasma lactate. This and the low permeability of the sheep placenta to lactate is probably responsible for the high plasma lactate concentration in the foetal sheep.

As observed with the glucose response to hypoxia there are no changes in the nature of the foetal triglyceride stores (Wensvoort, 1967) that would explain the sudden increase about 10 days before birth in the foetal free fatty acid response to hypoxia. The close correlation between the magnitude of the free fatty acid rise and the plasma catecholamine concentration at this time indicates that either plasma or locally released catecholamines are responsible for stimulating the break-down of tissue triglycerides. In addition, as plasma adrenaline is much more effective than noradrenaline at increasing the plasma free fatty acid concentration in the foetal, in contrast with the adult sheep (Bassett, 1970; Comline & Silver, 1972), and a change late in foetal life in the adrenaline response to hypoxia (Comline & Silver, 1961) would explain the free fatty acid responses to hypoxia. However the absence of a significant hypoxia-induced free fatty acid rise at 120–129 days is not easily explained as a rise in plasma adrenaline is observed at this time (Jones & Robinson, 1975). While there is no evidence to support the view, it is possible that changes in placental permeability to free fatty acids during hypoxia occur with increasing gestation. Cytologically all the adipose tissue investigated in the foetal sheep is of the brown adipose type (Wensvoort, 1967; Gemmell, Bell & Alexander, 1972). The lipolytic rate in brown adipose tissue is increased by adrenaline, but to a lesser extent than in white adipose tissue (Fain, Reed & Saperstein, 1967; Jones, 1976). Thus the results suggest that foetal brown adipose tissue will mobilize fatty acid in response to stimulation *in vivo* and the effects of hypoxia on free fatty acid release contrast with its effects on the noradrenaline-stimulated increase in oxygen consumption (Heim & Hull, 1966). The greater effect of adrenaline than noradrenaline on free fatty acid release indicates that in foetal sheep

plasma adrenaline may be more important than noradrenaline released by the local sympathetic system for the free fatty acid response to hypoxia.

The ketone body responses to foetal hypoxia follow very closely those of free fatty acids. Thus the appearance of significant ketone body concentration in response to foetal hypoxia during the two weeks before birth is probably a consequence of appearance of both a significant free fatty acid response to hypoxia and of the ability of the foetal sheep liver to produce significant quantities of ketone bodies from fatty acids (Alexander *et al.* 1973).

The rises in the concentration of α-amino nitrogen in foetal plasma associated with either a low blood pH or with hypoxaemia are probably caused by a reduced uptake or increased release of amino acids from the foetal tissues. The increase in the plasma urea concentration (Mellor & Slater, 1971) and in the urea production rate (Battaglia & Meschia, 1973) of the foetal sheep caused by surgery suggests that a rise in plasma amino acid concentration and in amino acid oxidation may be the usual foetal response to certain unfavourable physiological conditions.

I am grateful to Professor G. S. Dawes for his interest and encouragement, to Drs K. Boddy, J. W. K. Ritchie, J. S. Robinson and R. O. Robinson for handling the sheep and to Mr A. Stevens, Mr M. Pucklavec and Mrs Paula Webb for their expert technical assistance. The work was assisted by a grant to Professor Dawes from the Medical Research Council.

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