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ACETATE METABOLISM IN THE ISOLATED SHEEP FOETUS

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SUMMARY

1. Sheep foetuses over the age range 105–142 days and maintained on an extracorporeal circuit removed exogenous acetate at 2·0–4·9 mg/kg.min.

2. Urinary loss of acetate was less than 12·5% of the administered amount.

3. The rate of acetate removal appeared to be unaffected by the addition of glucose to the circuit. The removal of glucose also seemed to be unaffected by the presence of acetate.

4. A decline in the plasma concentration of free fatty acids and ketone bodies was also observed during the perfusion.

5. It is concluded that under these conditions acetate may have accounted for about one half of the oxygen consumption. The contribution of free fatty acids and ketone bodies must have been small. The relevance of these findings to the nutrition of the foetus *in utero* is discussed.

INTRODUCTION

It has been shown that the sheep foetus, detached from its placental connexions, may be maintained for at least 1 hr in the absence of a demonstrable concentration of glucose in the perfusate and further that the animal does not meet its metabolic requirements at the expense of the fructose which is also present (Alexander, Britton & Nixon, 1966*b*). In the adult ruminant it is known that acetate metabolism, derived from cellulose break-down, contributes substantially to supplying the energy requirements of the animal (Elsden & Phillipson, 1948; Bergman, Reid, Murray, Brockway & Whitelaw, 1965). It was of interest therefore to examine the possible uptake of acetate from the plasma of isolated sheep foetuses which were maintained on an extracorporeal circuit. In addition the plasma concentrations of free fatty acids and ketone bodies were also determined.

METHODS

Welsh mountain sheep bearing foetuses of known conceptual age were anaesthetized by the spinal administration of procaine supplemented with intravenous sodium thiopentone. The foetuses were exposed by Caesarian section and the foetal head enclosed in a polythene bag containing 0·9 % NaCl to prevent breathing. The femoral artery was catheterized under local anaesthesia (procaine) for blood pressure recording. In most of the experiments the urinary bladder was cannulated through a mid line abdominal incision also under procaine anaesthesia.

In one experiment, where the foetus remained attached to the placenta, catheters were placed in tributaries of an umbilical artery and vein for sampling and injecting respectively.

The extracorporeal circuit and the method of incorporating the foetus into this circuit have been previously described (Alexander, Britton & Nixon, 1964). Some 150–200 ml. of maternal blood were used to prime the circuit. A gas mixture of 97 % O_2 + 3 % CO_2 was used in the oxygenator. During the course of the metabolic observations, the loss of circulating volume through blood sampling and indirectly through urine loss was not made up by the addition of fresh blood.

Determination of plasma and urinary acetate concentrations were made using the method of Conway (1957); plasma ketone bodies by modifications of the methods of Michaels, Margen, Liebert & Kinsell (1951) and Mayes & Robson (1957); plasma free fatty acid concentration by the method of Trout, Estes & Friedberg (1960). The other determinations were carried out by methods previously described (Alexander *et al.* 1964).

No measurements were made of the utilization of these materials by the cells of the blood. Calculations of uptake will therefore include any utilization by these cells.

The haemodynamic status of the ten perfused animals is shown in Table 1. Metabolic investigations were stopped when the umbilical flow had dropped to 25 ml./kg.min.

TABLE 1. Mean values for circulatory parameters during the observation period on isolated sheep foetuses maintained by an extracorporeal circuit

Animal no.	Age (days)	Weight (kg)	Duration of observation (min)	Femoral arterial pressure (mm Hg)	Inflow pressure (mm Hg)	Heart rate (beats/min)	Umbilical blood flow (ml./kg.min)
169/64	105	1·06	128	40	8	224	41
34/65	106	0·76	278	39	—	171	46
137/64	107	1·04	136	40	14	180	92
167/64	108	1·04	135	—	8	214	37
134/64	111	1·14	74	52	6	208	33
148/64	111	1·10	123	49	7	177	41
149/64	121	1·60	133	59	—	197	126
160/64	140	3·30	174	42	12	174	31
154/64	142	2·16	310	56	10	217	30
164/64	142	2·31	169	63	13	197	40

RESULTS

Acetate removal from the plasma in the exteriorized foetus

A preliminary experiment was carried out on a sheep foetus with intact placental connexions to examine the disappearance of acetate from the circulation. In this experiment 1 g sodium acetate was injected into an umbilical vein of a foetus of 105 days (animal no. 57/58) and within 122

min the concentration had returned to its pre-injection level. The animal weighed 0·55 kg and over this period of time acetate was removed from the foetal plasma at a rate equivalent to approximately 10·8 mg/kg.min. The concentrations of glucose and fructose in the plasma appeared to be unaffected by the presence of acetate.

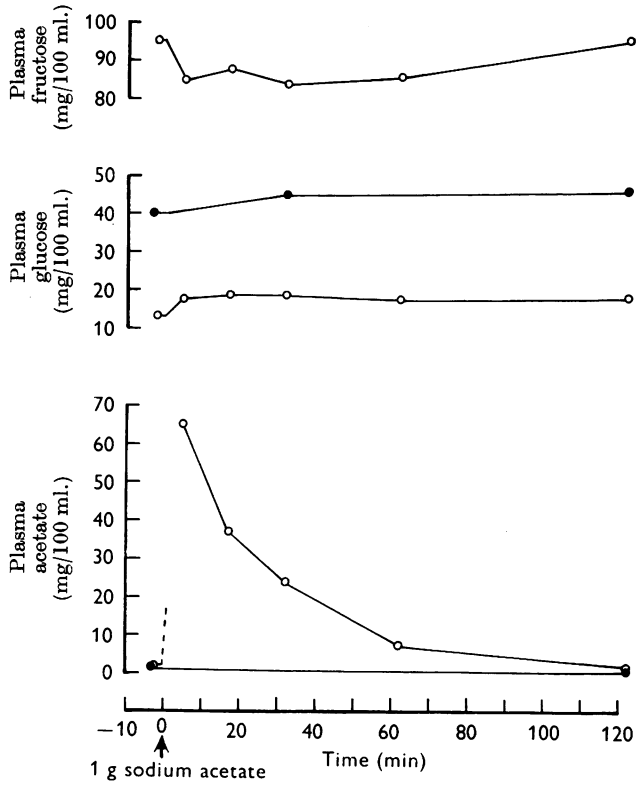

Fig. 1. Changes in the concentration of fructose, glucose and acetate in the maternal and foetal plasmas when acetate was injected into the foetal circulation of an exteriorized animal. ○ Foetal, ● maternal. Foetal age 105 days (animal no. 57/58).

The decline observed in the acetate concentration could be the resultant of several processes, namely transplacental passage into the maternal circulation (although the maternal arterial concentration was not elevated at the end of the experiment), placental utilization, urinary elimination and utilization by the foetus. In order to study further the possible removal of acetate by the foetal tissues subsequent investigations were carried out on foetuses detached from their placental connexions and maintained by an extracorporeal circuit.

Acetate removal from the plasma in the isolated foetus

Control animals. Three animals of 108, 121 and 140 days gestational age were maintained for between 133 and 174 min by means of the extracorporeal circuit. No acetate was added to the circulation in any of these animals but the 121-day animal had 1·5 g glucose added early in the perfusion.

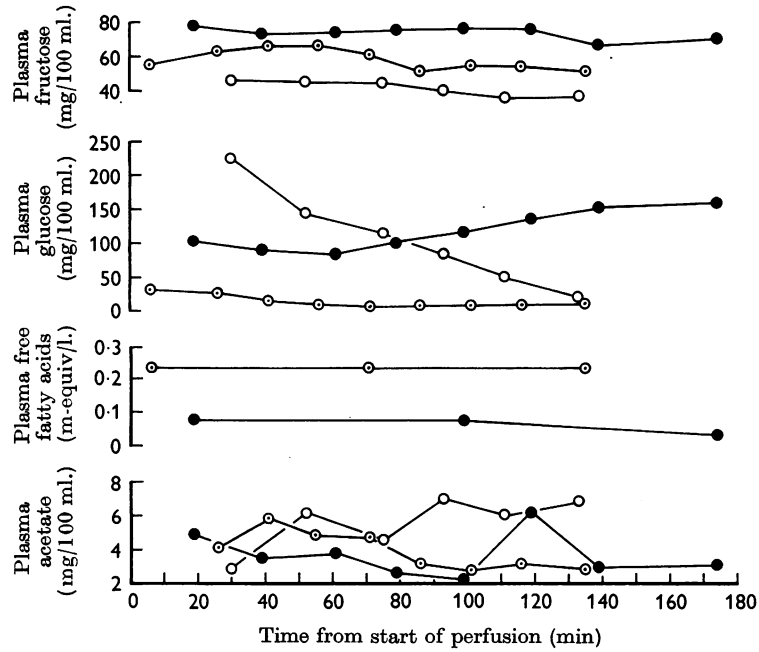

Fig. 2. The plasma concentration of fructose, glucose, free fatty acids and acetate in control sheep foetuses isolated and maintained by an extracorporeal circuit. Gestational ages were ⊙ 108; ○ 121 and ● 140 days (animals nos. 167/64, 149/64 and 160/64 respectively).

It will be seen from Fig. 2 that the apparent acetate concentrations in the perfusate of these animals were low and remained relatively constant over the observation period. Such small changes as did occur appeared to be unrelated to changes in the concentration of the other measured components of the perfusate and were of the same order as the variability of the method.

As previously seen in the artificially maintained foetus (Alexander *et al.* 1966*b*) there was a decline in glucose concentration of the younger foetuses. Using the methods described by these authors rates of uptake of 7·3 and 2·9 mg/kg.min were found for animals 149/64 and 167/64 respectively. The older animal was able to maintain its plasma glucose con-

centration. Fructose, on the other hand, irrespective of foetal age, showed only a slow decline in concentration. The rates of uptake by the foetal tissues (excluding urinary excretion) were 0·66 and 0·26 mg/kg.min for animals 167/64 and 160/64. The bladder was not cannulated in animal 149/64 so that no fructose uptake could be calculated.

The concentrations of free fatty acids and of ketone bodies in the plasma were determined in two of the animals (108 and 140 days gestation). A considerable difference in the concentration of free fatty acids existed between the two animals. No ketone bodies were detectable in either animal.

In these two animals urine collections were made and the samples estimated for acetate, glucose and fructose (Table 3). The total urinary loss of acetate was less than 1 mg in both animals.

Acetate removal following the addition of acetate to the perfusate. Seven foetuses of between 105 and 142 days were maintained on the extra-corporeal circuit for between 74 and 310 min.

The plasma acetate concentrations were elevated early in the course

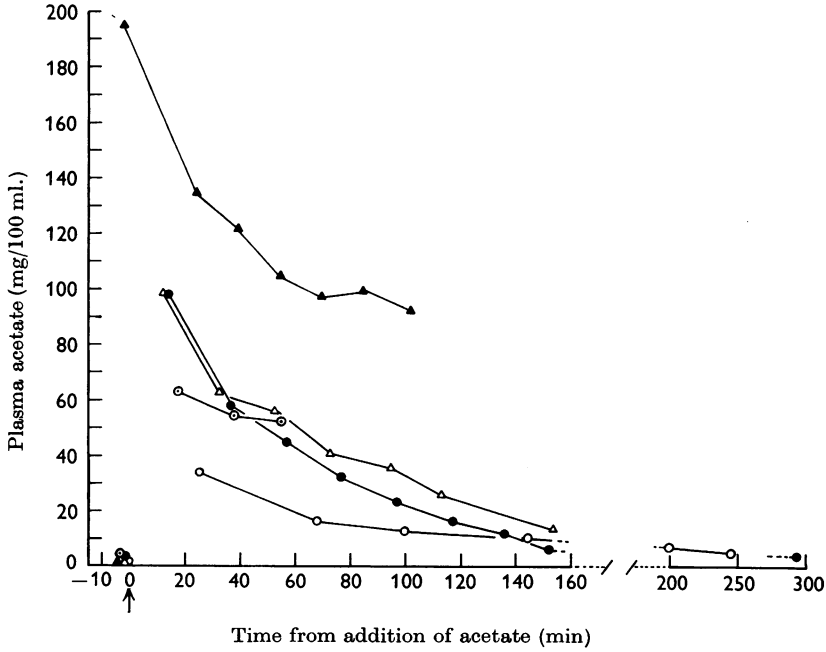

Fig. 3. The decline in the elevated acetate concentration of the plasma in five perfused sheep foetuses. △ (164/64), ▲ (148/64), ●, (154/64) received 2 g sodium acetate; ⊙ (134/64) received 1 g sodium acetate and ○ (34/65) 1 g sodium acetate $3H_2O$ + 300 mg glucose. (Gestational ages were 142, 111, 142, 111 and 106 days respectively.)

TABLE 2. Rate of loss of exogenous acetate from plasma and rate of urinary acetate excretion after acetate infusion in isolated foetal sheep maintained on an extracorporeal circuit

Animal no.	Age (days)	Weight (kg)	Duration of observation (min)	Acetate added (mg)	Time to return to pre-injection concn. (min)	Mean glucose concn. (mg/100 ml.)	Total removal rate of plasma acetate (mg/kg.min)	% of acetate administered eliminated in urine
169/64	105	1·06	128	720	210	82·8	3·2	5·8
34/65	106	0·76	278	430	280	24·4	2·0	Trace
137/64	107	1·04	136	720	220	2·5	3·2	—
134/64	111	1·14	74	720	210	2·7	3·0	2·9
148/64	111	1·10	123	1440	270	7·9	4·9	6·4
154/64	142	2·16	310	1440	160	98·3	4·2	12·5
164/64	142	2·31	169	1440	180	90·3	3·5	9·6

of the experiments, by a single injection usually of 1 or 2 g of anhydrous sodium acetate dissolved in 5–10 ml. of water.

In each animal the plasma acetate concentration declined with perfusion time (Figs. 3, 4). The removal rate from the plasma, obtained by extrapolation to the pre-elevation concentration, varied from 2·0 to 4·9 mg/kg.min (Table 2).

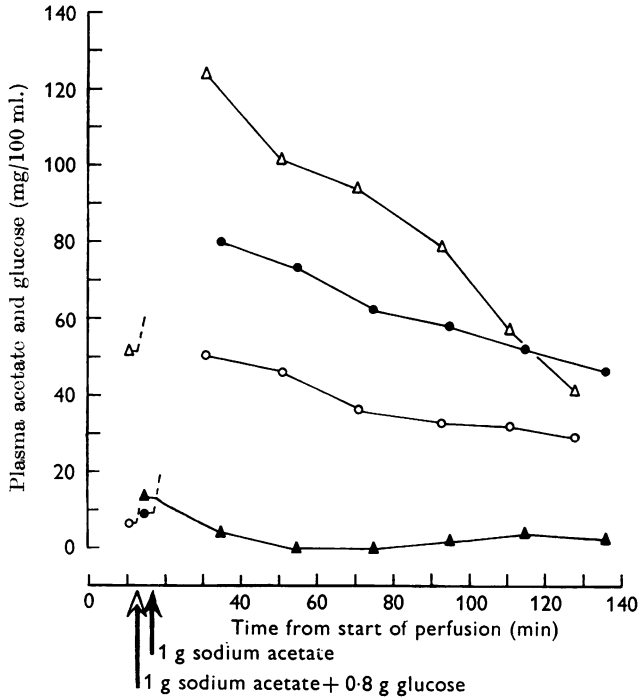

Fig. 4. The decline in the elevated acetate concentration of the plasma (○ and ●) in two perfused sheep foetuses in the presence of high (△) and low (▲) glucose concentrations. Open and filled symbols refer to animal 169/64 (105 days) and 137/64 (107 days) respectively.

In two of the younger animals (169/64 and 34/65) glucose was added to the perfusate at the commencement of the experiment. The data in Table 2 suggest that the removal rate of acetate from the plasma was independent of the plasma glucose concentration. The plasma acetate and glucose concentration in one of these animals is compared with the values obtained in another animal of comparable age, which did not receive any additional glucose (Fig. 4). It will be seen that a similar decline in plasma acetate concentration occurred in both animals in spite of considerable differences in their plasma glucose concentrations.

The decline in the glucose and fructose concentrations of the plasma

was similar in all of the foetuses to that previously observed in artificially maintained foetuses. The rate of uptake of glucose was 6·9, 2·9, 5·2, 4·1 and 4·5 mg/kg.min for animals 169/64, 34/65, 137/64, 134/64 and 148/64 respectively. Fructose uptake (excluding urinary excretion) was 0·0, 0·4, 0·18, 0·15 and 0·12 mg/kg.min for animals 169/64, 134/64, 148/64, 154/64 and 164/64 respectively. Glucose uptakes were not calculated for animals 154/64 and 164/64, since these older animals maintained their blood glucose. Fructose uptakes were not calculated for animals 34/65 and 137/64 since urinary data were not available. These rates of uptake therefore are similar to those found in the control animals and in previous studies on the isolated foetus (Alexander *et al.* 1966*b*).

The bladder was cannulated in six of these animals, and the urine estimated for acetate, fructose and glucose. In five of the animals between 2·9 and 12·5 % of the administered acetate was excreted in the urine; in the remaining animal, which was relatively anuric over the entire perfusion period, the urinary loss was only 0·003 %. The plasma clearance of fructose by the foetal sheep kidney represents about 70 % of the glomerular filtration rate (Alexander & Nixon, 1963, 1964), and upon this basis the tubular reabsorption of acetate, glucose and water has been calculated. It will be seen (Table 3) that between 30 and 49 % of the filtered acetate was reabsorbed, except in the oldest of the control animals where a value of 90 % was obtained. In these, as in previous experiments (Alexander *et al.* 1966*b*), the reabsorption of glucose was almost complete. In this respect the artificially maintained foetus resembles that of the exteriorized foetus with an intact placental circulation (Alexander & Nixon, 1963). Water reabsorption on the other hand was poor in those foetuses in which acetate was administered, suggesting that at these concentrations of acetate an osmotic diuresis was established.

Free fatty acid and ketone bodies. The free fatty acid concentrations in the plasma are shown in Fig. 5 and may be compared with the control

TABLE 3. Data on renal function in the isolated sheep foetuses maintained by an extracorporeal circuit; the values represent the mean of several collection periods. The glomerular filtration rate (G.F.R.) was calculated from the fructose clearance by assuming that the fructose clearance represented 70 % of the glomerular filtration rate

	Animal no.	Urine flow (ml./min)	G.F.R. (ml./min)	Acetate clearance (ml./min)	Percentage reabsorption		
					Acetate	Glucose	H_2O
Control	167/64	0·03	0·23	0·14	49	100	87
	160/64	0·05	0·54	0·06	90	98	91
Acetate added	169/64	0·36	0·80	0·69	42	85	55
	148/64	0·68	1·27	0·77	42	96	46
	134/64	0·53	0·94	0·57	39	100	44
	164/64	1·06	2·25	1·47	41	90	53
	154/64	1·33	3·02	2·24	30	95	56

values in Fig. 2. The high levels found initially in some animals (148/64, 137/64 and 134/64) may have been due to high levels in the maternal blood used to prime the circuit since in one case (164/64) the free fatty acid concentration was determined in the foetal plasma before detachment from the placenta, and was found to be only 0·036 m-equiv/l. Subsequently

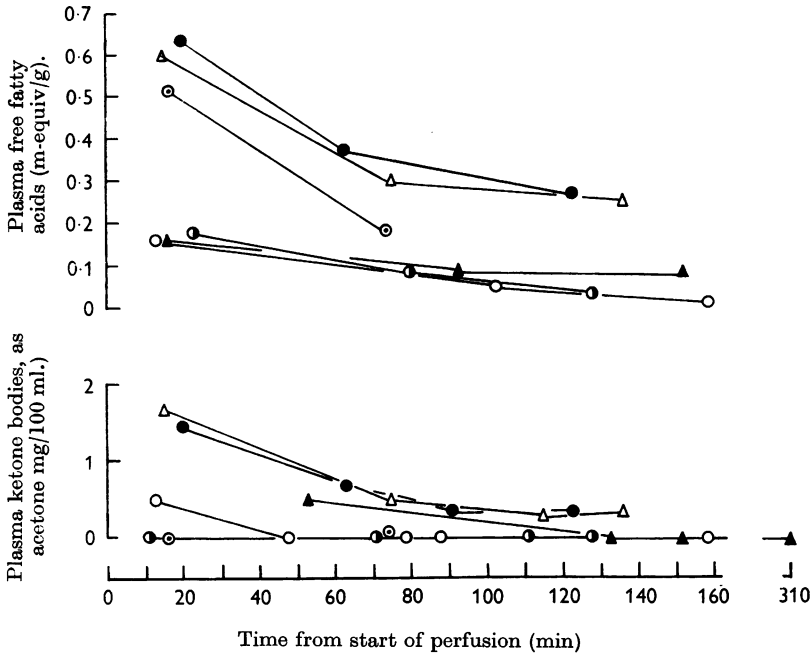

Fig. 5. The decline in the plasma concentration of free fatty acid and ketone bodies in perfused sheep foetuses. ◑ 169/64, ● 148/64, ○ 164/64, △ 137/64, ▲ 154/64 and ⊙ 134/64. (Gestational ages were 105, 111, 142, 107, 142 and 111 days respectively.)

values of 0·162, 0·054 and 0·018 m-equiv/l. were found 13, 103 and 169 min respectively after the start of the perfusion. This suggestion is also supported by Van Duyne, Parker, Havel & Holm (1960), who found mean concentrations of 0·1 and 1·0 m-equiv/l. in foetal and maternal plasma respectively. However, lipase activity has been reported to be present in the isolated sheep foetus (Alexander, Britton & Nixon, 1966*a*) and this may have released free fatty acids from triglycerides. Despite the rapid rates of fall in concentration that were seen in the animals that had a high initial level the rate of uptake by the foetus was calculated not to exceed $0{\cdot}8 \times 10^{-3}$ m-equiv/kg.min assuming that the free fatty acids were distributed in the plasma space alone.

The plasma ketone levels are also shown in Fig. 5 and may be compared with the zero values found in the two control animals. The zero values

probably arose from a correction being made for the lactic acid present, and it is possible that an over correction was applied. However even if no correction were made the concentrations would have been less than 1 mg/100 ml. and the trends would not be altered. Thus there was no evidence of any conversion of acetate into ketone bodies. The high initial levels may have been due to the presence of ketone bodies in the maternal blood used to prime the circuit. In animal 134/64, the maternal and foetal concentrations before detachment of the foetus were 4·0 and 0·9 mg/100 ml. respectively and in animal 164/64 the maternal and foetal concentrations were 1·2 and 0·5 mg/100 ml. while the maternal concentration in animal 154/64 was 0·9 mg/100 ml. The maximum rate of disappearance of ketones would indicate a ketone uptake of no more than about 0·15 mg/kg.min even if the ketones were distributed in total body water.

DISCUSSION

Quantitatively acetic acid is the most important of the volatile acids produced as the result of the action of the microflora upon cellulose in the rumen and caecum of the sheep (Elsden, 1946). Substantial concentrations of acetic acid may be found in the venous blood coming from these sites, although the concentration is much smaller in the arterial blood (Barcroft, McAnally & Phillipson, 1944). Intravenous acetate tolerance tests have shown uptakes of approximately 5·0 mg/kg.min in non-pregnant ewes (Jarrett & Filsell, 1960), and 3·3–5·0 mg/kg.min in pregnant ewes (Pugh & Scarisbrick, 1952). In lambs of less than 4 weeks rather higher uptakes of about 10 mg/kg.min were found (Jarrett & Filsell, 1960). To what extent the increased rate of uptake in the lamb is due to the increase in oxygen consumption at birth (Barcroft, 1946; Dawes & Mott, 1959) is not clear. Such large uptakes of acetate may represent a considerable source of metabolic energy, and Annison & Lindsay (1961) have shown in constant infusion experiments with radioactive acetate that 35% or more of the expired CO_2 may be derived from acetate at blood concentrations of about 13·2 mg/100 ml. (2·2 mM) acetate (a concentration of the order found in the fed animal). The rate of uptake of acetate by the tissues at this concentration was found by these workers to be 2·5 mg/kg.min and if the oxygen consumption of the sheep is taken to be about 4 ml./kg.min it follows that most of the acetate taken up must have been oxidized. At low concentrations (1·5–6·0 mg/100 ml.) in the starved animal only about 6% of the expired CO_2 was derived from acetate (Annison & Lindsay, 1961).

In the present paper, rates of uptake of 2·0–4·9 mg/kg.min have been found in foetuses from 105 days to term. These rates of uptake are thus similar to those that have been found in acetate tolerance tests on the

adult. Precise comparisons in such experiments are difficult because of the varying concentrations of acetate, but the range of acetate concentration used in the present study and in the studies mentioned above was similar. The oxygen consumption of the foetus is about 4·6–5·4 mg/kg.min (Dawes & Mott, 1964), and is comparable on a weight basis with the adult. If the acetate, which is taken up from the blood, is used for oxidative metabolism in the foetus, as the experiments of Annison & Lindsay (1961) have indicated in the adult, then an uptake of 6 mg/kg.min would be required to satisfy the oxygen consumption. The foetus thus appears to be able to take up acetate at a rate sufficient to account for about one half of the oxygen consumption.

The failure of glucose to alter the rate of acetate disappearance to any large extent may be compared with the variable results found by Annison & Lindsay (1961) in the adult sheep, and the increased rate of disappearance in the presence of glucose found by Jarrett & Filsell (1961) in single injection experiments on both adults and lambs. It was of interest that the presence of the acetate also did not influence the uptake of glucose or fructose.

In the foetus, relatively small amounts of acetate are excreted in the urine. This is also true of the adult and lamb (Annison & Lindsay, 1961; Jarrett & Filsell, 1960).

Although it appears that the foetus can use acetate it by no means necessarily follows that acetate is an important metabolite *in utero*. Pugh & Scarisbrick (1955) measured the umbilical arterial and venous concentrations in the exteriorized foetal sheep, and compared them with the maternal arterial concentrations which were elevated in some cases by acetate infusion. The foetal concentrations (1·1–7·8 mg/100 ml.) were always much less than the maternal (3·2–51·4 mg/100 ml.). The placenta therefore appears to be relatively impermeable to acetate: or alternatively, perhaps the placental tissues have a very large utilization of acetate. From the umbilical veno-arterial differences it was concluded that the acetate uptake of the foetus was probably equivalent to less than 10% of the oxygen uptake. The low rate of uptake was presumably related to the low concentrations in the foetal blood as in the starved adult.

The disappearance of free fatty acids from the plasma is of interest in so far as it indicates that the foetus is metabolically capable of handling these substances. However, the rate of disappearance indicated that free fatty acids probably contributed only a very small proportion to the metabolism. It is difficult to be sure that foetal metabolism has not been altered by the presence of heparin, lipase activity and other factors in the perfused foetus, but if the metabolism of the foetus when attached to the placenta is comparable it would suggest that free fatty acids can contribute little to

the metabolism *in utero*. This conclusion is not inconsistent with the work of Van Duyne *et al.* (1960), who found a small amount of labelling in the foetal free fatty acid when ^{14}C labelled palmitic acid was administered to the mother.

In the case of ketone bodies the rates of disappearance were very low and some of the apparent uptake may have represented urinary or respiratory loss. Since the concentrations in the early stages of the perfusions were of the same order as that found in the foetal blood before detachment from the placenta it would seem that ketone body uptake contributes little to foetal metabolism *in utero*. The present work also shows that even in the presence of acetate and low concentrations of glucose, the foetus is unable to produce sufficient ketone bodies to yield a ketosis. This is in contrast to the adult where high levels of acetate have been associated with ketonaemia (Jarrett & Potter, 1950; Pugh & Scarisbrick, 1952). In subsequent work Alexander Britton & Nixon (1966*c*) have shown that the foetus can metabolize large quantities of ketone bodies if the concentrations are sufficiently high, but it is prevented from doing so *in utero* by the relative impermeability of the placenta.

To summarize, therefore, the present work has indicated that the foetus from 105 days onwards can take up acetate at a rate which is substantial compared with oxidative metabolism. Ketone bodies and free fatty acids were also taken up but their contribution to the total metabolism would appear to have been small. It has frequently been suggested that *in utero* the foetus uses carbohydrate exclusively as a source of energy (see, for example, Kronfeld, 1958), and that it is for this reason that the pregnant ewe readily becomes ketotic. The fact that effectively the placenta is relatively impermeable to acetate supports this view. However, in the light of the present findings this impermeability of the placenta to acetate is interesting since it would have seemed mutually advantageous to both mother and foetus, if the foetus were allowed to use acetate.

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