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OBSERVATIONS ON THE ISOLATED FOETAL SHEEP WITH PARTICULAR REFERENCE TO THE METABOLISM OF GLUCOSE AND FRUCTOSE

By D. PAULINE ALEXANDER, H. G. BRITTON and D. A. NIXON

From the Physiology Department, St Mary's Hospital Medical School, London, W. 2

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SUMMARY

1. Isolated sheep foetuses of 72–146 days conceptual age (155–4840 g) have been maintained for periods up to 325 min on an artificial circuit where the placenta has been replaced by an oxygenator.

2. Measurements made during the period of observation included heart rate, femoral arterial pressure, umbilical blood flow, blood gases and pH; plasma and urine concentrations of glucose, fructose, lactic acid, urea, α amino nitrogen and electrolytes.

3. The circulatory, metabolic and renal conditions of the isolated foetus were found to be similar to those of the exteriorized foetus with intact placental connexions. However, in the later stages a terminal hypoxia developed. This was due to a progressive diminution in umbilical blood flow caused by umbilical arterial constriction.

4. The young foetuses removed glucose from the circulation and seemed unaffected by the consequent hypoglycaemia. If the glucose removed was completely oxidized it would account for much of the estimated oxygen consumption. The blood glucose concentration in the older foetuses, on the other hand, did not fall and sometimes rose. Renal excretion of glucose was very small.

5. Fructose was usually slowly removed from the circulation and under no conditions did a rapid removal occur. Renal excretion accounted for about half of the fructose disappearing from the apparent fructose space. It is therefore suggested that a small utilization of fructose occurs in foetal tissues but this could account for only a very small fraction of the estimated oxygen consumption.

INTRODUCTION

The foetal blood of the sheep contains, in addition to glucose, substantial quantities of fructose, but the significance of the latter in foetal metabolism is obscure. It is known that the placenta is the site of fructose synthesis (Alexander, Huggett, Nixon & Widdas, 1955; Britton, Huggett & Nixon, 1963), and that the placenta is permeable to fructose in the direction foetus to mother (Britton *et al.* 1963; Nixon, 1963). These factors make investigations of fructose metabolism difficult to interpret in a preparation in which the foetus, although exposed by Caesarian section, still remains attached to the placenta. The technique of maintaining the isolated foetus (Alexander, Britton & Nixon, 1964*b*) offers an experimental preparation in which metabolic requirements of the foetus may be examined without the complications introduced by the presence of placental connexion with the mother. In the study of fructose metabolism it is also necessary to assess renal loss, since this can be considerable (Alexander & Nixon, 1963) and this has also been measured in some of the perfused sheep foetuses.

While the main purpose of these investigations was to obtain information on whether or not the foetal sheep under these experimental conditions utilizes gross amounts of fructose the opportunity was taken to examine changes in other plasma components with perfusion time.

The results indicated that whereas a decline in plasma glucose was demonstrable only a slight fall in the fructose concentration was observed, some of which could be attributed to urinary loss. A preliminary account of some of these findings has been given by Alexander, Britton & Nixon (1964*a*).

METHODS

Foetuses in the conceptual age range 72–146 days (about term) and weighing between 155 and 4840 g were exposed by Caesarian section in ewes under procaine spinal anaesthesia which was supplemented with intravenous sodium thiopentone (Pentothal, Abbott Laboratories). Breathing was prevented by enclosing the head of the foetus in a polythene bag containing 0·9% NaCl. Under local anaesthesia (procaine) a foetal femoral artery was catheterized for blood pressure recording. In some experiments the foetal bladder was cannulated through a mid line abdominal incision (Alexander, Nixon, Widdas & Wohlzogen, 1958*b*). The details of the perfusion circuit and the method of introducing the foetus into the circuit have been described in a previous paper (Alexander *et al.* 1964*b*).

The initial volume of blood in the extra-foetal portion of the circuit varied in different experiments from 150 to 200 ml. Fluid lost either through blood sampling or in the urine was not replaced. The blood used to prime the circuit was obtained from the mother. Oxygenation was achieved in eight experiments by the use of a 97% O_2+3% CO_2 gas mixture, in one experiment (animal no. 98) by 95% O_2+5% CO_2 and in the remaining experiments (animals nos. S_2 and S_3) by 100% O_2.

In seven experiments the inflow pressures were recorded just before the bifurcation of the umbilical vein cannulae.

Metabolic investigations were restricted to the early parts of the experiments, that is before the umbilical blood flow fell too severely (below about 25 ml./kg.min). The animals often survived for a further period, but this part of the experiment was used to investigate haemodynamic aspects of the failing circulation; no data obtained during this period are reported here.

Methods used in the analysis of the perfusate were the same as those previously described (Alexander *et al.* 1964*b*).

RESULTS

Glucose and fructose

Control observations. The values obtained in three foetuses of 89, 110 and 120 days' gestation, which were observed for between 58 and 109 min, are shown in Fig. 1. During this time no marked reduction in the concentration of the plasma fructose was seen in any of the animals; on the other hand, in two (*A* and *C*) the glucose concentration declined considerably. The other animal (*B*) had no glucose detectable in the plasma and is of particular interest since even then the fructose concentration did not decline rapidly.

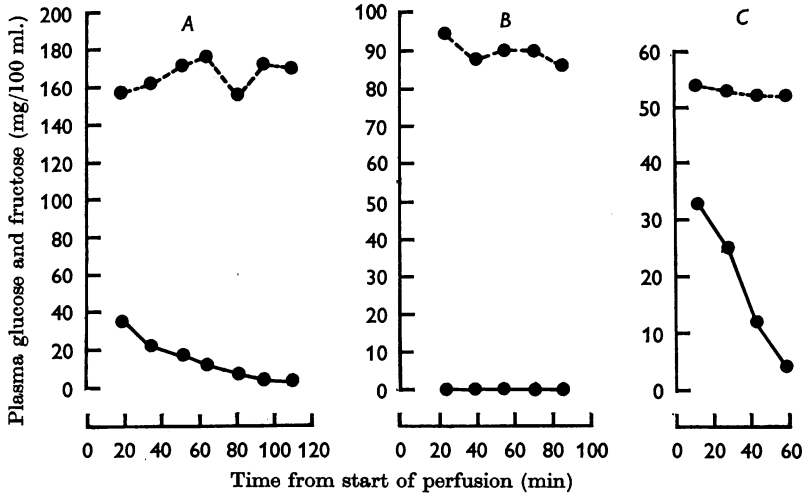

Fig. 1. Changes in the endogenous concentrations of glucose (●—●) and fructose (● - - - ●) in the plasma of three sheep foetuses during the course of perfusion. The gestational ages and terminal body weights were 89 days and 551 g (*A*), 110 days and 1153 g (*B*) and 120 days and 1400 g (*C*).

Two animals at 111 and 146 days' gestation (animal no. 94 and S_2) were maintained for 105 and 174 min respectively; in these animals the bladder was cannulated and the urine collected (Fig. 2). In the foetus of 146 days the plasma fructose fell at a rate of about 5 mg/100 ml. hr. It will be noted that a fall of fructose concentration at this rate would scarcely have been noticeable in the 111 days foetus shown in Fig. 2 or in the

experiments shown in Fig. 1 because of the shorter duration of perfusion. In the younger foetus the plasma glucose fell as it did in the 89- and 120-day foetuses shown in Fig. 1; however, in the 146-day foetus the glucose concentration was maintained initially and rose somewhat in the latter portion of the observation period.

In both foetuses the excretion rate of fructose in the urine was high. The total quantity eliminated was 70 and 113 mg in the 111- and 146-day foetuses respectively. The urinary loss of glucose which was measured in the older foetus was slight and amounted to 7·6 mg in spite of the rising plasma concentration.

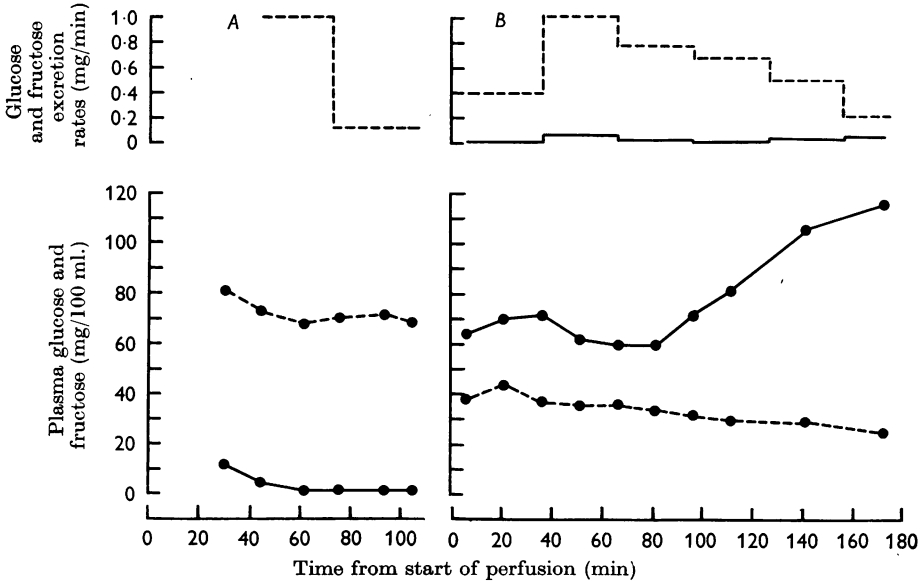

Fig. 2. Changes in the endogenous concentrations of glucose (●—●) and fructose (●- - -●) in the plasma and the excretion rates of glucose (——) and fructose (- - -) in two sheep foetuses during the course of perfusion. The gestational ages and terminal body weights were 111 days and 1332 g (*A*) and 146 days and 3800 g (*B*).

Addition of glucose and fructose. The effect of adding glucose and a mixture of glucose and fructose in equal quantities to the perfusate of foetuses (nos. 27 and 98) isolated at 72 and 110 days respectively, is shown in Fig. 3. In the 72-day foetus a removal of glucose from the perfusate at a rate of about 0·1 mg/100 ml.min is seen in the first 60 min of perfusion. On the addition of 300 mg of glucose to the circuit the concentration was elevated from 20 to 115 mg/100 ml. which subsequently fell at a rate of about 0·38 mg/100 ml.min. A further addition of 200 mg of glucose resulted in a rise in concentration from 67 to 126 mg/100 ml. and this was

followed by a decline in the concentration at a rate of 0·36 mg/100 ml. min. This fairly rapid removal of glucose is in marked contrast to the relative stability of the fructose concentration.

In the foetus of a 110 days' gestation the plasma glucose concentration showed a decline over the first 30 min while a slight rise in the plasma fructose occurred. The simultaneous administration of 850 mg of both glucose and fructose resulted in the plasma concentration attaining values of 93 and 252 mg/100 ml. respectively after 10 min. Over the next 70 min the plasma glucose concentration fell to reach approximately the pre-elevation concentration. Since this foetus weighed 1·26 kg the results suggested that the rate of glucose removal was about 9·6 mg/kg body wt. min. The elevated fructose concentration after equilibration showed no significant decline.

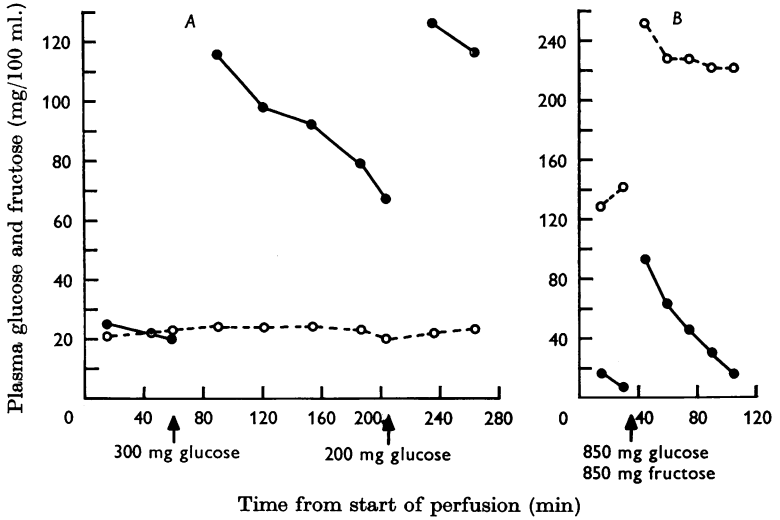

Fig. 3. Changes in the concentration of glucose (●—●) and fructose (●– – –●) in the plasma of two sheep foetuses following the addition of glucose and glucose plus fructose. The gestational ages and terminal body weights were 72 days and 155 g (*A*) and 110 days and 1265 g (*B*).

The results obtained on adding glucose and fructose to the perfusing blood in three foetuses (nos. 121, 99 and S_3) in which the bladder was cannulated are shown in Fig. 4. It will be seen that in the 100- and 125-day foetuses the plasma glucose concentration declines rapidly, whereas the fructose concentration falls more slowly. In the younger foetus the total renal loss of fructose was 52 mg and in the older animal it was 101 mg. In contrast, the renal loss of glucose was only 1·1 and 1·5 mg respectively. A similar result (not illustrated) was obtained in another foetus also of 125 days gestation (no. 128). In this animal the plasma glucose concen-

tration rose from 57 to 150 mg/100 ml. and the fructose concentration from 78 to 212 mg/100 ml. as the result of adding 1·5 g of each sugar to the perfusate; after 59 min the plasma concentrations were 72 and 184 mg/100 ml. for glucose and fructose respectively. Urinary loss of fructose over this period of time amounted to 228 mg while that of glucose was only 7·2 mg.

In the foetus of 146 days the priming blood was supplemented with glucose before the attachment of the animal to the perfusion circuit and this contributed to the high plasma glucose concentration initially present.

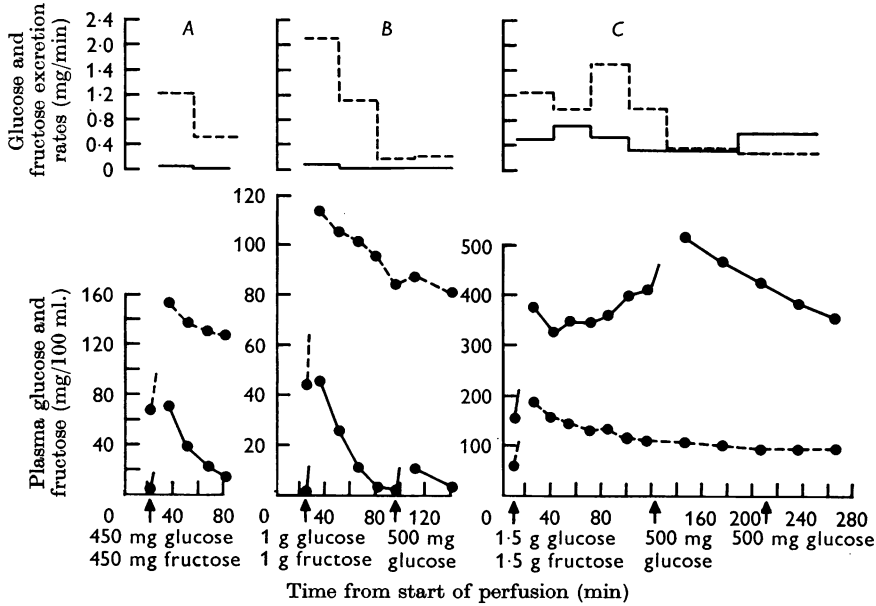

Fig. 4. Changes in the concentrations of glucose (●—●) and fructose (●– – –●) in the plasma and the excretion rates of glucose (——) and fructose (– – –) in three sheep foetuses following the addition of glucose and fructose. The gestational ages and terminal body weights were 100 days and 765 g (*A*), 125 days and 2225 g (*B*) and 146 days and 4840 g (*C*).

In contrast to the younger animals in this group no decline in plasma glucose was seen over the first 120 min of the perfusion following the addition of 1·5 g of glucose to the circuit. The rise in concentration of plasma fructose after the addition of 1·5 g of fructose was followed by a slow decline in the plasma concentration. Although the plasma glucose concentration was substantially higher than that of the fructose the excretion rate was, except in the last collection period, lower than that of fructose. The urinary loss of fructose over the 238 min following the addition of 1·5 g of fructose to the circuit totalled 179 mg; over the same period of time only 116 mg of

glucose were excreted despite the addition of a total of 2·5 g of glucose to the circuit.

The uptake of glucose and fructose by the foetus. In four of the experiments which have been described the glucose concentration returned to a value close to its initial value after the administration of glucose. A mean rate of uptake of the sugar could therefore be calculated: these values were 7·4, 8·4, 6·3 and 8·5 mg/kg.min for sheep nos. 121, 98, 99 and 128 respectively. This method could only be used in a few cases for glucose and was not applicable at all to fructose. Uptake rates have therefore also been estimated from the rate of fall of concentration of the sugar. Tangents were drawn to the appropriate curves ignoring points taken shortly after the addition of sugar. For glucose, the remainder of the curve was not always straight since uptake seemed to fall off at very low glucose concentrations (Fig. 4*A* and *B*) and in these cases the tangent was drawn to the curve in the region of 20–40 mg/100 ml. To calculate the rate of uptake the equilibration space for the sugar is also required. This was determined in some cases from the change in glucose and fructose concentration when these sugars were administered. The two spaces were found to be similar in volume as is apparent from Figs. 3*B* and 4*A* and *B*, and therefore the fructose space has been used for all calculations. Where the spaces were not measured interpolations have been made. The results of these calculations are given in Table 1. It will be appreciated that the values obtained cannot be very precise but they should give an indication of the order of magnitude. As would be expected the over-all uptake rates given at the beginning of the paragraph differ somewhat from those calculated from the slope of the appropriate curves.

Lactic acid, pH, O_2 saturation and P_{CO_2}

The initial lactic acid concentration of the perfusing blood was very variable, 19–78 mg/100 ml.; but previous work showed that lactic acid concentration in foetal blood with intact foeto-placental circulation lay within a similar range (Barker & Britton, 1957). In four of the preparations there was an initial decline in the concentration but the more usual picture was for the concentration in an individual foetus to maintain its initial level for 60–120 min and then to rise (Fig. 5). In three animals values of over 100 mg/100 ml. were obtained by the end of the observation period; two of these animals were a few days short of term and the third animal (110 days) was perfused for the longest time in this reported series of experiments.

The pH values of the blood reflected fairly closely the blood lactic acid concentration. In the two oldest animals the pH dropped below 7·0.

The oxygen saturations in the blood returning to the foetus were between

TABLE 1. Rates of glucose and fructose uptake compared with the urinary output. The calculated total uptake includes urinary excretion

Animal no.	Foetal age (days)	Period of observation (min)	Equilibration space (ml.)	Glucose		Fructose	
				Total foetal uptake (mg/kg.min)	Urinary output (mg/kg.min)	Total foetal uptake (mg/kg.min)	Urinary output (mg/kg.min)
27	72	264	(290)	1·9, 7·1, 6·7	—	0	—
107	89	109	(590)	4·3	—	0	—
121	100	80	690	7·7	0·005	2·9	1·2
S_1	110	103	(810)	—	—	0·6	—
98	110	325	720	6·0, 2·9, 3·7*	—	1·8	—
94	111	165	(890)	3·1	—	0·77	0·43
106	120	58	(790)	3·7	—	0	—
99	125	200	1290	4·5, 1·8*	0·0014	0·96	0·17
128	125	72	920	6·5*	0·06	0	1·2
S_2	146	174	(880)	—	0·012	0·19	0·18
S_3	146	265	1070	—	0·1	0·35	0·22

Volumes in parentheses are interpolated values.

* Rates obtained when blood lactic acid concentration was rising rapidly.

68 and 100 %. The umbilical arterial saturation varied in different animals and during the course of the perfusion. As would be expected the saturation typically became less as the umbilical blood flow fell.

The plasma P_{CO_2} of the umbilical arterial blood was also variable and the values tended to be high when the O_2 saturations were low. However, the P_{CO_2} was also dependent upon the gas mixture supplied to the oxygenator.

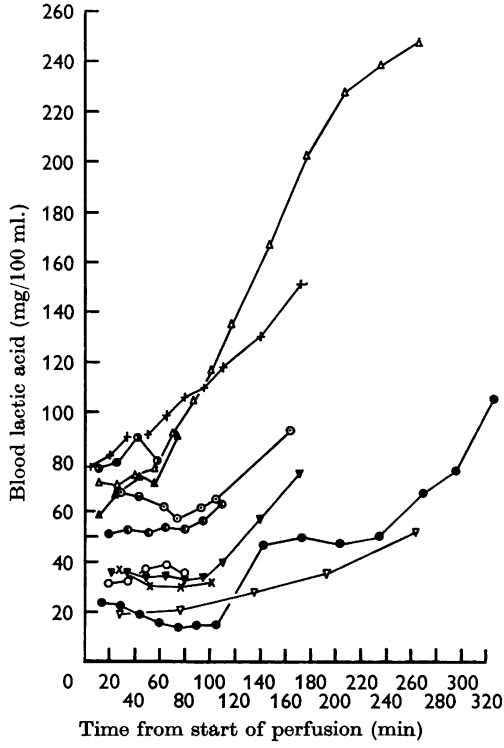

Fig. 5. Changes in the concentration of lactic acid in the blood of sheep foetuses during the course of perfusion. The symbols denote individual animals whose reference numbers are in parentheses. + (S_2), ◑ (106), △ (S_3), ⊙ (94), ▲ (128), ◐ (107), × (S_1), ▼ (99), ○ (121), ● (98) and ▽ (27).

The values obtained for the parameters reported in this section are shown in Tables 2 and 3. Similar values to these were reported by Alexander *et al.* (1964*b*) in animals which were allowed subsequently to survive after a short period of perfusion.

Oxygen consumption

In some animals, where haemoglobin concentrations and umbilical venous oxygen saturations were determined, it was possible to calculate

TABLE 2. Values obtained for blood lactic acid and plasma K^+, Na^+, Cl^-, inorganic P, α amino nitrogen, urea, umbilical arterial P_{CO_2} and pH in the isolated foetus

Sheep no.	27	107	121	S_1	98	94	106	99	128	S_2	S_3
Foetal age (days)	72	89	100	110	110	111	120	125	125	146	146
Obs. period (min)	264	109	80	103	325	165	60	200	72	174	265
K^+ (m-equiv/l.)											
Mean	—	4·5	4·8	—	5·1	—	5·5	4·5	5·5	5·0	7·0
Range	—	4·4–4·8	4·5–5·1	—	4·5–5·6	—	4·8–6·0	4·1–5·3	5·3–5·7	4·2–5·7	4·3–9·7
No. of obs.	—	7	5	—	12	—	4	9	5	9	13
Na^+ (m-equiv/l.)											
Mean	—	160	186	—	163	—	162	247	151	166	150
Range	—	156–164	176–210	—	159–176	—	160–164	200–320	145–156	155–176	140–164
No. of obs.	—	2	5	—	3	—	4	9	5	9	13
Cl^- (m-equiv/l.)											
Mean	—	120	108	—	113	—	109	148	112	117	106
Range	—	116–130	102–113	—	101–118	—	108–112	135–163	112–113	112–121	105–108
No. of obs.	—	7	5	—	14	—	4	9	5	9	13
Inorganic P (mg/100 ml.)											
Mean	—	7·1	5·5	—	7·9	—	9·5	6·8	9·0	10·2	13·0
Range	—	6·4–8·0	5·0–7·0	—	6·0–14·4	—	6·0–16·0	6·0–8·2	7·1–14·3	8·2–11·8	8·8–17·6
No. of obs.	—	6	5	—	12	—	4	10	5	10	13
α amino N_2 (mg/100 ml.)											
Mean	—	5·3	5·1	5·0	4·5	7·5	9·3	5·5	6·4	6·1	7·7
Range	—	4·6–5·9	4·4–6·2	4·8–5·3	3·9–5·6	6·9–9·1	8·7–10·1	4·4–6·3	5·9–7·7	5·7–6·5	5·5–12·0
No. of obs.	—	7	5	5	14	6	4	10	5	9	13
Urea (mg/100 ml.)											
Mean	—	45·8	22·4	66·6	38·3	72·6	51·1	45·8	60·7	390	70·8
Range	—	36–50	22–24	65–68	35–42	70–76	49–52	41–51	47–72	353–470	60–89
No. of obs.	—	7	5	5	11	7	4	10	5	10	13
Lactic acid (mg/100 ml.)											
Mean	31·4	55·4	35·5	32·6	41·3	67·6	82·3	42·4	73·7	105	141
Range	19–53	52–65	31–39	30–37	14–106	57–92	78–91	34–75	61–92	78–151	70–248
No. of obs.	5	7	5	4	14	7	4	9	5	10	13
Umb. art. P_{CO_2} (mm Hg)											
Mean	33	34	35	43	55	56	30	50	55	39	37
Range	—	30–37	34–36	37–49	46–61	45–63	28–32	46–55	53–58	32–57	32–47
No. of obs.	1	6	5	4	10	6	4	7	4	9	12
Umb. art. pH											
Mean	7·35	7·29	7·37	7·28	7·21	7·09	7·08	7·19	7·19	7·11	7·02
Range	—	7·26–7·33	7·36–7·38	7·25–7·32	7·08–7·29	7·05–7·15	7·06–7·12	7·11–7·24	7·16–7·21	6·94–7·20	6·58–7·25
No. of obs.	2	6	5	4	11	6	4	7	4	9	12

TABLE 3. Values for heart rate, femoral artery and inflow pressures, haematocrit, haemoglobin concentration, O_2 saturation of umbilical vein and artery, blood flow and O_2 consumption. Heart rate, blood flow and femoral artery and inflow pressures were read at 5 min intervals from a continuous record

Sheep no.	27	107	121	S_1	98	94	106	99	128	S_2	S_3
Foetal age (days)	72	89	100	110	110	111	120	125	125	146	146
Obs. period (min)	264	109	80	103	325	165	60	200	72	174	265
Heart rate (beats/min)											
Mean	170	145	176	148	205	170	155	160	170	155	220
Range	160–197	129–160	172–180	80–159	145–210	124–210	136–200	88–220	114–228	97–193	126–275
Fem. art. press. (mm Hg)											
Mean	—	—	—	25	45	77	65	60	65	55	77
Range	—	—	—	11–37	37–65	75–79	59–78	36–72	59–75	42–80	67–95
Inflow press. (mm Hg)											
Mean	7	—	13	—	—	15	—	22	11	13	39
Range	2–17	—	8–18	—	—	5–30	—	12–33	5–20	5–24	7–68
Haematocrit (%)											
Mean	24	29	33	37	34	41	41	34	37	40	38
Range	23–28	28–31	28–34	33–41	30–38	39–43	39–44	29–36	35–39	36–42	33–44
No. of obs.	10	7	5	5	13	7	4	10	5	10	11
Haemoglobin (g/100 ml.)											
Mean	—	—	10·1	—	10·5	12·6	12·3	—	—	11·6	11·0
Range	—	—	8·5–10·8	—	9·8–11·0	12·0–13·5	11·8–13·0	—	—	11·2–12·3	9·9–12·7
No. of obs.	—	—	5	—	6	7	4	—	—	10	12
Umb. vein O_2 satn. (%)	—	97	100	95	96	68	98	—	—	—	71
Umb. art. O_2 satn. (%)											
Mean	67	72	64	53	67	22	80	22	23	17	51
Range	39–98	63–84	45–83	41–60	56–83	15–36	72–90	8–47	15–34	5–37	28–61
No. of obs.	5	5	5	3	6	6	4	8	5	9	8
Blood flow (mg./mg.min)											
Mean	150	43	99	92	140	70	175	77	69	50	91
Range	35–320	16–76	59–134	74–130	30–260	34–116	155–260	42–133	27–104	21–89	54–116
O_2 consump. (ml./kg.min)											
Mean	—	—	4·5	—	4·2	5·7	4·5	—	—	—	2·8
Range	—	—	3·1–5·7	—	1·6–8·3	2·8–10·6	2·0–6·8	—	—	—	1·6–4·3

the oxygen consumptions and these are shown in Table 3. The values obtained were similar to those found by Acheson, Dawes & Mott (1957). There was a tendency for the oxygen consumption to fall as the blood flow declined, a feature in keeping with the observation of Dawes & Mott (1964). However, no allowance has been made in the present calculations for the dissolved oxygen in the blood leaving the oxygenator. This may have been appreciable at the low blood flows with the gas mixtures used.

Plasma α amino nitrogen and urea

The mean concentration of plasma α amino nitrogen in ten preparations was 6·5 mg/100 ml. In five of the animals (nos. 98, 121, 128, 94 and S_2) no consistent trend in concentration was observed, in three animals (nos. 99, 107 and S_1) the concentration declined while in two animals (nos. S_3 and 106) an increase was seen over the perfusion period. Except in one animal (no. S_3) where an increase from 5·5 to 12·0 mg/100 ml. occurred over 265 min these changes were probably not significant.

The initial concentrations of urea were very variable, ranging from 22 to 407 mg/100 ml. Only in one perfusion was there a consistent rise in concentration from 36 to 50·4 mg/100 ml. (no. 107) which took place over 90 min. In one animal (no. S_2) an exceptional value of 407 mg/100 ml. was found but this fell during the course of the perfusion to 366 mg/100 ml.

The mean and range of the concentrations of α amino nitrogen and urea for the individual animals are shown in Table 2.

Plasma electrolytes

The average and range of concentration found for K^+, Na^+, Cl^- and inorganic phosphorus are also given in Table 2. The K^+ concentration tended to rise during the perfusion but the rise was less than 1·5 m-equiv./l. hr. The highest value of 9·7 m-equiv/l. occurred at the end of a 265 min perfusion in a 146-day foetus (no. S_3). The other electrolytes measured showed no consistent trend with perfusion time. The concentrations of Na^+, Cl^- and inorganic P in the plasma of the perfused animals were similar to those previously observed in the foetal and pregnant sheep with one exception (no. 99) where both Na^+ and Cl^- concentration were found to be exceptionally high. The values obtained for K^+, with the exception of no. S_3, were below the previously reported concentrations (Alexander, Nixon, Widdas & Wohlzogen, 1958*a*) in spite of some haemolysis.

Haemodynamics

Some of the haemodynamic measurements made on the perfused foetus are shown in Table 3. The pulse rate and femoral blood pressures were

within normal limits when compared with previous experience with the exteriorized foetus with intact umbilical circulation and with the data of Dawes & Mott (1964). The umbilical flow varied considerably and in a typical experiment tended to fall progressively after the first 30–40 min of perfusion, apparently due to umbilical arterial constriction. As a consequence the mean flows in some cases were rather below those found by Cooper, Greenfield & Huggett (1949), and Dawes & Mott (1964). The high oxygen saturation of the inflowing blood, however, in many cases appeared to prevent the umbilical arterial blood oxygen saturation from falling excessively. The inflow pressure varied quite widely but, in general, when the umbilical flow was of the order of 100–200 ml./kg.min, it was about 10–30 mm Hg. Dawes (1962) reported an abdominal umbilical venous pressure of 10 mm Hg in foetuses with intact placental connexions. It does not follow, however, that the abdominal umbilical venous pressure is higher in the isolated foetus since the inflow pressure included the pressure drop through the venous cannulae and along the external part of the umbilical vein. When the umbilical circulation fell the inflow pressure usually, but not always, decreased. There were some variations in haematocrit but these were not systematic and in particular there was no evidence of haemoconcentration.

Renal function in the isolated foetus

Glomerular filtration. In earlier investigations where the placental circulation remained intact, the renal plasma clearance of fructose was used as an index of glomerular filtration rate (Alexander *et al.* 1958*b*). Calculations based on the data of Alexander & Nixon (1963, 1964) show that the fructose clearance is about 70% of the glomerular filtration rate as measured by inulin clearance. However, the fructose clearances in the present series of experiments give values mainly similar to those previously obtained, and thus provide a basis for assessment of renal function in the isolated foetus. Considerable fluctuations in the clearance of fructose occurred during any one experiment but similar changes have been noted in the foetus with intact placental connexions (Alexander *et al.* 1958*b*). Fluctuations in glomerular filtration rate have also been observed in adult sheep (Alexander & Nixon, 1963, 1964). Some fall in the fructose clearance is observed in exposed foetuses with intact placental circulations after a few hours and this also occurred in the isolated foetuses.

Tubular function. Approximately 95% of the filtered glucose was re-absorbed in four animals where the plasma glucose concentration varied between 2 and 130 mg/100 ml. The fifth animal (no. S_3), which had a plasma concentration 350–500 mg/100 ml., re-absorbed 82% of the filtered glucose. The highest rate of glucose re-absorption on a body weight basis

was encountered in this animal and was 0·88 mg/kg.min. This is below tubular maximum (Alexander & Nixon, 1963).

The rate of water re-absorption in four of the animals (94, 99, 128, S_2) was low. In these animals the oxygen saturation of the umbilical arterial blood was also markedly lower than in the other foetuses in the present series.

The proportions of sodium and chloride re-absorbed from the filtrate were in the range 73–89 % and 66–90 % respectively (except for animal no. 128 which showed poor tubular activity in general apart from glucose re-absorption). These rates of sodium and chloride re-absorption are higher than the corresponding values for water. The calculated potassium re-absorption gives lower, more consistent, values independent of the rate of water re-absorption. These findings are similar to those previously obtained with intact placental circulation (Alexander *et al.* 1958*b*).

Inorganic phosphate excretion is variable but high in comparison with previous data (D. P. Alexander & D. A. Nixon, unpublished), where frequently a total re-absorption of phosphate was observed.

The proportion of filtered lactate that is re-absorbed appears to decrease when the blood pH falls. Values for α amino nitrogen re-absorption were also determined. There are, however, no previous data available for any comparison to be made.

A summary of the findings is shown in Table 4.

DISCUSSION

It is difficult to assess the physiological condition of the foetuses but they had normal blood pressures and heart rates and showed occasional spontaneous natural movements. Painful stimuli elicited withdrawal reflexes. In some of the more acidotic foetuses respiratory movements were also seen. The umbilical flows fell progressively due to umbilical arterial spasm and it appeared to be the resultant hypoxia which eventually led to foetal death rather than any other factor.

The disappearance of glucose from the perfusate in the younger foetuses was not associated with any obvious change in the condition of the animal. Foetal movements persisted and there was no evidence of convulsive activity. Further, there seemed to be no fall in oxygen consumption as might possibly have been expected if glucose were an essential metabolite. It would appear therefore that these foetuses are not critically dependent on glucose at least for short periods.

It has been demonstrated in the younger foetuses that when glucose is present it is taken up and it may be of interest to compare this uptake with the oxygen consumption. The oxygen consumption measurements varied

TABLE 4. Renal function in the isolated foetus. The calculated percentage re-absorption is based upon the fructose clearance

Animal no.	Age in days	Period of observation (min)	Urine flow (ml./min)	Fructose clearance (ml./min)	Urea clearance (ml./min)	Calculated percentage re-absorbed from filtrate							
						H_2O	Glucose	Na^+	Cl^-	K^+	Inorganic P	Lactic acid	α amino nitrogen
121	100	80	0·2	0·65	0·54	70	94	84	84	72	28	94	60
94	111	165	0·47	0·87	—	51	—	—	—	—	—	—	—
99	125	200	0·26	0·67	0·63	65	93	73	66	65	79	73	51
128	125	72	0·66	1·68	1·2	48	96	18	50	3	72	51	39
S_2	146	174	0·53	1·82	0·84	71	96	78	82	50	0	22	35
S_3	146	265	0·09	0·86	0·46	87	82	89	90	64	33	76	59

somewhat, but were comparable with the figure of about 4·5 ml./kg.min found by Acheson *et al.* (1957) for intact sheep foetuses. If such an oxygen consumption were used entirely to oxidize glucose to carbon dioxide and water an uptake of 6 mg/kg.min would be expected. From the results it is apparent that the glucose uptake could account for a large part, although not necessarily all of the oxygen consumption. An increase in glucose uptake might have been expected when glycolysis was occurring but there was no evidence of this from observations made when the lactic acid concentration was rising steeply. On the other hand, hyperglycaemia probably increased glucose uptake, since the decline in glucose concentration often followed a curved rather than a linear time course. It is difficult, however, to be sure that the curvature was not partly due to equilibration of glucose concentration within the glucose space. Such an increase in glucose uptake with hyperglycaemia might explain the beneficial effects in hypoxia of glucose infusions (when combined with alkali) found by Dawes, Mott, Shelley & Stafford (1963), and Dawes, Jacobson, Mott, Shelley & Stafford (1963). The urinary excretion of glucose was negligible.

It appears that the young foetuses may depend upon a transplacental passage of glucose of about 5 mg/kg.min to maintain their blood concentration. Nixon (1963) has shown that the perfused uterus and placenta at a conceptual age of 120 days metabolizes glucose at about 20 mg/min. The total requirement of the pregnant uterus at this age with a foetus of 1·5 kg may therefore be about 28 mg/min or 40 g/day.

In the older foetuses the blood glucose did not fall and in some cases rose, particularly when the foetus was becoming hypoxic. This is consistent with the report of Dawes, Mott & Shelley (1959), who found that the blood glucose rose when the cord was tied in older foetuses. It would thus seem that in the older foetuses endogenous glucose production can keep pace with glucose utilization. Towards term the glycogen concentration of the sheep foetal liver is known to rise steeply (Shelley, 1960). It is therefore possible that the rise in blood glucose observed in the older foetuses reflects a break-down of this glycogen store due to hypoxic stress.

In contrast to the glucose it was found that fructose uptake could account, at most, for only a small part of oxidative metabolism. Further, there was no evidence of any change in fructose disappearance from the blood during hypoglycaemia or during hypoxia when glycolysis was occurring, as might have been expected if fructose were a reserve carbohydrate. Dawes & Shelley (1962), suggested that renal excretion in mature foetuses might account for most if not all of the fructose that disappeared from the circulation when the umbilical cord was tied. However, in our experiments, although excretion of fructose was considerable, in most cases

it did not account for all of the fructose that disappeared. There is thus suggestive evidence for some utilization of fructose, although it is probably extra-hepatic, since Andrews, Britton, Huggett & Nixon (1960) and Andrews, Britton & Nixon (1961) were unable to demonstrate fructose removal in the perfused liver until a few days after the normal gestation period.

In the terminal stages of the perfusions there was a progressive hypoxia associated with an acidosis and a rising lactic acid. Before this occurred, however, there seemed to be no significant changes in the plasma constituents other than those discussed above. It is difficult to assess to what extent this reflected foetal homoeostasis and insufficient time being available for changes to develop, but it is perhaps of interest that the foetus can be independent of the placenta for some hours without gross abnormalities developing.

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