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THE PLACENTAL TRANSFER OF SUGARS IN THE SHEEP: STUDIES WITH RADIOACTIVE SUGAR

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In 1951, Huggett, Warren & Warren demonstrated that experimental hyperglycaemia in the pregnant sheep or in the foetus is followed by hyperfructosaemia confined to the foetus, in which there normally is fructose (Bacon & Bell, 1948). Huggett *et al.* found too that the source of the new fructose is the placenta, and that the foetus plays no part in its production. Further, glucose passes across the placenta in both directions but fructose moves only from the mother to the foetus and at a slower rate than does glucose.

Widdas (1952) reviewed the kinetics of the passage of glucose across the placenta in the sheep. He suggested that this can be explained if the mechanism is one of active transport and not simple diffusion. The exact nature of the process is, however, unknown.

The question arose as to whether there is any fructose formation by the placenta in the absence of hyperglycaemia, that is, whether fructose formation can proceed at low or normal blood glucose levels. The experiments described in this paper show that hyperglycaemia is not essential for fructose production; they also demonstrate that glucose molecules can pass from the foetus to the mother against a concentration gradient.

Two types of investigation have been used, namely perfusion of the umbilical circulation and the injection of radioactive ^{14}C-glucose. This paper is confined to a study of the intravenous injection of ^{14}C-glucose into the mother or the foetus.

The methods used were to determine the concentrations of both glucose and fructose in maternal and foetal bloods and also the distribution of the ^{14}Carbon in the glucose and fructose. This latter determination was effected by converting the sugars of the deproteinized blood filtrate to carbon dioxide by fermentation with yeast before and after destroying the glucose with glucose

oxidase (notatin). The radioactive carbon dioxide was collected as barium carbonate and counted with a Geiger counter. One would expect the counts on carbon dioxide, obtained by fermentation after treatment with glucose oxidase, to be due to fructose which is fermented by yeast but is not oxidized by glucose oxidase.

In practice it was found that if a pure radioactive glucose standard were twice fermented with yeast, once before and once after treatment with glucose oxidase, there was an evolution of radioactive CO_2 on both occasions, and this occurred with more than one strain of yeast. It would appear therefore that the yeast in the second fermentation was acting on some substance not present until the glucose oxidase had acted on the glucose. This material could be a product like gluconic acid formed by the oxidation with notatin but there is no evidence on the point.

The residual counts obtained when fermenting the glucose standards after notatin oxidation need to be allowed for when assaying the blood specimens. In practice, aliquots of ^{14}C-glucose standards, as well as blood specimens, were fermented with yeast in an atmosphere of nitrogen with and without prior oxidation by notatin in an atmosphere of oxygen.

The experiments fall into three classes:

(i) Intravenous injection of ^{14}C-glucose to the mother without the production of maternal hyperglycaemia to determine if ^{14}C-fructose is, in this circumstance, formed in foetal blood.

(ii) Intravenous injection of ^{14}C-glucose to the foetus to see if it goes back to the mother.

(iii) Intravenous injection of ^{14}C-glucose to the mother with an elevated concentration gradient of glucose from mother to foetus to determine the resulting glucose uptake by umbilical blood.

Two strains of yeast were used, *Saccharomyces cerevisiae* and *Torulopsis mannosa*. Experiments with the second yeast gave greater constancy of results.

METHODS

The caesarean section, blood sampling, deproteinization and sugar determinations were essentially the same as described by Huggett *et al.* (1951). A difference was introduced in the determination of the total reducing substances (T.R.S.). This was effected by Somogyi's modified tartrate reagent (1952) for which we are indebted to Dr Michael Somogyi who kindly supplied us with a preview of his manuscript before publication. The fructose estimation was by Cole's modification of Roe's method (1934) as described in Bacon & Bell (1948). Corrections were applied for the influence of each on the determination of the other when both were present.

Radioactive sugar determinations

The fermentation by yeast of the filtrates and the evolution and trapping of the $^{14}CO_2$ as barium carbonate was carried out in a closed circuit apparatus shown in Fig. 1.

The three-way tap T on the fermentation flask F_1 connects either the side tube A or the thistle funnel to a tube leading below the surface of the liquid in the fermentation flask. The second side

tube *B* with standard taper and tap can be connected to a pump used for drawing gas (O_2 or N_2 as required) through the flask before adding the glucose oxidase or the yeast suspension via the thistle funnel.

This ground joint can also be connected with a second flask F_2, shaped like an inverted closed funnel, containing baryta water. This arrangement is used with a pump, employing a roller working on rubber tubing, which circulates gas from the fermentation flask through the baryta water and back to the fermentation flask.

After collection of the CO_2 as barium carbonate the second flask is temporarily closed while being inverted over a Seitz filter (sterimat) for filtration under reduced pressure.

The radioactive glucose of known specific activity was weighed and dissolved in water to give a known concentration usually between 1 and 5%. If necessary this solution was then mixed with inactive carrier glucose (concentration 5 g/100 ml.) before injection. About 200 μc were required for an experiment.

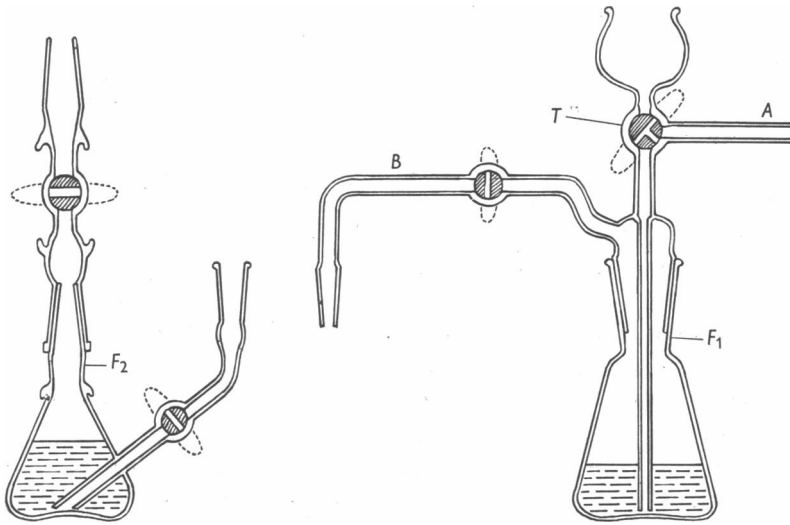

Fig. 1. Apparatus for fermentation of total sugars and oxidation of glucose. Description in the text.

Samples were taken from the maternal and foetal bloods as desired. The filtrates from blood samples of 1·25 ml., deproteinized by barium hydroxide and zinc sulphate, were used for yeast fermentations as previously described. Fermentation was carried out at 37° C for 40 min. The filtered barium carbonate on the sterimat was transferred to a desiccator and when dry counted with a thin-window Geiger counter.

A second sample of the filtrate from 1·25 ml. of blood was incubated with 0·5 mg of glucose oxidase together with a catalase preparation and a stream of oxygen bubbled through for 40 min. The oxygen in the fermentation flask was then replaced by nitrogen which was brought to a pressure of about 450 mm Hg, a suspension of yeast added and fermentation continued for 40 min. The subsequent collection of CO_2 as barium carbonate was as described above.

RESULTS

In control experiments of the earlier series, using aqueous sugar solutions and *S. cerevisiae*, it was found that the counts given by the barium carbonate were proportional (with a maximal error of 15%) to the number of milligrams of

radioactive sugar fermented in the range 0·5–4·0 mg. Thus the counts/min divided by the weight in mg of the sugar in a specimen could be taken as a measure of the specific activity of the sugar. The counts from one experiment to another, however, varied considerably, and it is more convenient to refer such activities to that of one specimen and this has been done in giving quantitative values to the results which follow. The specific radioactivity of one specimen in each experiment has been fixed at an arbitrary value of 100 and values given for other specimens are their activities relative to this standard.

To determine the allowance to be made for the activity after oxidation by notatin it was found that in six experiments using pure glucose standards the average residual counts were 15% of those obtained from the product of yeast fermentation alone. In four experiments with maternal sheep's blood the average residual counts were $\simeq$22%. In these experiments, therefore, counts from foetal blood, after oxidation of the glucose, which are in excess of about 22% of the counts from yeast fermentation alone are taken to indicate incorporation of ^{14}C into fructose, whereas counts of the same order as 22% or less are regarded as not so indicating. An estimate of the activity due to fructose was obtained by calculation from two counts, (1) the count obtained from the CO_2 resulting from yeast fermentation of a blood filtrate which had not been treated with glucose oxidase (a measure of radioactive glucose and fructose), and (2) the count obtained from a second sample of blood filtrate which had been treated with glucose oxidase before yeast fermentation (a measure of radioactive fructose and residual activity).

The activity per mg of fructose could be arrived at from this measurement of activity, together with the quantities of fructose and T.R.S. estimated by chemical methods.

The results showed that the slow intravenous injection of radioactive ^{14}C-glucose into the ewe or the foetus did not significantly affect the pre-injection levels of sugar in the blood of either, but the counts from foetal specimens after oxidation of glucose were significantly higher than could be accounted for by the residual glucose counts in the $\frac{1}{4}$, $\frac{1}{2}$, 1 and 3 hr samples. Further, when the activity was estimated on the assumption that it was all in the foetal glucose, values were obtained which were not only greater than those for maternal glucose but also in some cases implied an impossible concentration of ^{14}C-glucose relative to ^{12}C-glucose in foetal blood.

Fructose formation in the absence of maternal hyperglycaemia

The figures for radioactivity in the bloods of ewe and foetus, derived from three experiments in which ^{14}C-glucose was injected into the ewe without causing maternal hyperglycaemia, are summarized in Table 1.

In the later series of experiments using the yeast *Torulopsis mannosa* and

carrying out fermentations in an atmosphere of N_2 with azide added to the medium it was found that the residual counts obtained from ^{14}C-glucose standards when fermented, following oxidation of the glucose with notatin, were somewhat less than in the *Saccharomyces cerevisiae* experiments but they were still of the order of 5–15% of the counts obtained on fermentation without prior oxidation. In experiments of short duration (20 min), in which

TABLE 1. Sheep 526, 539, 513, 659 and 806. Measurement of radioactivity in maternal and foetal bloods showing formation of foetal fructose during injection of ^{14}C-glucose into ewe while avoiding maternal hyperglycaemia

	Relative counts/min derived from yeast fermentation			
Time sample taken	Of 1·25 ml. maternal blood A	Of 1·25 ml. foetal blood B	Following glucose-oxidase action on foetal blood C	Post-oxidase counts (%) i.e. 100C/B D
	Sheep 526 (foetus 3·0 kg, 134 days gestation age)			
¼ hr	100 (2)*	40	15	37·5
½	67	65	45	69
1	45	72†	94	>100
3	55	40	9·4	23·5
	Sheep 539 (foetus 3·2 kg, 134 days gestation age)			
¼ hr	100 (21·4)*	41	20	49
½	75	71	53	75
1	61	109	74	68
3	21	71	66	93
	Sheep 513 (foetus 0·5 kg, 95 days gestation age)			
¼ hr	100 (21·7)*	48	32	67
½	89	84	42	50
1	38	126	69	55
3	15	72	56	78
	Sheep 659 (foetus 2·1 kg, 124 days gestation age)			
5 min	19 (4)	19	2	9·5
10	100 (22)*	33	3	9·1
20	110 (36)	49	10	20
30	89	32	18	56
	Sheep 806 (foetus 3·6 kg, 140 days gestation age)			
¼ hr	100 (5)*	27·5	—	—
½	100 (5)	37·5	10·4	28
1	69 (14)	33·5	18·3	55
3	6 (1·9)	25·5	20·5	80

Times measured from beginning of injection of radioactive sugar.

* Reference specimen for each experiment. Figures in brackets (column A) indicate residual counts on a second specimen after glucose oxidation by oxidase. In sheep 526, 539 and 513 the 1 hr foetal specimens give higher counts than do maternal samples although containing less glucose, indicating the presence of radioactive foetal fructose. The post-oxidase counts which averaged 21% on the maternal samples are significantly higher in foetal specimens. In these sheep fermentation was accomplished with *S. cerevisiae*. In sheep 659 the post-oxidase counts from foetal specimens exceed what may be expected from residual counts at 30 min. In this sheep and in sheep 806 fermentation was with *Torulopsis mannosa*.

† The low relative count of 72 is due to an error in technique which yielded the high value of >100 in column D (actually 130%).

foetal blood was sampled at 5, 10, 20 and 30 min following the commencement of slow infusion of ^{14}C-labelled glucose into the maternal blood, the activity of foetal blood samples after glucose oxidation by notatin was not significantly above what might be attributed to residual counts except in the 30 min sample. The results of such an experiment are shown in Table 1 and Fig. 2. Thus the method proved to be insufficiently sensitive to follow the rate of formation of ^{14}C-fructose in the early stages of an experiment. The activity per mg of the foetal fructose at 30 min in two such experiments was only 2·8 and 7·6% of the activity of the maternal glucose at 10 min (sheep 615 and 659 of 110 and 124 days foetal age respectively).

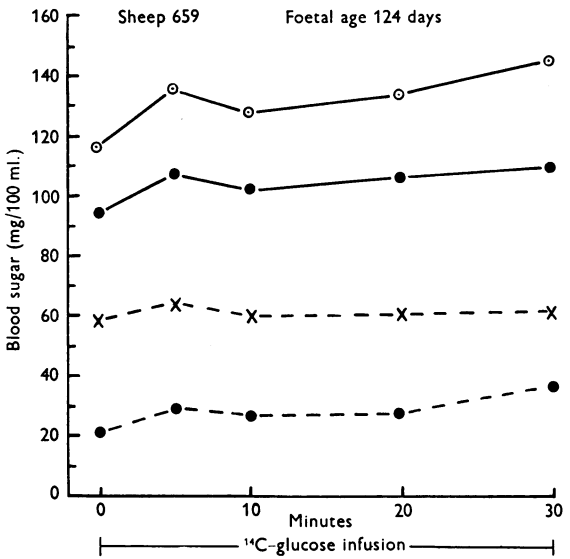

Fig. 2. Data from sheep 659 illustrating the quantitative results of the slow infusion experiments in which radioactive glucose is injected into the maternal blood without carrier glucose. Although the glucose concentration of the maternal blood exceeds that of foetal blood, as is usual, the combined foetal sugars (glucose and fructose) exceed in concentration that of the maternal glucose. ● —— ●, foetal fructose; ● - - - ●, foetal glucose; ⊙ —— ⊙, foetal total reducing substance, T.R.S.; × - - - ×, maternal glucose.

In an experiment (sheep 806), of 3 hr duration instead of 30 min and similar to those described above using sheep 615 and 659, the residual counts on glucose standards and maternal blood at $\frac{1}{4}$ and $\frac{1}{2}$ hr were only 5% of the original counts but were of the order of 20 and 30% at 1 and 3 hr (Table 1).

In this experiment the activities of the foetal fructose at $\frac{1}{2}$, 1 and 3 hr relative to that of the maternal glucose in the $\frac{1}{4}$ hr specimen were 2·3, 7·8 and 11·6% respectively, when allowance is made for residual glucose counts of 20%. The same calculations made for residual glucose counts of 5% give

activities of 5·0, 11·0 and 12·2% at ½, 1 and 3 hr respectively. The best estimate which these results permit us to make is that about 2·3–5·0% of the foetal fructose was derived from maternal glucose during the first ½ hr period and about 5·5–6·0% during the second half hour period.

Assuming no radioactive fructose was destroyed during the same period, an estimate of the rate of fructose synthesis could be obtained if the total mass of fructose were known.

Fructose space

To estimate the total mass of fructose in the foetus and placenta it is necessary to know the fructose space—that is the combined foetal and placental fluid volume within which fructose becomes evenly distributed. There have been a number of experiments carried out by Huggett *et al.* (1951) and by the present authors in which fructose was injected into the umbilical vein and a specimen taken 5 min later. If it be assumed that this time allows even distribution throughout the fructose space but is so short as to permit of no significant removal of fructose and, as has been observed (Hitchcock, 1949; Goodwin, 1954), the sugar is evenly distributed between red blood cells and plasma, then the volume of the fructose space can be estimated in litres from the formula

$$\frac{W \text{ mg injected}}{\text{(increase in concentration mg/100 ml. of fructose in foetal blood resulting from injection)}} \div 10,$$

i.e.

$$\frac{W \text{ mg}}{10\,(C_2 - C_1) \text{ mg/100 ml.}}.$$

The results of a number of such experiments are given in Fig. 3 showing the dependence of the value obtained on foetal age.

Fructose synthesis

Using the values for the fructose space taken from Fig. 3 and the relative specific activity of the foetal fructose at 30 min, following a slow infusion of ^{14}C-glucose as described in the above experiments, it is calculated that the minimum rate of new synthesis of fructose in the sheep's placenta of 110 days gestation age lies between 2·3 and 7·7 mg/min and the single experiment of 140 days gestation age (sheep 806, Table 1) gives a value of 2–5·3 mg/min.

This value is derived as follows: the fructose space is taken as 1·5 l. and the total mass of fructose therefore $177 \times 15 = 2660$ mg. But 2·3–5·0% was derived from ^{14}C-labelled glucose within the first ½ hr and a further 5·5–6·0% during the second ½ hr. These amounts range from 61 mg (2·3%) to 160 mg (6·0%) so, assuming no loss of radioactive fructose, new production must be of the order 2·0–5·3 mg/min.

In spite of the limitations of the radioactive method, due to the residual counts found after oxidation of pure glucose, these estimates are of the same order as those of direct perfusion experiments to be described in a subsequent paper.

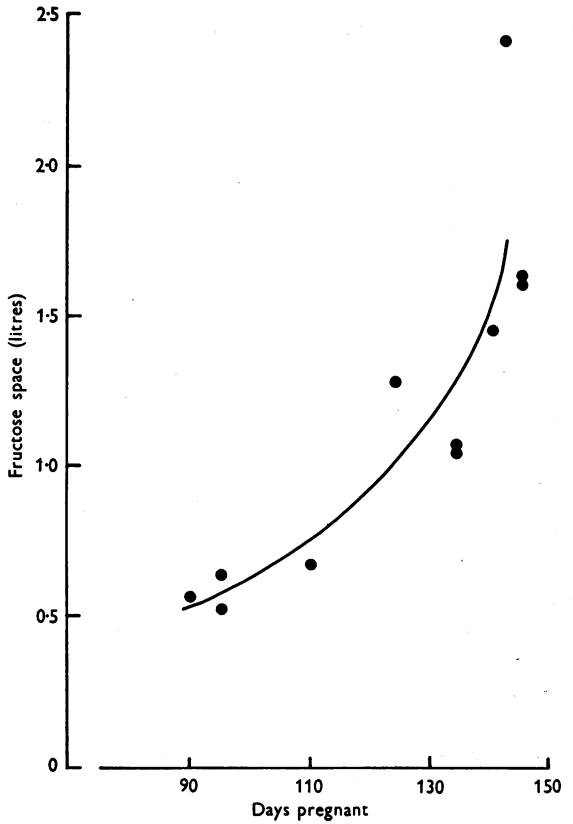

Fig. 3. Fructose space in sheep foetus and placenta at different foetal ages. The volume was calculated from the formula

$$V = \frac{\text{fructose injected (mg)}}{\{C\ (5\ \text{min}) - C\ (\text{initial})\} \times 10}.$$

Effect of foetal hyperglycaemia

In experiments with ^{14}C-glucose in which a foetal hyperglycaemia was produced by injecting additional non-radioactive carrier with the radioactive glucose into the umbilical vein, it was possible to confirm the transfer of glucose from the foetal to maternal circulation.

This is illustrated by the results of two experiments (sheep 509 and 518 of foetal ages 104 and 110 days) shown in Tables 2 and 3. In these experiments the activity of the mixture of carrier and radioactive glucose in the injected

solution was taken for reference (i.e. as 100) and activities of the other specimens are relative to this value.

Since the counts in the two experiments have been scaled down to compare with a standard whose activity is taken as 100 counts/min for each mg of

TABLE 2. Sheep 509 and 518. Measurement of radioactivity of maternal and foetal bloods during injection of ^{14}C-glucose with ^{12}C-glucose as carrier into the umbilical vein to cause foetal hyperglycaemia and fructose formation

	Relative counts/min derived from yeast fermentation			
Time sample taken (hr)	Of 1·25 ml. of maternal blood A	Of 1·25 ml. of foetal blood B	Following glucose-oxidase action on foetal blood C	Post-oxidase counts (%), i.e. 100C/B D
	Sheep 509 (foetus 0·9 kg, 104 days gestation age)			
½	22	95	55	58
1	17·5	78	50	64
3	7·6	51	49	96
	Sheep 518 (foetus 1·3 kg, 110 days gestation age)			
½	3·5	51	36	71
1	3·2	47·5	36	76
3	1·7	26	26	100

Rate of injection 2 g/kg foetal weight. In these experiments the counts have been scaled down in proportion to the injection glucose such that each mg of injected glucose gave 100 counts/min on yeast fermentation.

TABLE 3. Sheep 509 and 518. Quantities of labelled glucose present in 100 ml. of maternal and foetal blood as a result of injecting ^{14}C-glucose and ^{12}C-carrier glucose into the umbilical vein in quantity to cause foetal hyperglycaemia

	Maternal blood				Foetal blood	
Time sample taken (hr) (1)	Total glucose mg/100 ml. (2)	Labelled sugar present mg/100 ml. (3)	Glucose mg/100 ml. (4)	Fructose mg/100 ml. (5)	Total sugar mg/100 ml. (6)	Labelled sugar present mg/100 ml. (7)
	Sheep 509 (foetus 0·9 kg, 104 days gestation age)					
Initial	47	—	52	168	220	—
½	70·8	18	116	172	288	76
1	93·6	14	86·5	196	282·5	62
3	56	6	47	200	247	41
	Sheep 518 (foetus 1·3 kg, 110 days gestation age)					
Initial	26·6	—	25	68	93	—
½	60·4	3	55	92	147	41
1	56·4	2·6	37	108	145	38
3	45	1·4	39	132	171	21

mixed glucose (radioactive and carrier) in the injected solution, the amount of such mixed glucose in 100 ml. of the various specimens can be calculated by multiplying the counts from 1·25 ml. by 80 and dividing by 100. This has been done for columns A and B of Table 2 and the results included in Table 3, columns 3 and 7.

The high values of the post-oxidase counts in these hyperglycaemic experiments, compared with the non-hyperglycaemic, indicate that as the foetal glucose is progressively mixed with the larger quantity of non-radioactive glucose in the maternal circulation and extracellular fluid the activity left in foetal blood is more and more due to foetal fructose.

The measurements of radioactivity confirm the rapid flow of injected glucose across the placenta to the maternal circulation (Table 2, column A).

Effect of maternal hyperglycaemia

Another aspect of placental physiology on which it was considered ^{14}C-glucose experiments might throw some light was the rate of glucose transfer across the placenta under conditions of maternal hyperglycaemia.

In experiments with this object in view it was possible to modify the techniques in two respects. First, the maternal blood sugar was raised by a slow injection of non-radioactive glucose and time allowed for the blood sugar concentration to reach a plateau in both maternal and foetal circulations; then a slow continuous injection of ^{14}C-labelled glucose was added to the infusion of non-radioactive glucose into a maternal vein for 20–30 min during which period samples of foetal blood were taken. Secondly, as a short-term experiment only was planned and as the incorporation of ^{14}C into foetal fructose was found to be small within the first 30 min, the glucose oxidase separation technique could be discarded.

In one experiment (sheep 626, Table 4), therefore, yeast fermentations without oxidase were carried out, and as a parallel check planchettes were prepared with 0·1 ml. plasma and dried. In a second experiment (sheep 645, Table 5) duplicated samples of dried plasma were used. Although counts obtained on dried plasma are lower than those with yeast fermentation, due to the small volume of plasma used, the errors in manipulation and technique are minimized and for the present purpose the results are probably more reliable.

The results of these experiments are summarized in Tables 4 and 5. In both sheep the slow infusion of non-radioactive glucose was effective in establishing a plateau concentration in both maternal and foetal blood although a concentration difference from mother to foetus of about 500 mg/100 ml. was observed in each case.

In the results of sheep 626 the activity in maternal blood containing 800 mg/100 ml. is equal to 70 counts/min/0·1 ml. dried plasma over the first 5 min of the ^{14}C-glucose infusion. The 11 counts/min given by foetal plasma must represent 126 mg ($\frac{11}{70} \times 800$) of maternal glucose per 100 ml. acquired in 5 min for every 270 mg of glucose in the foetal concentration. If equilibration at this level has been complete within the 5 min then 25 mg/min of maternal glucose has transferred to each 100 ml. of foetal blood. Between 5 and 10 min

the uptake of maternal glucose averages 30 mg/min for every 100 ml. of blood, assuming no loss of radioactivity by backflow.

Since the umbilical blood flow in a foetus of this age is about 200 ml./min (Cooper, Greenfield & Huggett, 1949) it follows that a placental transfer of maternal glucose of at least 60 mg/min was occurring in this experiment.

TABLE 4. Sheep 626. (Foetus 2·2 kg, 126 days gestation age.) Placental transfer of maternal glucose. Maternal hyperglycaemia established by prolonged intravenous infusion of ^{12}C-glucose; additional slow injection of ^{14}C-glucose after 60 min

	Maternal blood			Umbilical vein blood			
Time of sample	Glucose mg/100 ml.	Counts derived from 1·25 ml. blood	Counts/min 0·1 ml. dried plasma	Glucose mg/100 ml.	Fructose mg/100 ml.	Counts derived from 1·25 ml. blood	Counts/min 0·1 ml. dried plasma
Initial	7·8	—	—	0	47	—	—
After 60 min ^{12}C-glucose infusion and at start of ^{14}C injection	768	—	—	279	88	—	—
+ 5 min	800	163	70	270	92	35	11
+10 min	782	223	125	289	88	61	28
+15 min	740	289	192	331	95	96	Plasma lost
+20 min	806	282	194	321	96	118	71

Counts not corrected relative to the count of any standard blood sample.

TABLE 5. Sheep 645. (Foetus 0·92 kg, 107 days gestation age.) Placental transfer of maternal glucose. Maternal hyperglycaemia established by prolonged intravenous infusion of ^{12}C-glucose; additional slow injection of ^{14}C-glucose after 30 min

	Maternal blood		Umbilical artery blood			Umbilical vein blood		
Time of sample	Glucose mg/100 ml.	Counts/min 0·1 ml. dried plasma	Glucose mg/100 ml.	Fructose mg/100 ml.	Counts/min 0·1 ml. dried plasma	Glucose mg/100 ml.	Fructose mg/100 ml.	Counts/min 0·1 ml. dried plasma
Initial	62·5	—	—	—	—	38	163	—
After 30 min ^{12}C-glucose infusion and at start of ^{14}C injection	705	—	152	181	—	124	202	—
+ 5 min	793	74	183	175	4	187	181	11
+10 min	790	115	193	173	12	207	186	23
+15 min	838	130	209	181	19	228	183	30
+20 min	802	208	207	181	21	239	195	44

Counts not correlated relative to the count of any standard blood sample.

In sheep 645, samples of umbilical artery and vein blood were taken simultaneously and the arteriovenous (A–V) difference in radioactivity can be used to estimate the uptake of maternal glucose by the foetus on the same lines as

described above. The results are shown in Table 6 and indicate that each 100 ml. of foetal blood passing through the placenta is taking up over 70 mg of maternal sugar. This means that in this experiment about one-third of the total glucose in the foetal blood is derived from maternal glucose at each passage through the placenta.

TABLE 6. Sheep 645 (Table 5). Calculation of maternal glucose entering the umbilical circulation

Time of sample (1)	Maternal blood mg/100 ml. (2)	Maternal blood counts/min from 0·1 ml. plasma (3)	Umbilical blood A–V difference in counts/min per 0·1 ml. plasma (4)	Maternal glucose entering umbilical venous blood mg/100 ml. $\frac{\text{coln. (4)}}{\text{coln. (3)}} \times \text{coln. (2)}$ (5)
+ 5 min	793	74	7	75
+10 min	790	115	11	75·5
+15 min	838	134	11	69
+20 min	802	208	23	89
				Av. 77·1

The determination of radioactivity treated in this way gives no information about the net transfer from mother to foetus since the glucose flux from foetus to mother is unknown. However, if the process was one of simple diffusion the forward and back transfers would be proportional to the plasma glucose concentrations. In the foetal blood the sugars are approximately evenly distributed between cells and plasma, whereas in the adult the sugar is mainly in the plasma and plasma concentrations are approximately 1·5 × whole blood concentrations (Goodwin, 1954). Therefore the approximate plasma glucose concentrations are given by

$$\text{maternal plasma} : \text{foetal plasma} = 800 \times 1{\cdot}5 : 200 = 6 : 1.$$

On a basis of diffusion and with a forward flux of 70 mg/100 ml. of blood the retroflux should be 12 mg/100 ml. and the net transfer of glucose should be 60 mg and a concentration difference of this order would therefore be expected between umbilical venous and arterial blood. The actual concentration differences observed in the 5, 10, 15 and 20 min samples respectively were 4, 14, 19 and 32 for glucose alone and 10, 27, 21 and 46 if both glucose and fructose are taken into account. These values are considerably below the 60 mg needed for a diffusion mechanism.

The evidence of these results confirms the inability of simple diffusion to account for glucose transfer and, assuming an active transfer mechanism exists, gives an indication of the maximum transfer which could be effected by the mechanism if it were fully saturated and working unidirectionally.

In fact, in a carrier transfer system such as postulated by Widdas (1952), when measuring with a radioactive tracer technique there would be found an increase in glucose flux but not net transfer, since the back flux would rise too.

This back flux not only explains the absence of equilibration between maternal and foetal glucose concentrations but also the fact that the blood glucose level of the foetus is only a third of that of the mother.

DISCUSSION

It would appear from the literature (Huggett & Hammond, 1952) that traces of fructose probably occur in the foetal blood of most mammals, but the ruminants and the whales (Goodwin, 1954) are the only groups so far investigated in which the foetal fructose exceeds the foetal glucose and in which fructose is indeed quantitatively the chief foetal sugar.

The present experiments show that fructose is being formed while the glucose concentrations in ewe and foetus are at physiological levels, and it seems there must be a dynamic equilibrium in which the rate of formation of fructose in the placenta is balanced by the rate of removal either by foetal or placental tissues.

The estimated rate of fructose production, 2–7 mg/min, appears small compared with the large quantities of glucose which can be transferred through the placenta under conditions of hyperglycaemia, but these conditions are physiologically abnormal. The fructose production, however, may play an important part in foetal or placental metabolic processes as suggested by Huggett *et al.* (1951) in view of the low glucose concentrations frequently met with in the sheep.

As regards glucose transfer, the present experiments demonstrate a high capacity for transfer either from maternal to foetal circulation or in the reverse direction when hyperglycaemia is produced experimentally. Considerable variation may exist from animal to animal but a flux of 70 mg/min appears to be minimal at high glucose concentrations. The observed backflow of ^{14}C-glucose from foetus to mother is what might be expected of a non-secretory process by which transfer of sugar can occur in both directions. That this backflow occurs even against a gradient has been shown in recent studies by Chinard, Danesino, Huggett, Paul & Reynolds (1955) in the monkey.

Although observed glucose transfer cannot be reconciled with simple diffusion (Widdas, 1952), the process would not appear to be an active secretion of glucose and our experiments suggest that the direction of net transfer depends on the relative magnitudes of the maternal and foetal concentrations.

It is possible to visualize a transport mechanism whereby molecules are transferred from a region of higher to one of lower concentration, not by a simple process of diffusion but by a chain of reactions (Danielli, 1954). The chain, in this instance, would be one in which glucose is involved in the formation and breakdown of complexes whose motion is such that the direction of net transfer of glucose is determined by the relative active masses of the sugar at the ends of the chain. If this be so the concentration of glucose in the

foetal blood must normally be lower than in maternal blood. In fact the maternal blood glucose concentration does usually exceed the foetal blood glucose concentration in all species (Huggett & Hammond, 1952). The hypothesis would account for these observations and further it offers an explanation of the passage of labelled glucose molecules in a direction opposite to that of net transfer.

An enzymic pathway whereby glucose is converted into fructose, does not necessarily suffer such limitations and there would appear to be continued synthesis of fructose even though its concentration is higher than that of glucose in either the maternal or foetal bloods. Thus fructose production is in keeping with a secretory function on the part of the placenta. This function, coupled with the relative impermeability of the placenta to fructose (Huggett *et al.* 1951), presumably accounts for the raised foetal fructose concentration.

The use of a radioactive technique to investigate the placental fructose production is limited by the need to separate glucose and fructose in foetal blood specimens. No simple method suitable for the quantities required for radioactive assay is available, and the indirect method described in this paper loses in sensitivity as a result of the residual counts given on yeast fermentation by glucose standards even after glucose oxidation by notatin. A paper chromatographic method was considered, but in view of the small quantities of solute separated on paper it would be necessary to use injection solutions of very high activity. The present results, however, suggest that the rate of fructose production and placental glucose may be determinable also by direct perfusion experiments and these will form the subject of a subsequent paper.

SUMMARY

1. ^{14}C-fructose can be identified and determined in the presence of ^{14}C-glucose by differential fermentation with yeast before and after destroying the glucose with glucose-oxidase, the $^{14}CO_2$ being trapped and counted.

2. The method is found to be somewhat insensitive since the oxidase residues fermented with yeast yield $^{14}CO_2$ counts equal to approximately one-fifth of those obtained from pure glucose standards. Allowance has been made for these residual counts.

3. When ^{14}C-glucose is injected into the pregnant sheep both ^{14}C-glucose and ^{14}C-fructose are found in the foetal blood even though there is no maternal hyperglycaemia.

4. Experiments with ^{14}C-glucose also demonstrate the backflow of glucose from foetus to ewe when a foetal hyperglycaemia is produced.

5. The magnitude of the glucose flux from ewe to foetus during a maternal hyperglycaemia was found to be over 70 mg/min. The net transfer was considerably less than would be expected on a basis of diffusion.

6. The evidence suggests that under physiological conditions, the formation and withdrawal of fructose must be in dynamic equilibrium such that fructose production by a secretory-like process is balanced by its removal or utilization by the foetus or placenta or both.

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