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THE TRANSMISSION OF HEXOSES ACROSS THE PLACENTA IN THE HUMAN AND THE RHESUS MONKEY (*MACACA MULATTA*)

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The blood sugars of the foetus appear to be two in number, glucose which is always present and fructose, which is only present in certain species. The role of foetal fructose and the evolution of our knowledge concerning it have already been reviewed up to 1946 (Huggett & Hammond, 1952). Since then it has been shown that when present in the foetal blood of sheep it is also present in the amniotic and allantoic fluids (Cole & Hitchcock, 1946; Barklay, Haas, Huggett, King & Rowley, 1949). Davies (1952) emphasizes its appearance in the allantoic fluid of the sheep embryo before implantation.

It appears that fructose in foetal blood in any quantity is restricted to sheep, goats, pigs, horses and whales (Goodwin, 1953, 1954) and deer (Walker, 1954), and in these species its concentration exceeds that of glucose. It is absent or present only in traces of 5 mg/100 ml. or less in the human foetus and in certain other mammalian foetuses (Karvonen, 1949*a*, *b*; Hagerman & Villee, 1952; Villee, 1953; Goodwin, 1954; Davies, 1954; Huggett, unpublished observations).

Karvonen (1949*a*, *b*) has suggested, on the basis of exclusion experiments, that the placenta is probably the site of formation of fructose, and that it is not made in the foetal body. Huggett, Warren & Warren (1951), with evidence from twin foetuses and placental perfusion by the umbilical circulation, showed that fructose is formed by the placenta and also that in the second half

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of pregnancy the sheep foetus does not make fructose, thereby confirming Karvonen. They also showed that in the foetal sheep hyperfructosaemia follows hyperglycaemia: and that, while the placenta is permeable in both directions to glucose, fructose passes only from the mother to the foetus, and then only in experimental maternal fructosaemia—in other words, foetal blood fructose does not pass back to the mother. Alexander, Andrews, Huggett, Nixon & Widdas (1955), using placental perfusion techniques, demonstrated that the sheep placenta synthesizes the foetal fructose from glucose at a constant rate of about 10 mg/min which rate is independent of the concentrations of glucose in the foetal or maternal bloods. Further, it appeared that the immediate source of the fructose is the umbilical blood glucose; the maternal blood glucose is only a secondary source to the extent that it passes to the foetus across the placenta. It appeared that the hyperfructosaemia in the foetus following hyperglycaemia, while the production rate of fructose was unaltered, was probably due to diminished rate of fructose removal by the placental and foetal tissues. This, in turn, was probably due to preferential utilization of glucose when present in increasing amounts, resulting in diminished utilization of the fructose. Meanwhile, Widdas (1951, 1952) had shown that the passage of glucose across the sheep placenta, while reversing with reversal of gradient, could not be adequately accounted for by a diffusion mechanism, and he postulated an active facilitated transport system within the placental cells for which he calculated the kinetics. It was therefore of acute interest to determine how these findings were applicable in the primate placenta. For this purpose two species were studied, *Homo sapiens* and *Macaca mulatta*: a preliminary report of the findings has already been made (Huggett, 1954).

METHODS

The principle used in the experiments was the infusion or injection of inactive or radioactive glucose or fructose into the maternal or foetal bloods before and during Caesarean section, and the removal of serial blood samples from the mother and foetus. These samples were assayed for glucose, fructose and radioactivity. The experiments involving radioactive sugars were restricted to the monkeys. The collection of 'foetal' samples after ligature and section of the cord was directly from the newborn infant, and sometimes from the stagnant blood of the placenta immediately after cord section, a technique of limited value first introduced by Boyd & Wilson (1935).

Human experiments. The human cases were all patients upon whom classical Caesarean section was necessary. The technique outlined by Dieckmann & Kramer (1944) was used. Operation was conducted under local anaesthesia with lignocaine ('Xylocaine', Duncan, Flockhart) and adrenaline, a frequent practice in Johns Hopkins Hospital. Clinical experience has shown that under these conditions the infant has the optimum chance of resuscitation should asphyxia neonatorum supervene. No cases of this condition occurred, all the babies being in excellent condition on delivery and thereafter. Intravenous infusion of 10 % glucose or fructose to the mother was effected during the operation by a needle in the vein of the antecubital fossa. If the placenta was on the anterior surface of the uterus the investigation was impracticable and the operation proceeded without any

experiment. If, however, the placenta was posterior, then the cord was delivered through a small incision in the front of the uterus. The umbilical vein was punctured and 5 ml. blood withdrawn through a 3-way tap into a heparinized syringe. The needle was left *in situ* and the tap turned to a saline drip just adequate to stop clotting in the needle and cord. The syringe with blood sample was removed and replaced by a second heparinized syringe. Heparin powder and not liquid heparin was used. The surgeon watched the foetus and foetal pulse so long as there was no evidence of foetal distress. Slowing of the pulse was taken as a sign of such distress: when this occurred 2 ml. more of blood were taken at once and the removal of the infant was completed. An effort was made to have an interval of 15 min between the two foetal samples as was achieved by Dieckmann & Kramer (1944), but in fact 11 min was the maximum interval obtained. The maternal blood samples were removed from the hand veins simultaneously with the taking of the foetal samples. Eleven cases were investigated. There were no untoward incidents or mishaps to either mother or infant.

Monkey experiments. All monkeys were anaesthetized with an initial 5 ml. of 5 % (w/v) intravenous thiopentone ('Pentothal', Abbott Laboratories) given very slowly through a polythene catheter kept in the saphenous vein. Through this catheter further 0·5 or 1·0 ml. injections were made when necessary. Previous experiments upon sheep, both in London and Baltimore, had shown that 'Pentothal' produced no change of blood sugar on injection. This was confirmed for the monkey. In the first monkey experiment pentobarbitone ('Nembutal', Abbott Laboratories) was used and produced considerable fluctuation of the maternal blood sugar which did not occur with pentothal. Changes in the reactivity and tone of muscle indicated the need for supplementary doses of 'Pentothal'. A cannula was put into the carotid artery for maternal blood samples and a catheter in the second saphenous vein for intravenous injection or infusion. Occasionally maternal blood was obtained from a uterine vein tributary.

Foetal blood was obtained by two techniques. In the first two experiments a purse-string suture was put in the uterus and the top of the head carefully delivered through an incision within it: the suture, being adjacent to the bony points of the skull, was tightened round the head sufficiently to prevent loss of amniotic fluid but not enough to stop venous return from the head. The majority of foetal blood samples were obtained, however, from the interplacental artery as described by Reynolds, Paul & Huggett (1954). The principle upon which this method depends is that 80 % of *Macaca* monkeys have a double placenta, a primary and a secondary, joined by two interplacental arteries and veins which are branches of the main umbilical artery and vein and proceed on the surface of the primary or main placenta to the secondary and smaller placenta. These vessels being external to the amniotic sac but within the uterine wall, it is possible to expose them by dissection and to insert a polythene catheter within the artery, leaving it in position for withdrawal of foetal blood samples so long as desired. In some cases collections were made for 5 hr. The only difficulty lies in location of the interplacental vessels. This was accomplished in earlier experiments by pushing a child-size cystoscope through an incision in the uterine wall within a purse-string suture. In later experiments a pocket torch was used. In a darkened room the transillumination of the uterine wall showed up the opacity of the placenta and the interplacental vessels. When the cystoscope was used it not only did this but also helped by illuminating the foetus, placenta and the umbilical vessels and their interplacental branches. The suture being drawn tight on the cystoscope there was no loss of amniotic fluid and no fall of intra-uterine pressure. The transillumination procedure served our purpose better, however. Both techniques ensured that there would not be expulsion of the foetus and placental separation, which is certain to occur when the vessels are sought by exploratory operation.

Sugar determinations. Fructose was determined by Cole's modification of Roe's (1934) resorcinol method as described by Bacon & Bell (1948). Glucose was determined directly by colour formation with benzidine (McCance, 1926) and made quantitative by the method of Jones & Pridham (1953, 1954). Glucose determination in the presence of fructose as the difference between copper values for total reducing substances and resorcinol values for fructose can give fallacious results needing correction. These errors can be obviated when glucose is determined directly by chromogenesis

with aniline (Gardell, 1951) or benzidine (Jones & Pridham, 1953). Benzidine was found the more suitable in these present studies.

In adapting this method to whole blood the main points of technique were:

(i) Protein of whole blood was precipitated by mixing 1 part of blood and 9 parts of absolute alcohol or of 10 % trichloracetic acid. The former was preferable when counts of radioactivity were to be made on the filtrate because of the difficulty of drying trichloracetic filtrates before introduction to the windowless gas flow counter.

(ii) Boiling was effected in a Gardell bath (1951).

(iii) Resorcinol chromogenesis was effected at 100° C in the same Gardell bath. At this temperature there is 11 % less colour formation than at 75–79° C, but with standard and blanks at 100° C there is no loss in accuracy and considerable gain in convenience, flexibility and time.

(iv) There is a linear relation between optical density of the colour produced and sugar concentration. For each series of glucose determinations with benzidine two glucose standards were set up together with two fructose standards which yielded benzidine values (very small) for fructose. Similarly, with resorcinol there were two glucose standards in addition to the fructose standards. This enables one to allow with accuracy for the other hexose when determining one of them.

(v) The reagents were: benzidine 3 % (w/v) in glacial acetic acid; resorcinol 0·15 % (w/v) in 95 % ethyl alcohol mixed immediately before use with an equal volume of ferric chloride 0·75 % (w/v) in concentrated hydrochloric acid.

(vi) The final methods were: (*a*) for glucose; to 1 ml. of protein-free filtrate add 1 ml. of 3 % benzidine in glacial acetic acid; boil for 10 min and cool in bath of ice and water; read in Beckmann Spectrophotometer (Model B) at 400 mμ; in addition use two water blanks and two standards of 10 and 25 mg % respectively both of glucose and of fructose. (*b*) For fructose; to 1 ml. of protein-free filtrate add 3 ml. of resorcinol-ferric chloride mixture; boil for 20 min and then estimate as for glucose, reading at 480 mμ. Increased accuracy was obtained by keeping reagents and protein-free filtrates in ice-water till mixed and transferring immediately to bath at 100° C.

Radioactivity determinations. Of the alcohol filtrates, 1·0 ml. aliquots were dried in metal cups at room temperature with a final drying under an infra-red lamp. The sample cups were then introduced into a windowless gas flow Geiger detector; the disintegrations per minute were recorded by means of a conventional scaler. The number of counts, N, was such that the error, $\sqrt{(N)}/N$, had values considerably less than 0·04 for all but the background counts and the samples with the lowest activities. No sample was counted for more than 15 min. It is assumed that the ^{14}C activity originated from the particular hexose injected; no attempt was made to separate glucose from fructose before the radioactivity determinations.

RESULTS

No fundamental difference was detected between the monkey and the woman in regard to the passage of sugars from the mother to the foetus. Therefore experiments upon both species will be described collectively, differentiation being based upon functional experimental variations rather than upon the morphological differences or characteristics. The total number of primates operated upon was twenty, eleven women and nine monkeys. Of these, sixteen yielded results of value.

The experiments to be described fall into three groups: (i) Experimental hyperglycaemia in the mother. (ii) Experimental fructosaemia in the mother. (iii) Saturation of the carrier system with inert glucose and determination of the effect upon passage of labelled sugars.

The resting maternal blood glucose concentrations in all these cases, both human and simian, are high, being greater than 150 mg/100 ml. and often greater than 200 mg/100 ml. This is attributed to the excitement engendered before anaesthesia and to the fact that local anaesthetics containing adrenaline will not diminish but may even intensify the maternal blood glucose concentration.

Experimental hyperglycaemia in the mother

In this group were four monkeys, M1, M2, M3 and M4 and three women, two at full term, W9 and W10, and the third, W11, who required a therapeutic abortion, at the third month (foetal age 84 days). In W9 and W11 the glucose was infused intravenously before the uterus was opened and, in M1, 9·4 g of glucose was infused over a period of 10 min after the head was delivered and the first foetal sample taken from the superior longitudinal sinus. In the four remaining cases (M2, M3, M4 and W11) glucose was administered by quick intravenous injection. The effects of maternal hyperglycaemia are best shown by monkeys M2 (Fig. 1) in which 25 ml. of 25% glucose was injected into the saphenous vein, M4 (Fig. 2) which had 25 ml. of 25% glucose and by patient W9 who received an infusion of 10% glucose at rates shown in Fig. 3.

The protocol of M2 is given in full since it illustrates the general procedure (Table 1). Monkey M2 was the first case in which the cystoscope was used. The time from the first injection of Pentothal to the catheterization of the interplacental artery branch of the umbilical artery was 1 hr 49 min. Technical difficulties in this first experiment were overcome in later experiments and the time cut down to 45 min. The sugar curves obtained are shown in Fig. 1.

Fig. 2 shows an experiment upon monkey M4 similar to that of Fig. 1, but in which the fall in the foetal blood glucose from the peak height is slower and that in the maternal blood is faster. In consequence, the maternal glucose concentration becomes less than the foetal glucose values as the glucose injected into the maternal blood is rapidly distributed throughout the extracellular space.

Patient W11 required hysterotomy and therapeutic abortion for psychiatric reasons. The foetal age was computed clinically as 84 days. The infusion of the patient with glucose solution (50 g/100 ml.) was begun at 2.13 p.m. and completed at 2.33 p.m., 25 g glucose having been given intravenously. The foetus and placenta were removed intact at 2.33 p.m. and the maternal blood sample taken at the same time. The foetal blood sample was taken immediately after from the foetal heart. The maternal blood contained 258 mg/100 ml. of glucose and no fructose. The foetal blood contained 192 mg/100 ml. of glucose and 6·0 mg/100 ml. of fructose. It would appear therefore that at the third month of intra-uterine life the human foetus and placenta behave similarly to the full-term foetus and placenta in their response to maternal hyperglycaemia.

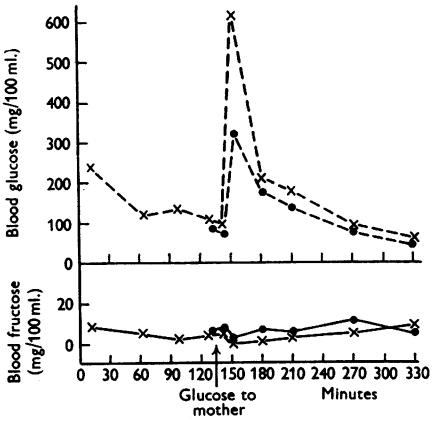

Fig. 1

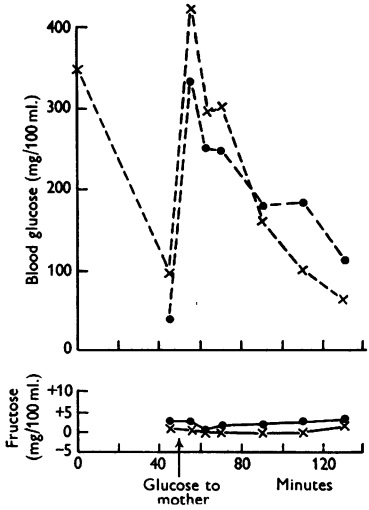

Fig. 2

Fig. 1. Monkey M 2. Foetal age 156 days. 'Pentothal' anaesthesia. Maternal and foetal blood glucose and fructose concentrations. Effect of intravenous injection of mother with 6·25 g of glucose (25 ml. of 25 g/100 ml.). ×---×, Maternal glucose; ×——×, maternal fructose; ●---●, foetal glucose; ●——●, foetal fructose.

Fig. 2. Monkey M 4. Foetal age 143 days. 'Pentothal' anaesthesia. Maternal and foetal blood glucose and fructose concentrations. Effect of intravenous injection of mother with 6·25 g of glucose (25 ml. of 25 g/100 ml.). ×---×, Maternal glucose; ×——×, maternal fructose; ●---●, foetal glucose; ●——●, foetal fructose.

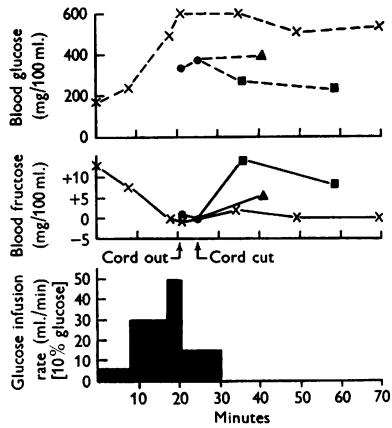

Fig. 3. Patient W 9. Caesarean section at term. Local anaesthesia, 'Xylocaine' and adrenaline. Maternal, foetal and post-section umbilical vein blood sugars. Effect of maternal infusion with 10% glucose solution at rates indicated in lower third of the figure. ×---×, Maternal glucose; ×——×, maternal fructose; ●---●, foetal glucose; ●——●, foetal fructose; ■---■, infant glucose; ■——■, infant fructose, ---▲, post-section umbilical vein or placental glucose; ——▲, post-section umbilical vein or placental fructose.

TABLE 1

Monkey M 2. Weight 6300 g, foetal age 156 days.

After experiment: foetal weight 390 g; placental weight 113 g. Anaesthetic: intravenous thiopentone ('Pentothal', Abbott Laboratories) 125 mg/ml. concentration.

Time	
9.34	1·0 ml. 'Pentothal'
9.36	Polythene catheter inserted right saphenous vein
9.41	Maternal blood 1 ml. taken from left saphenous vein
9.43–10.31	Fourteen injections of 'Pentothal', total vol. 8·0 ml. in 48 min = 0·16 ml./min.
9.43–10.32	Cannula inserted in maternal left carotid artery. Abdomen opened. Uterus examined and placental site located
10.33	Maternal blood 1 ml. taken from left carotid artery
10.36–10.40	Three injections of 'Pentothal', total vol. 1·25 ml.
10.45–10.48	Purse-string suture in uterus over amnion
10.49	Cystoscope inserted
10.50–11.21	Three injections of 'Pentothal', total vol. 1·5 ml.
10.50–11.17	Location of interplacental artery with cystoscope and its exposure
11.18	Maternal blood 1 ml. taken from carotid artery
11.23	Catheter inserted in interplacental artery
11.23–11.54	Maternal blood samples (0·5 ml.) at 11.38; 11.52. Foetal blood samples (0·5 ml.) at 11.41; 11.52. 'Pentothal' at 11.38; 11.54, 0·5 ml. each
11.55–11.57	25 ml. 25% glucose in saline. Mean time 11.56
11.58–15.00	Maternal blood samples at 12.01; 12.30; 13.00; 14.00; and 15.00. Foetal blood samples at 12.03; 12.31; 13.00; 14.00 and 15.00. Thirteen injections of 'Pentothal', total vol. 6·0 ml.
15.01	Monkey killed by air embolism

Experimental fructosaemia in the mother

The experiments in this group were the first to be performed. Observations were confined to eight human patients, no fructosaemia being produced in the monkey. The number was large for two reasons: first because several new procedures and team techniques were being utilized and a small number of cases was investigated before the techniques were considered adequate; and secondly because the number of cases suitable for Caesarean section having a placenta on the posterior wall of the uterus was less than expected. Of the patients W 1 to W 8, in whom maternal fructosaemia was produced, W 6 and W 8 are selected since they were two patients in which the best technique was achieved. Figs. 4 and 5 illustrate the findings in these two cases.

In Fig. 4 the maternal fructose was raised to an average concentration of about 90 mg/100 ml. When the umbilical cord was exposed it was possible to take four foetal blood samples during a period of 11 min. These showed that in the 39 min of maternal infusion the foetal blood had acquired a fructose concentration of about 15 mg/100 ml. The blood of the infant 35 min after severance of the umbilical cord contained 30 mg/100 ml. of fructose. This extra 15 mg of course could not have been obtained by placental passage and

at the moment our knowledge of foetal carbohydrate metabolism does not enable us to do more than record the finding. It is of interest that in this period, after cutting the cord, the infant blood shows rising values of both fructose and glucose concentration. When the fructose infusion was ended the mother no longer played any part in the experiment. The theatre staff now replaced fructose by glucose in the maternal infusion in accordance with the routine therapeutic procedure after Caesarean section. The maternal blood glucose rose and simultaneously the maternal blood fructose fell.

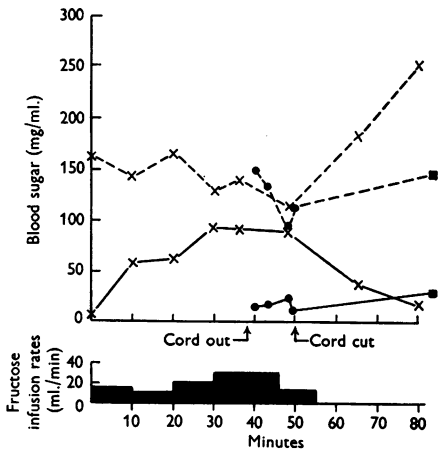

Fig. 4

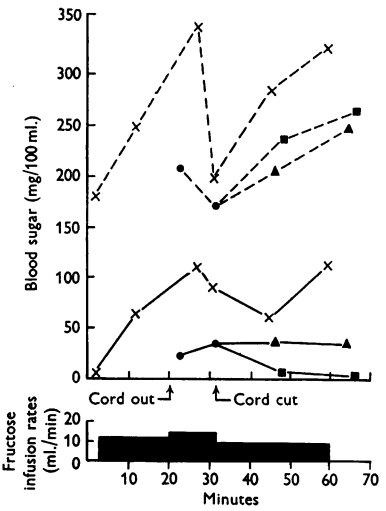

Fig. 5

Fig. 4.¶[Patient W 6. Caesarean section at 38th week. Local anaesthetic, 'Xylocaine' and adrenaline. Maternal, foetal and infant blood sugar concentrations. Effect of maternal infusion with 10% fructose solution at rates indicated in lower part of the figure. ×---×, Maternal glucose; ×——×, maternal fructose; ●---●, foetal glucose; ●——●, foetal fructose; ---■ infant glucose; ——■, infant fructose.

Fig. 5. Patient W 8. Caesarean section at term. Local anaesthesia, 'Xylocaine' and adrenaline. Maternal, foetal, infant and post-section umbilical vein (placental) blood sugar concentrations. Effect of maternal infusion with 10% fructose solution. ×---×, Maternal glucose; ×——×, maternal fructose; ●---●, foetal glucose; ●——●, foetal fructose; ■---■, infant glucose; ■——■, infant fructose; ▲---▲, placental glucose; ▲——▲, placental fructose.

In the case of patient W 8 (Fig. 5), it was possible to infuse the mother with fructose over a period of 55 min, during which time two samples of foetal blood were obtained with fructose concentrations (22 and 37 mg/100 ml.), both outside the range of fructose found normally or even after glucose infusion of the mother. It was possible in this case to determine the concentrations of the two sugars both in the blood of the infant and in the stationary

blood in an umbilical vein deep within the placenta. It is of interest that the fructose concentration steadily fell to normal values in the infant but kept at foetal level in the placental blood. On the other hand, the maternal blood glucose was 178 mg/100 ml. initially and rose to values fluctuating round 300 mg/100 ml. with a drop at the opening of the uterus. In this case the infusion of fructose to the mother was continued after the cord was cut and the infant removed. The maternal fructose in consequence was maintained high and the maternal glucose showed a high value of 325 mg/100 ml. Presumably the high maternal glucose results from the conversion in the maternal liver of fructose to glucose by way of fructose-6-phosphate and glucose-6-phosphate.

In contrast with these changes in the mother, the blood fructose of the infant fell after the cord was cut while the blood glucose rose from a foetal value of 165 mg/100 ml. to a high value of 262 mg/100 ml. We think that the maternal glucose resulting indirectly from the infused fructose caused in the foetus not only a rise of blood glucose but also an active storage of glycogen: and that after the cord was cut and the supply of maternal glucose cut off, the glycogen formed from the glucose, and possibly from the fructose, passed back into the blood as glucose.

In the placenta stationary blood steadily increases in glucose concentration with fructose concentration falling. The source of this glucose must be the continuing active transport of glucose by the still living placental cells. There is no suggestion that any of this glucose is converted to fructose, which is in striking contrast with what has been observed in the ungulate.

It would appear therefore that fructose given to the mother passes through the placenta to the foetus. It has not been practicable experimentally to test the existence of the reverse transfer. It appears also that glucose in the mother can form glucose in the placenta, and the continuation of this transfer in the placenta after placental removal suggests a continuation of the process of active transport. The accumulation of glucose in the placenta suggests also that in it fructose is not formed from this glucose in any appreciable amounts.

Experiments with radioactive sugars

It is clear from these experiments that the placenta of the primate does not to any appreciable degree convert glucose to fructose. It was of interest within the limitation of the primate material to test two questions: (1) does glucose given to the infant appear in the mother and if so does it pass from a region of low to a region of high concentration as might be expected with carriers maintaining a state of disequilibrium; (2) is the fructose passage from mother to foetus dependent or not upon the glucose transport system of the placenta? For both purposes radioactive sugars labelled biologically with ^{14}C were used and the experiments were therefore confined to the monkey.

The passage of glucose from foetus to mother. In the two monkeys, M 6 and M 9, an experimental maternal hyperglycaemia was established which was thought to be adequate to saturate the placental carrier system for glucose. Radioactive glucose was injected into the blood stream of each foetus before the hyperglycaemia and again while it was present. It was hoped that by comparing the rates of rise of radioactivity in the maternal blood following two similar injections of ^{14}C-glucose to the foetus it would be possible to say if the saturation of the glucose transport mechanism influenced the transfer of glucose from foetus to mother.

In the experiment with M 6, the first injection of ^{14}C-glucose into the foetus was made when the maternal glucose was 145 mg/100 ml. and the second when it had been raised to approximately 300 mg/100 ml. by the intravenous infusion of glucose to the mother for 46 min. The infusion resulted also in a rise in foetal blood glucose from 158 to 230 mg/100 ml. The radioactive glucose used for each injection was 35 μc in 0·5 ml., a quantity inadequate to cause a rise in blood glucose concentration measurable by ordinary chemical means.

The value obtained for radioactivity of foetal blood before the first injection was not above background and after the injection a single value of 17,000 counts/min was obtained. The activity of the maternal blood was raised from zero (background) to about 760 counts/min in 15 sec. The radioactivity of the foetal blood before the second injection had fallen to approximately 2000 counts/min, but after the injection a single value of 15,200 counts/min was obtained. The activity of the maternal blood rose from about 400 counts/min to 3500 counts/min in about 40 sec. The rate of rise of radioactivity of maternal blood in the first instance is about 3000 counts/min/min and in the second, 4500 counts/min/min. It should be emphasized that the values of 17,000 and 15,200 counts/min obtained in the foetal blood after the injection being single figures cannot be regarded as strictly comparable. They do not represent completely the rapid changes in the radioactivity of the foetal blood. This point will be further considered in the discussion.

This experiment with M 6 was repeated with monkey M 9 with the difference that the two injections of ^{14}C-glucose, while still of equal quantity were considerably reduced (4·5 μc in 2·5 ml.) and an attempt was made to saturate the glucose carrier system by giving the mother a single intravenous injection of glucose, 5·9 g in 200 ml. (1·0 g/kg body weight) in place of continuous infusion. The maternal blood glucose was raised by this single injection from 124 to 526 mg/100 ml. after 7 min: the peak concentration, immediately after injection, must have been considerably higher. The foetal blood glucose rose from 136 to 388 mg/100 ml. in 13 min.

The maternal blood counts before and 3·5 min after the first injection were zero to 71 counts/min in a period of 5 min. The foetal counts before and 4 min after the second injection were 160 and 2800 counts/min respectively, while

the maternal count rose by 45 counts/min in 1·5 min. The rate of rise of radioactivity of the maternal blood before the hyperglycaemia was, therefore, 14 counts/min/min and during the hyperglycaemia 30 counts/min/min.

Sugars in the amniotic fluid. The fresh amniotic fluid in M1, M3 and M4 was analysed and glucose found in concentrations of 54, 68 and 103 mg/100 ml. respectively. No fructose was found in M1 and M4 but 9 mg/100 ml. was found in M3. At the end of the experiment with M9, in which radioactive glucose had been twice injected into the foetal blood, the amniotic fluid was

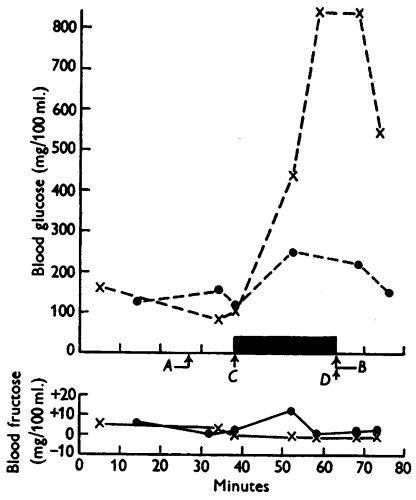

Fig. 6

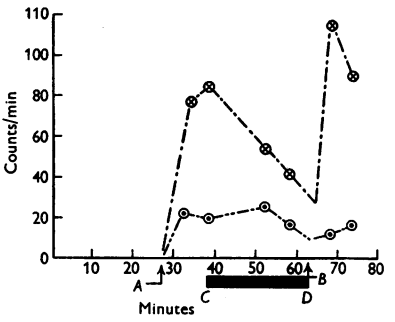

Fig. 7

Fig. 6. Monkey M7. Foetal age 153 days. 'Pentothal' anaesthesia. Maternal and foetal blood sugar concentrations. Effect of glucose infusion to the mother between *C* and *D* between two maternal injections of 4·2 μc of ^{14}C-fructose at *A* and *B*. ×– – –×, Maternal glucose; ×——×, maternal fructose; ●– – –●, foetal glucose; ●——●, foetal fructose.

Fig. 7. Monkey M7. Counts/min in blood filtrates of mother and foetus following two injections of 4·2 μc of ^{14}C-fructose to the mother, one before (at *A*) and one after (at *B*) maternal infusion with ^{12}C-glucose between *C* and *D*. ⊗–·–·–⊗, Maternal counts/min; ⊙–·–·–·–⊙, foetal counts/min.

radioactive to the extent of 58 counts/min, at which stage the maternal blood had 232 mg/100 ml. of glucose with 136 counts/min and the foetal blood had 292 mg/100 ml. of glucose with 1044 counts/min: but at this stage, despite these high blood glucose concentrations, the amniotic fluid had only 38 mg/100 ml.

The passage of fructose from mother to foetus. Radioactive fructose was in this instance injected into the maternal blood of monkey M7 before and after attempting to saturate the glucose carrier system, mother to foetus, with glucose. The rate of rise of the ^{14}C-fructose counts in the foetal blood was determined. The findings are seen in Figs. 6 and 7.

DISCUSSION

It would appear that these primates differ from the sheep in that in them there is little or no fructose formation. From available data the fructose of the foetal primate may be said to range from zero to 5 mg/100 ml. On only four occasions in all this series of experiments with primates did the concentration of fructose in the foetal blood exceed 10 mg/100 ml. and in no instance did it exceed 20 mg/100 ml. in the absence of experimental maternal fructosaemia. The magnitudes of these four isolated values lie well outside the range of experimental error and they have no apparent experimental or biological cause. The bloods with high concentrations were not examined chromatographically or by any means other than by the routine analysis described and they stand as unsupported evidence that fructose may appear transiently in primate foetal blood in moderate quantity for reasons unknown. In the ungulate and whale there is a marked difference: fructose is always present in quantity in the foetuses of these animals and is accompanied by glucose always in lesser concentration.

It is clear that, as in the sheep, when glucose is injected or infused into the mother there follows a rise in foetal blood glucose, and equilibrium is not attained (Figs. 1–3). With the possible exception of monkey M9, in no case was a second sample of amniotic fluid taken after producing a maternal hyperglycaemia. It was, therefore, not possible to ascertain if the glucose of the amniotic fluid was related to or derived from the maternal or foetal blood glucose. These results throw no light on the source of the amniotic fluid, whether foetal or maternal. It might be either or both.

The evidence that fructose infused into the maternal blood passes into the foetus is shown in Figs. 4 and 5, and is supported unequivocally by other experiments in this series. In this respect the woman appears to be similar to the sheep. The passage of fructose from mother to foetus was not tested in the monkey, but it is reasonable to suppose that the monkey resembles the human in this respect.

The legitimacy of accepting the blood taken after birth from the umbilical vein deep in the placenta as identical with foetal blood is open to question. This procedure was adopted in the case of patient W8. The procedure was first used by Boyd & Wilson (1935) when examining the exchange of lipids in the human umbilical circulation at birth. They inferred that there was active cellular transport by the placenta. The comparison we have made between the placental and infant bloods, in the instance of patient W8, suggests that the placenta in the half hour after birth and while still warm is in some degree at least exercising a prenatal function and maintaining a secretion of the previously injected fructose and apparently forming some glucose. The foetus, on the other hand, is not forming fructose: its blood

fructose falls while the placental blood fructose is maintained. Put briefly, therefore, our findings support the view of Boyd & Wilson that the placenta actively transmits nutrients.

The experiments upon monkeys M6 and M9 were designed to see if, as in the sheep, the glucose transfer system can be heavily loaded with glucose in the direction mother to foetus and yet readily transfer tagged glucose molecules back to the mother against the concentration gradient. It is well known that the rate of distribution of glucose from the blood stream to other parts of the extracellular fluid is very rapid during the first few minutes after it has been added to the blood stream and as, in these experiments, neither the foetal blood volume nor the extracellular space available to the sugar is known, it is not possible from the very few measurements that could be made to get a reliable estimate of the radioactivity of the foetal blood to compare with the rise of radioactivity of the maternal blood. The quantity of radioactive sugar added to the foetal blood is, however, so small that it seems justifiable to assume that it causes no appreciable disturbance of osmotic relations and leaves the foetal blood volume and the volume of the extracellular fluid unaffected. A second injection of ^{14}C-glucose made when the radioactivity of the foetal blood is again at a low value can reasonably be assumed to cause the same rise in the radioactivity of this blood as did the first: and in considering the results of these experiments, in absence of any other disturbance of blood volume or distribution of sugar, comparison need be made only of the rates of rise of radioactivity in the maternal blood. There is, however, a source of change in blood volume, both of the foetus and of the mother, which has to be borne in mind, namely the effect of infusion in the one instance and injection in the other of a hypertonic glucose solution to the mother before the second injection of ^{14}C-glucose to the foetus. Although it is difficult to assess the effect of this administration of glucose to the mother, the values obtained for the radioactivity of the maternal blood strongly suggest that the transfer of glucose from foetus to mother is unimpaired by the large transfer in the opposite direction, and it is difficult to see how an increase in blood volume of mother, of foetus or of both, as a result of the infusion or injection of hypertonic glucose solution, could do other than support this conclusion.

The rationale of the experiment on monkey M7 by which it was hoped to determine if glucose and fructose are transported by the same carrier system is this: it is argued that, if the carrier mechanism is saturated in the direction mother to foetus as the result of a maternal hyperglycaemia, the movement of ^{14}C-fructose in the same direction may be affected if a common mechanism is involved. The figures for the radioactivity of the maternal and foetal bloods in this experiment (Fig. 7) do no more than suggest a slower rate of transfer of the radioactive fructose during the maternal hyperglycaemia: and this one experiment, which unfortunately could not be repeated, cannot be accepted

as adequate proof of a common mechanism. It is probable, however, that the carrier of glucose is essential and common to the transfer of both sugars. In other words, glucose and fructose are probably competitive for transfer when the concentration of one or both is high.

SUMMARY

1. Techniques are described for studying the passage of hexoses across the placenta (i) in women during Caesarean section, and (ii) in the *Macaca* monkey at term or earlier, and for longer periods than are permitted during human Caesarean section.

2. A method is described for the determination of glucose in the presence of fructose, based upon the method of Jones & Pridham (1954), in which colour formation with benzidine is used.

3. The primate differs from the sheep in that the placenta appears not to form fructose from glucose when foetal hyperglycaemia is produced, and the normal primate foetal blood hexose appears to be glucose without fructose.

4. Glucose appears to pass freely across the primate placenta in both directions.

5. Fructose goes freely from the mother to the foetus when injected into the maternal circulation.

6. The experiments suggest that the kinetics of transfer of sugars advanced by Widdas (1952) for the sheep placenta may also hold for the primate and that the transfer system can carry glucose in both directions.

7. Some part of the glucose carrier system seems able to transmit fructose from the mother to the foetus and there is a suggestion that an excess of glucose can impede the flow of fructose.

8. Radioactive glucose given to the foetus appears in the maternal blood with ease, but in the amniotic fluid only in small quantities and there is no evidence as to the route by which it arrives.

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