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FRUCTOSE METABOLISM IN THE ISOLATED PERFUSED LIVER OF THE FOETAL AND NEW-BORN SHEEP

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While fructose is known to be a major component of the foetal blood in all species of Ungulata and Cetacea which have been examined, the mechanism by which it is formed is uncertain. Huggett, Warren & Warren (1951), Alexander, Andrews, Huggett, Nixon & Widdas (1955) and Alexander, Huggett, Nixon & Widdas (1955) showed that glucose was probably a precursor in its formation and that the site of synthesis was centred in the placenta. It was postulated that intermediate phosphate esters would participate in the synthesis of the free fructose. However, Hers (1957*a*) obtained evidence that fructose was formed from glucose in the seminal vesicle without the intervention of phosphorylated intermediates. In this pathway glucose is reduced to sorbitol by the enzyme aldose reductase, and the sorbitol is subsequently oxidized to fructose by the enzyme ketose reductase. Evidence was also obtained that aldose reductase, but not ketose reductase, was present in the sheep placenta. Since foetal liver slices were able to convert sorbitol to fructose, it was postulated that the fructose in the sheep foetus was formed in two stages: in the first stage sorbitol was formed from glucose in the placenta, and in the second stage sorbitol was converted to fructose in the foetal liver. This mechanism is shown schematically in Fig. 1, DPN and TPN referring to di- and tri-phosphopyridine nucleotides respectively.

The work of Alexander, Andrews *et al.* (1955) did not exclude the possibility that some foetal fructose is formed by the mechanism indicated above, and it was thought desirable to confirm the second stage of the Hers hypothesis on the perfused foetal liver. At the same time the uptake of fructose by this preparation was examined. This latter aspect was investigated since preliminary experiments had suggested that the foetal liver was unable to utilize fructose, in contrast to the adult liver where conversion to glucose may take place (Cori, Ochoa, Slein & Cori, 1951).

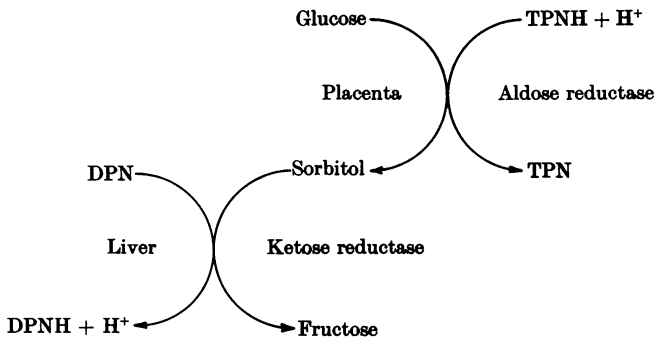

Fig. 1.

METHODS

Apparatus. The apparatus used to perfuse the livers of lambs was similar to that described by Andrews, Hecker & Maegraith (1956). The portal vein was supplied from a small, constant-level reservoir, the portal pressure was about 8 cm saline. The perfusing fluid was blood obtained from the lamb, supplemented with maternal blood. Thermometers placed between lobes of the liver registered 35·5–36° C.

Foetal livers were perfused with the apparatus illustrated in Fig. 2. Blood from the liver flowed to the reservoir from where it was pumped to a bubble oxygenator (moist gas, 5 % CO_2 + 95 % O_2), and allowed to overflow into a collection chamber which was coated with Antifoam A (Midland Silicones, Ltd.). The blood passed into a constant-level device which also acted as a bubble trap, and thence, via a heating coil, to the liver. A manometer was incorporated near the liver and the inflow pressure was controlled to 8 cm saline by a screw clip. The overflow from the constant-level device was returned to the reservoir. The foetus and reservoir were placed in a lagged bath of saline at 37° C. The apparatus was primed with heparinized maternal blood.

Operative technique. Lambs were given pentobarbitone (20 mg/kg body wt.) intraperitoneally as a basal anaesthetic and then anaesthetized with intravenous thiobarbitone. The trachea was cannulated and artificial respiration begun. A mid-line incision was made, avoiding the umbilicus, the sternum was divided in the mid line, and the ribs retracted laterally. The thrombosed umbilical vein was ligated and the rumen divided between ligatures. The portal vein was cleaned for about 1·5 cm of its length immediately cephalad to the pancreas. A cannula was inserted into the thoracic part of the posterior vena cava, pointing towards the liver, and blood from the posterior part of the body was allowed to flow into the reservoir. Immediately afterwards the portal vein was cannulated and the perfusion begun. Tributaries to the portal vein between the cannula and the porta hepatis were ligated. When the level of blood in the reservoir became constant the vena cava was clamped just posterior to the liver. The edges of the incision were drawn together. The total time for operation was about 25 min.

With the foetus, the head was submerged in saline, and 2 ml. of 2 % procaine was injected parenterally to prevent pain. A mid-line incision was made as in the procedure for lambs; the umbilical vein and thoracic posterior vena cava were cannulated; a clamp was applied to the vena cava posterior to the liver and the perfusion was started. Ligature of the portal vein appeared to be unnecessary. The time taken for the operation was approximately 6 min.

In both the lamb and foetal preparations a certain amount of mottling of the surface of the liver occurred, especially where the ribs appeared to be exerting slight pressure. Some

dilatation of the vessels took place during the first hour of the perfusion, since the rate of flow increased when the pressure was kept constant—most of this increase occurred in the first 20 min. Therefore 30 min was allowed to elapse between the beginning of the perfusion and the commencement of metabolic experiments. An injection of Indian ink, made at the end of the experiment, demonstrated that the inflowing blood rapidly penetrated to all areas of the liver in both preparations.

The volume of the perfusate was between 250 and 500 ml. and the rate of flow varied from 100 to 280 ml./min in different preparations. In the foetal liver preparations the weight of the organ ranged from 51 to 80 g, and in the neonatal livers from 150 to 190 g.

Chemical determinations. Fructose was determined by the method of Chinard, Danesino, Hartmann, Huggett, Paul & Reynolds (1956); glucose by the method of Huggett & Nixon (1957); lactic acid by the method of Barker & Britton (1957), except that the concentration of semicarbazide in the buffer solution was 0·5 g/100 ml.; and inositol by the microbiological method of Campling & Nixon (1954).

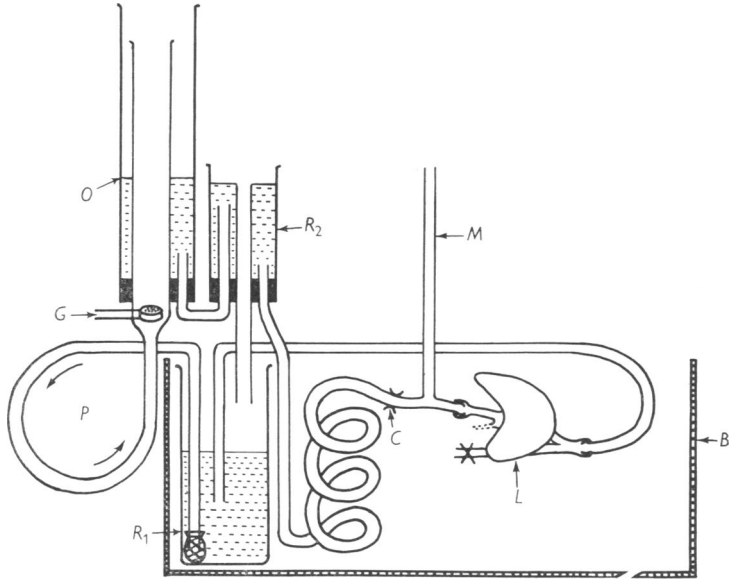

Fig. 2. A schematic diagram of the foetal or neonatal liver perfusion circuit. *B*, saline bath; *C*, screw clip; *G*, 95% O_2+5% CO_2; *L*, liver; *M*, manometer; *O*, oxygenator; *P*, pump; R_1, reservoir; R_2, constant-level reservoir and bubble trap.

RESULTS

Observations on perfused foetal livers

In the five foetal livers perfused, ranging from 122–140 days of foetal age, the ability of the liver to convert sorbitol to fructose was consistently demonstrated. Neither the addition of glucose nor of meso-inositol significantly influenced the level of the circulating fructose in the preparation, whereas the introduction into the circuit of an equivalent quantity of sorbitol resulted in a prompt production of fructose (Fig. 3*a*). The

magnitude of this conversion was in several cases such as to suggest a complete conversion of the added sorbitol to fructose. In one experiment the addition of galactose was also without effect upon the fructose concentration.

The blood used to prime the circuit was obtained from an adult ewe and therefore the foetal liver was subjected to a perfusate having a concentration of fructose lower than that encountered in sheep foetal blood. It might therefore be suggested that the sorbitol–fructose conversion only proceeded under conditions of a low concentration of blood fructose, and that the reaction was a mechanism ensuring the characteristic high blood fructose concentration encountered in the sheep foetus. However, the

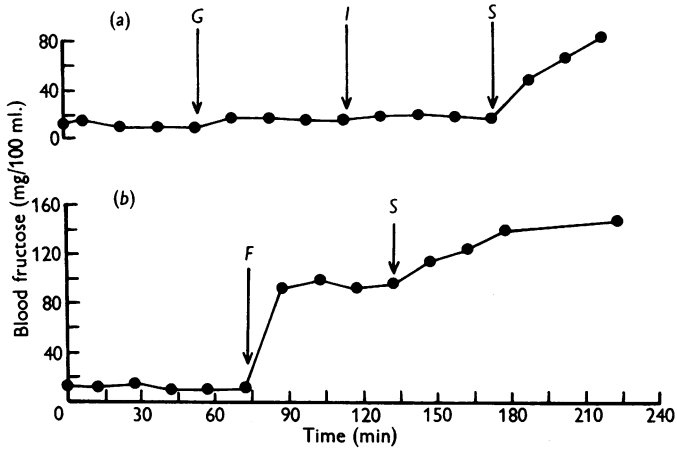

Fig. 3. The elevation of the fructose concentration in the perfusate of the foetal sheep liver preparation following the addition of sorbitol. (*a*) Concentration of fructose in the perfusate following the addition of 500 mg of glucose, *G*, inositol, *I*, and sorbitol, *S*. Foetal age 122 days. Liver weight 61 g. Terminal perfusate volume 410 ml. (*b*) Concentration of fructose in the perfusate following the addition of sorbitol (*S*, 500 mg) subsequent to the elevation of the fructose concentration by the addition of 500 mg of fructose, *F*. Foetal age 140 days. Liver weight 80 g. Terminal perfusate volume 500 ml.

ability of the liver to convert sorbitol to fructose still took place when the concentration of fructose was elevated to a level comparable to that found in the normal foetus (Fig. 3*b*).

The initial rate of conversion of sorbitol to fructose ranged from 4·2 to 13·3 mg/min, and was unrelated to foetal age. The glucose concentration in the perfusing blood showed an increase with time. The initial concentrations ranged from 84 to 232 mg/100 ml. (mean 116) and during the course of the experiments the maximum increase observed was 124 mg/100 ml.

In two experiments the concentration of lactic acid in the perfusing blood was measured. In both cases the values showed a fall in concentration 60–80 min after the start of the perfusion, with restoration of the initial value after a further 60 min. In one experiment the initial value was 72, the lowest value 32 and the final value 99 mg/100 ml. at 205 min. In the other experiment the initial value was 25·2 mg/100 ml. and the final value, after 200 min, was 28·5 mg/100 ml. (illustrated in Fig. 4).

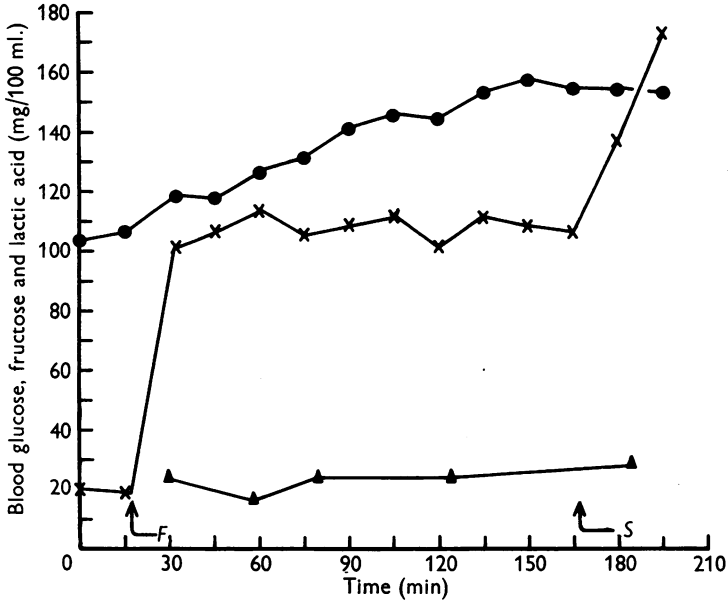

Fig. 4. Concentrations of glucose, fructose and lactic acid in the perfusate of the foetal liver after the addition of 500 mg fructose, *F*, and 500 mg sorbitol, *S*. Foetal age 134 days. Liver weight 51 g. Terminal perfusate volume 460 ml. ×, Fructose; ●, glucose; ▲, lactic acid.

It will be seen from Fig. 3*b* that the elevated fructose concentration remained constant over the 60 min before the introduction of sorbitol. The inability of the perfused foetal sheep liver to utilize fructose under these experimental conditions was confirmed in two further preparations, where the elevated fructose concentration was observed over a longer period of time, one of which is shown in Fig. 4. In the other preparation the concentration fluctuated between 90 and 94 mg/100 ml. over 120 min.

An increase in the inositol concentration of the perfusing blood was observed in the three experiments where it was determined. The rate of production over the first hour varied from 5·0 to 7·5 mg inositol/hr. This apparent synthesis of inositol by the liver is possibly an artifact, since it is to be remembered that the perfusion circuit was primed with maternal

blood, considerably lower in inositol concentration than foetal blood (Campling & Nixon, 1954); thus the observed rise may merely represent the elution of inositol from a tissue initially in equilibrium with a higher inositol concentration. When inositol was added to the perfusate the concentration subsequently showed no significant change (Fig. 5).

The additions of sorbitol, glucose or fructose were without significant effect upon the inositol concentrations present in the perfusing blood.

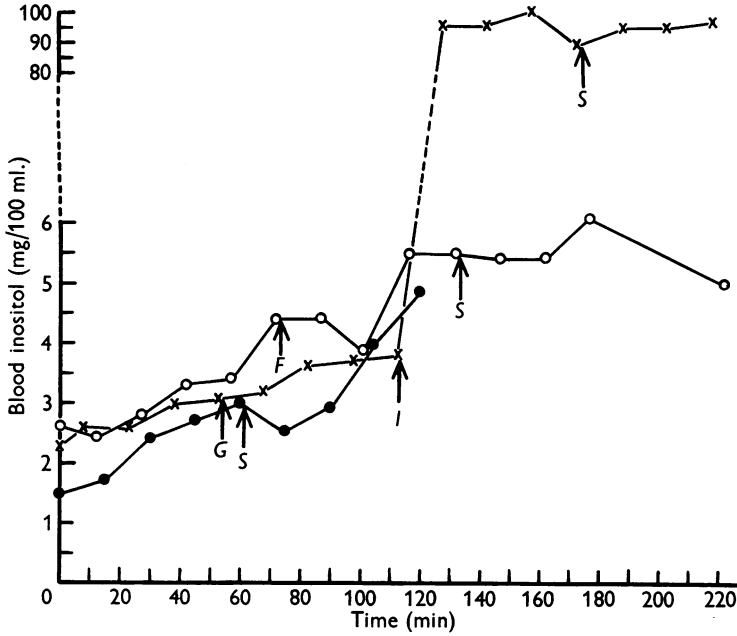

Fig. 5. Concentration of meso-inositol in the perfusate of foetal livers. At *G*, *S*, *F* and *I* 500 mg of glucose, sorbitol, fructose or meso-inositol respectively were introduced to the perfusate. ×, Foetal age 122 days. Liver wt. 61 g. Terminal perfusate vol. 410 ml. ●, Foetal age 138 days. Liver wt. 75 g. Terminal perfusate vol. 400 ml. ○, Foetal age 140 days. Liver wt. 80 g. Terminal perfusate vol. 500 ml.

Observations on perfused lamb livers

The inability of the preparation to utilize fructose to a substantial degree was still present in a lamb 3 days after birth. By the fifth day, however, fructose utilization appeared to be well established (Fig. 6). A similar prompt decline in the elevated blood fructose was also observed in livers from 9- and 15-day-old lambs.

Lactic acid concentrations in the perfusate were found to be lower than those encountered in foetal preparations. A substantially higher concentration of blood glucose was present in all the lamb perfusions,

compared with the foetal liver preparations. The response of the liver to sorbitol was again the production of fructose, although the concentration rise was smaller due to its metabolism.

In one experiment on the intact anaesthetized lamb, 10 days after birth, fructose was rapidly removed from the circulation following its experimental elevation. Renal loss of fructose over the initial 74 min following the administration amounted to 17·4% of the injected dose. Ligation of the renal pedicles did not greatly influence the decline of blood fructose concentration, suggesting that the main loss of the

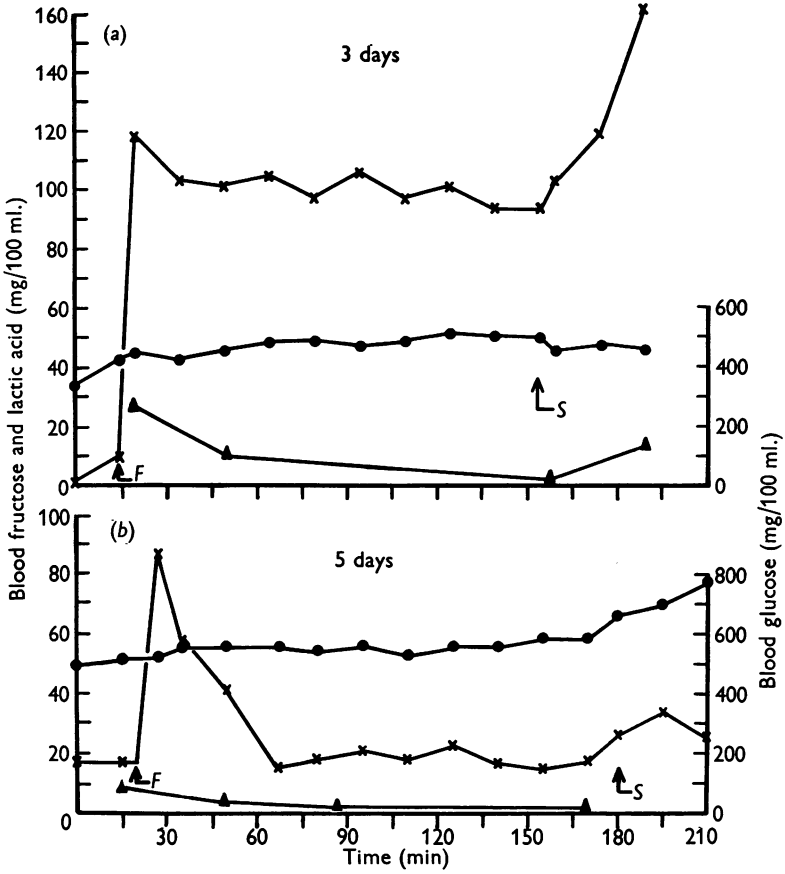

Fig. 6. Fructose and glucose and lactic acid concentrations in the perfusate following the addition of 500 mg fructose, *F*, and 500 mg sorbitol, *S*, to neonatal preparations. (*a*) Response 3 days after birth. Liver weight 150 g. Terminal perfusate volume 380 ml. (*b*) Response 5 days after birth. (The apparent high control values for fructose are due to interference by the high concentration of glucose and other chromatogenic substances in the perfusate.) Liver weight 165 g. Terminal perfusate volume 350 ml. ×, Fructose; ●, glucose; ▲, lactic acid.

administered fructose was extra-renal. An elevation of the fructose concentration again occurred following the injection of sorbitol. The elevation of the glucose concentration following ligature of the renal pedicles was probably a reflexion of the trauma induced by the procedure.

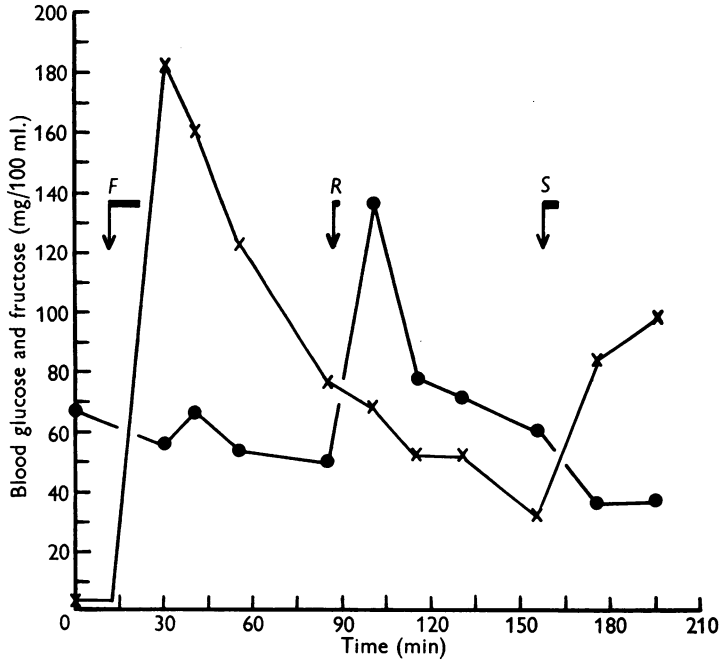

Fig. 7. Fructose and glucose concentrations in the blood of an anaesthetized 10-day new-born lamb following the intravenous injection of 3·8 g fructose, *F*, and 3·8 g sorbitol, *S*, and ligation of the renal pedicles, *R*. ×, Fructose; ●, glucose.

DISCUSSION

Embden & Griesbach (1914) demonstrated a production of fructose from sorbitol in the perfused dog liver. The results presented in this paper, obtained by using a perfused foetal sheep liver preparation, indicate that this conversion of sorbitol to fructose can occur in foetal life and thus confirm the observations of Hers (1957*a*) who used foetal sheep liver slices. Although the conversion has been shown to be possible it, of course, does not imply that the fructose present in sheep foetal blood is formed by the liver from sorbitol. Studies on the perfused sheep placenta show that little sorbitol is formed compared to the amount of fructose produced, at least in the initial period of the perfusion (Andrews, Britton & Nixon, 1959). Thus in a typical experiment the fructose concentration in the perfusion circuit rose by 54 mg/100 ml. in the first hour of perfusion while

the sorbitol concentration increased by 10 mg/100 ml. This suggests that the amount of fructose formed by the liver from sorbitol, in the intact animal, is small.

Blakley (1951) showed that the conversion of sorbitol to fructose in the adult was brought about by the enzyme ketose reductase (sorbitol dehydrogenase). A similar enzyme has been demonstrated in the foetal sheep liver from 90 days onward by H. G. Britton (unpublished), using the method of Neil, Walker & Warren (personal communication).

The inability of the foetal liver to take up fructose contrasts strongly with the neonatal liver a few days after birth. It is believed that the first step in the metabolism of fructose is the phosphorylation of the sugar to fructose-1-phosphate by adenosine triphosphate in the presence of fructokinase (Hers, 1955), and it is perhaps relevant that Hers (1957*b*) was unable to demonstrate fructokinase activity in the foetal sheep liver. Whether the uptake of fructose by the lamb liver is paralleled by the appearance of this enzymic activity in the tissue is, however, not known.

The constancy of the lactic acid concentrations suggests that foetal livers were able to remove lactic acid produced by glycolysis occurring in the blood. Since the blood lactic acid concentrations in the lamb liver perfusions were lower than in the foetal liver preparations the ability of the foetal liver to take up lactic acid may be limited. If this is the case it may provide an explanation for the fact that lactic acid concentration in the foetal blood usually exceeds that of the mother (Barker & Britton, 1958), and also for the maintenance of high lactic acid concentration induced by hypoxia (Britton, Nixon & Wright, 1959).

From the perfused liver experiments, where no hormone additives are present, there would appear to be a developmental pattern in the establishment of hepatic metabolism. Thus, by the 122nd day of foetal life the liver is capable of converting sorbitol to fructose, yet the ability of the tissue to remove fructose to any substantial degree does not appear to be present until after birth.

SUMMARY

1. Perfusion circuits suitable for metabolic investigations have been developed for the foetal or neonatal sheep liver.

2. It has been shown that sorbitol can be converted to fructose by the preparation. Glucose, galactose and inositol did not increase the fructose concentration in the perfusate.

3. Under these experimental conditions the foetal liver appears to be unable to metabolize fructose, but rapid utilization cannot be detected in the perfused neonatal liver before the fifth day after birth.

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