J. Physiol. (1956) 132, 146–156

DIVISION OF THE COMMON MAMMALS INTO TWO GROUPS ACCORDING TO THE CONCENTRATION OF FRUCTOSE IN THE BLOOD OF THE FOETUS

By R. F. W. GOODWIN

From the Department of Veterinary Clinical Studies, Madingley Road, Cambridge

(*Received* 1 *November* 1955)

The presence of fructose in the fluids of the foetus, described by Needham (1931) as 'the most enigmatic aspect of embryonic carbohydrate metabolism', has posed many problems, and stimulated in recent years several experimental studies that have sought to explain the means whereby fructose reaches the foetal circulation and the role that this hexose plays in foetal life.

Bacon & Bell having established (1946, 1948) that the Seliwanoff-positive material in the blood of the foetal sheep was very largely free D-fructose, Huggett and his associates working with the same species have made a prolonged study of the pathways that maintain the fructose content of foetal blood (Huggett, Warren & Warren, 1951; Widdas, 1952; Alexander, Andrews, Huggett, Nixon & Widdas, 1955; Alexander, Huggett, Nixon & Widdas, 1955). Hitchcock (1949) and Karvonen (1949*a*) also worked mainly with the pregnant ewe, so that apart from one study using pregnant guinea-pigs (Karvonen & Räihä, 1954) and another using monkeys (Chinard, Danesino, Huggett, Paul & Reynolds, 1955) most experimental work has been on the sheep. This concentrated study of one species, while yielding much new information on the transfer of hexoses across the placenta of the ruminant, has not, however, revealed the function of fructose during gestation.

To investigate this last question specifically, a study with a fresh approach —that of the comparative physiology of the whole animal—was undertaken in Cambridge in 1950. By this means a comprehensive explanation has been offered for the presence of fructose in the foetus and, by analogy, a reason suggested for the use of hexoses other than glucose in seminal fluid and milk (Goodwin, 1954). This concept postulates first that some species of mammals with a relatively low maternal blood glucose concentration cannot supply their foetuses with a sufficient concentration of blood sugar without the intervention

of the fructose mechanism, and it thus confirms the hypothesis advanced tentatively by Winterton in 1949.

Alexander, Huggett, Nixon & Widdas (1955) have discussed their recent findings on the placental transfer of sugars in the sheep in the light of the work of Goodwin (1954), and it appears that these two widely differing experimental approaches to the fructose problem have yielded results that harmonize satisfactorily.

The first stage in the comparative approach was to establish whether a distribution pattern for foetal fructose occurred among the more readily available mammals, as the existing literature presented only a confused answer to this question. This confusion had arisen partly from the misreading of early literature, but mainly from a failure to appreciate that fructose does not occur at a similar concentration in the foetuses of different species.

The first report of fructose in the foetus appears to be that of Bernard (1855), who did not define clearly the fluid analysed. Consequently some writers believed that he had been working with human (Needham, 1931) or canine (Itizyô, 1934) material, but as he had just referred to a diagram of the foetal calf and also spoken of allantoic fluid it is almost certain that he had examined bovine samples. Gürber & Grünbaum (1904) found fructose in both the amniotic and allantoic fluids of the cow, pig and goat, and produced the methylphenylosazone 'in several cases'. Some authors have attributed to Gürber & Grünbaum the demonstration of fructose in other species (the horse and sheep). Paton, Watson & Kerr (1907) claimed that the amniotic and allantoic fluids of the sheep and cow contained fructose, but they also discussed other species in a way that is difficult to follow. There can be little doubt that these authors believed that their findings for the sheep and cow applied to the foetus generally and thus, in addition to allowing several different interpretations of their results (Orr, 1924; Needham, 1931; Bacon & Bell, 1948; Barklay, Haas, Huggett, King & Rowley, 1949; Huggett & Hammond, 1952), they engendered an assumption that fructose was a universal feature of foetal life. This assumption has been re-stated by Yamada (1933*a*), Itizyô (1934), Okamura (1938) and others. Orr (1924) believed that both human blood at birth and blood from a kid shortly after birth contained appreciable quantities of fructose. Takata (1922) and Suzuki (1925) found fructose to be the only sugar in the foetal fluid from a Sei Whale and a Sperm Whale respectively. Yamada (1933*b*) found small amounts of fructose in the mixed embryonic fluids of the incubated hen's egg, but once again it was not appreciated that the earliest reports such as those of Gürber & Grünbaum were concerned with fructose in high concentration and not with the trace amounts then being described. There was thus a tendency to seek fructose even at very low concentrations in foetal structures, and in this manner Itizyô (1934) and Masuko (1940) added their support to the contention that human foetal fluid contained fructose.

With the advent of experimental work on the sheep foetus, the foetal blood was examined by several workers (Cole & Hitchcock, 1946; Hitchcock, 1949; Karvonen, 1949*a*; Barklay *et al.* 1949; Huggett *et al.* 1951) and, using quantitative Seliwanoff reactions, average values for fructose concentration of about 70–100 mg/100 ml. were reported. Karvonen (1949*b*), however, examined human cord blood taken at delivery in the same way and, finding fructose concentrations of 1 mg/100 ml. or less, concluded that by comparison with the sheep, fructose could be taken as absent. This was a change of outlook from that which had accepted positive fructose results from almost all samples and, by separating the two mammals (man and sheep) according to the concentration of fructose, the problem was given perspective and the way prepared for the work described here. Huggett (1950) confirmed the finding of Karvonen.

This paper provides a more detailed and, by including findings for the foetal whale, slightly enlarged account of the work that was published in summarized form in 1952.

MATERIALS AND METHODS

The origin of the biological samples in the various species is given with the results. In general, foetal blood was examined rather than the foetal fluids usually selected by previous workers, although a few figures for the fructose content of foetal fluids are quoted. Blood analyses were preferred because they allow a more justifiable comparison between species. Furthermore, little is known of the metabolic functions of the foetal fluids, whereas much is known of the behaviour of sugar in adult blood. Obtaining fresh blood samples from foetuses is difficult in the larger mammals and, consequently, many such samples were obtained at birth. They were thus not strictly foetal in origin but were probably more physiological, being taken from the living animal.

Blood and other samples were usually collected over potassium oxalate and deproteinized at once with sodium hydroxide and zinc sulphate. Later, however, barium was substituted for sodium and the samples were collected over dried heparin. Thus a few of the pig samples, together with the samples for the third rabbit and the ferret, were handled in this second manner. The foal samples were also deproteinized by the barium method but the anticoagulant was usually sodium fluoride, a fact that is discussed in the results.

Fructose was estimated colorimetrically, with a modification of the Seliwanoff reaction in which glycerol acted as solvent and allowed the reaction to proceed in a boiling water-bath, and copper sulphate provided the catalyst (Goodwin, 1954). Unknown sugar solutions were boiled with the reagents for 12 min and water blanks (two) with the same reagents were always included. The orange-red colour that developed was read with an Ilford 601 Filter in a Spekker Photo-electric Absorptiometer (H. 560). But for the estimations made with samples from the foetal sheep, standard solutions of fructose were included with every series of unknowns. The method has been tested repeatedly and has a standard deviation of about 1 mg/100 ml. on twenty or so similar fructose standards made up at a concentration comparable to that found in filtrates from foetal (ungulate) blood and estimated together. Glucose standards were included periodically when estimating fructose, and solutions equivalent to 150 mg/100 ml. of blood glucose registered fructose colour equivalent to an average concentration of 1·5 mg/100 ml. of blood fructose.

Total reducing substances were estimated with the Somogyi (1945) reagent but with a slightly modified procedure (Goodwin, 1954). Fructose gave an average reduction of 92% compared with glucose, and this factor was used in the calculations arising from the estimation of fructose and

glucose in one solution. The method, tested repeatedly, had a standard deviation of about 1 mg/100 ml. on twenty similar standard solutions estimated together.

Basic standards (both fructose and glucose) were made up as 5% solutions of sugar (Kerfoot) by weight in saturated benzoic acid and then checked by polarimetry (4 dm tubes). They were re-checked after constant use and showed a maximum concentration change for fructose and glucose of less than 1 and less than 2% respectively. These strong solutions were diluted until equivalent to blood filtrates on the day the unknowns were estimated.

In correcting for the influence of each hexose upon the other, the Seliwanoff colour registered by glucose was allowed for only when the total Seliwanoff colour was very weak, while the unequal reduction of fructose and glucose was taken into account only when fructose was present in high concentration. Thus no correction was made for the presence of glucose in ungulate blood or for the presence of fructose in non-ungulate blood.

The Seliwanoff reaction is not specific for fructose but any specific method is both laborious and only roughly quantitative. However, now that it is known that the Seliwanoff-positive material in the blood of the foetal sheep is mainly D-fructose, a strong Seliwanoff colour in other species has been taken as similarly representing fructose.

The reducing material in blood and other filtrates, in excess of the equivalent reduction for the fructose concentration, has been recorded as glucose. This is probably a less justifiable procedure, as Bacon & Bell (1948) had indications that non-fermentable reducing material existed in the sodium hydroxide/cadmium sulphate filtrates of foetal blood from the sheep. Their work was not conclusive and would not be applicable to blood samples deproteinized by other means, or to reagents other than the ferricyanide reagent used by them, but their findings must be borne in mind. The main concern of these analyses, however, was to estimate the concentration of fructose, and minor inaccuracies in the estimation of glucose have no bearing upon the main conclusions.

RESULTS

Ungulates

Samples from the horse, pig, sheep, ox and goat have been analysed for their fructose content. The findings in the sheep accorded with those recently reported by others and they are not, therefore, detailed here. The results for the remaining species are summarized in Table 1. The foal samples were of cord blood from twenty-one thoroughbred mares. It is now known that the concentration of anticoagulant (sodium fluoride) was insufficient to arrest glycolysis completely during the delay between collecting and deproteinizing the blood and, therefore, the actual fructose concentration at birth was often a few mg/100 ml. higher than shown by the analyses. In the pig, cord blood samples were taken from sixty-six newborn animals, but in only sixty-one of these was glucose estimated also. These pigs were born during thirteen farrowings and

TABLE 1. Concentration of sugar in the blood of newborn ungulates

	Sugar in mg/100 ml.			
	Fructose		Glucose	
Species	Mean	Range	Mean	Range
Ox	108 (1)	—	105 (1)	—
Goat	50 (1)	—	58 (1)	—
Horse	111	57–160 (21)	60	33– 84 (21)
Pig	49	23–110 (66)	65	32–188 (61)

Figures in parentheses denote the number of young sampled.

were out of eleven different sows. Although only one sample (venous blood) has been analysed from a calf at birth, several calves have been bled shortly after birth and fructose in appreciable concentration was present in all. For the goat, mixed tail blood was taken from a kid that could have been up to 3 hr old when bled, but was probably rather less. The fructose content would almost certainly have been higher in an earlier sample.

The whale

Whereas all the foetuses of ungulates so far examined contain both fructose and glucose in their blood, the foetal fluid of the whale has been reported on two occasions to contain only fructose. This anomaly, therefore, required further investigation.

TABLE 2. Concentration of sugar in the foetal blood of whales

Species	Time (hr) between killing cow and sampling foetus	Foetus		Remarks	Sugar in mg/100 ml.	
		Sex	Length (ft.)		Fructose	Glucose
Fin	6	M.	9	Killed with electric harpoon. Blood from abdom. vein	113	24·5
Fin	About 8	M.	14	Blood from heart region	98	27
Humpback	11½	M.	4½	Blood from jugular vein	72	30
Blue	4½	F.	10	Blood from heart region	228	60

Blood samples were taken from four foetuses representing three species of whales (Fin: *Balaenoptera physalus*, Humpback: *Megaptera nodosa* and Blue: *Balaenoptera musculus*). Details of the foetuses and the manner in which the samples were taken are given in Table 2, together with the analytical results. At least three parallel blood samples were taken from each foetus and some of these were examined in duplicate, so that in most cases the figures quoted are the mean values for several estimations. The samples were deproteinized at once in the Antarctic and the filtrates frozen. They were not liquefied again until just before the analyses were performed.

It is apparent that fructose is present in high concentration in the foetal blood of the whale but, in addition, there is a reducing substance at a lower concentration. This has not been shown to be glucose but can be accepted as such on comparative grounds: it is at the concentration that might be expected with such samples, and shows an increased concentration in one foetus when the value for fructose was also particularly high.

Non-ungulates

Analyses for sugar have been made on samples from the cat, dog, ferret, guinea-pig, rabbit and rat. In the cat and the dog, samples were taken from newborn animals by decapitating them at birth after wiping them dry. In the

ferret, guinea-pig, rabbit and rat, laporotomy was performed and the foetuses bled in the same manner after being removed from the uterus as rapidly as possible. To obtain enough blood for a satisfactory analysis, it was necessary to mix the samples from several foetuses in the ferret and to pool the blood from all eight foetuses in the rat. The first two pregnant rabbits were anaesthetized with pentobarbitone (Nembutal). The guinea-pigs were anaesthetized with ether, and as the maternal samples were not taken until all the foetuses had been sampled they show maternal hyperglycaemia. The third rabbit, the ferret and the rat were stunned.

TABLE 3. Concentration of sugar in the blood of newborn puppies and kittens, and in the blood of foetal ferrets

Species	Young (birth order)	Sugar in mg/100 ml. Fructose	Glucose
Dog	1	0·7 (2)	68
	2	0·7 (4)	97 (2)
	3	0·3 (6)	81 (3)
	4	2·8	74
Cat (no. 1)	2	1·3 (2)	72
	3	2·3 (2)	110 (2)
	4	0·7 (2)	75 (2)
	5	0·2 (2)	91
Cat (no. 2)	2	0 (3)	66
Ferret	1 to 7 (mixed blood)	1·3	73
	8 to 10 (mixed blood)	3·2	85
		2·1 (2)*	105 (2)*

Figures in parentheses denote the number of parallel analyses.
* Mixed maternal blood.

When analysing the samples from the dog, cat, guinea-pig and the first two rabbits, correction for the presence of glucose was made after noting the fructose colour produced by glucose standards estimated at the same time as the unknowns. With the remaining samples, glucose standards were not estimated by the fructose method and the standard correction factor of 1% was used instead. All negative fructose concentrations have been taken as zero in calculating mean values.

The results for the non-ungulates are shown in Table 3 (carnivores) and Table 4 (guinea-pig and rabbit). The values for the rat are included in Table 5, which summarizes all the analyses. From this last table it can be seen that, in each of the three species where the comparison was made, the concentration of Seliwanoff-positive material in the foetal and maternal blood was almost identical.

TABLE 4. Concentration of sugar in the foetal blood and foetal fluid of the guinea-pig and rabbit

Species	Foetal age (days)	Sample	Sugar in mg/100 ml. Fructose		Glucose
Guinea-pig 1	31	Foetus 1. Amniotic fluid	5	4*	104
		Foetus 2. Amniotic fluid	5	4	97
		Foetus 3. Amniotic fluid	5·5	5	95
		Maternal heart blood	0·9	1·7	209
Guinea-pig 2	31	Foetus 1. Amniotic fluid	10·1	9·4	94
		Foetuses 2 and 3. Amniotic fluid	4	3	82
		Maternal heart blood	0	0·3	178
Guinea-pig 3	Near term	Foetus 1. Blood	2·5	0	81
		Foetus 1. Amniotic fluid	0·7	0	77
		Foetus 3. Amniotic fluid	1·7	0·4	84
Guinea-pig 4	Near term	Foetus 1. Amniotic fluid	0·6	0	80
		Foetus 1. Blood	0	0	65
		Foetus 2. Amniotic fluid	0	0	70
		Foetus 2. Blood	0	0	40
Guinea-pig 5	41	Foetus 1. Blood	0	0	—
		Foetus 2. Amniotic fluid	1·4	2·7	—
		Foetus 3. Blood	0	1·8	—
		Foetus 3. Amniotic fluid	1	2·4	—
		Foetus 4. Amniotic fluid	0	1·1	—
Rabbit 1	29	Foetus 1. Blood	3·3	—	93
		Foetus 3. Blood	1·2	—	65
		Foetus 4. Blood	2·5	—	90
		Foetus 5. Blood	2·1	—	87
Rabbit 2	29	Foetus 1. Blood	0·6	—	37
		Foetus 2. Blood	1·6	—	34
		Foetus 3. Blood	0·9	—	27
		Foetus 4. Blood	0	—	35
		Foetus 5. Blood	0	—	32
		Foetus 6. Blood	0	—	27
Rabbit 3	28	Maternal throat blood after stunning	1 0·3	3·1 2·4	79
		Foetuses 1 to 4. Mixed blood	0·8	2·9	60
		Foetus 5. Blood	2·4	4·5	75
		Foetus 6. Blood	0·5	2·6	56
		Foetus 7. Blood	2·1	4·1	55
		Foetus 8. Blood	0·9	3·0	73
		Mixed fluids	2·5 1·2	4·6 3·4	36

* Second column records figures derived using alternative (duplicate) blank reading.

TABLE 5. Summary of results for non-ungulate mammals

Species	No. of pregnant females	Fructose in mg/100 ml. Foetal blood Mean	Foetal blood Highest	Amniotic fluid Mean	Amniotic fluid Highest	Maternal blood. Mean
Guinea-pig	5	0·4 (5)	2·5	2·8 (13)	10·1	0·7 (2)
Rabbit	3	1·6 (18)	4·5	2·9	—	1·7 (1)
Rat	1	0·1 (8)	0·1	—	—	—
Cat	2	0·9 (5)	2·3	—	—	—
Dog	1	1·1 (4)	2·8	—	—	—
Ferret	1	1·9 (10)	3·2	—	—	2·1 (1)

Figures in parentheses denote the number of foetuses or mothers sampled and not the number of parallel estimations.

DISCUSSION

It is known that virtually all the material that gives a positive Seliwanoff reaction in the blood of the foetal sheep is free D-fructose. If it is accepted that this finding can reasonably be applied to all mammalian foetuses, then the present results indicate that fructose also occurs in high concentration in the foetal blood of the horse, pig, ox, goat and whale. Although the foetal blood also contains glucose, it may be concluded that in all these species under normal conditions the concentration of glucose is considerably less than the concentration of fructose, for such is the case in the pig (representing the non-ruminants), as shown by Goodwin (1954), the sheep (representing the ruminants) and the whale.

The non-ungulate mammals contrast sharply with the ungulates, for in the foetal blood of the carnivores and rodents (for convenience, the rabbit is grouped with the rodents) fructose was present only in trace amounts and at concentrations comparable with those existing in maternal blood. Whether the weak Seliwanoff-colour registered in these species represents the presence of fructose is not clear, as there is no information on the non-hexose blood chromogens that might produce a similar trace of colour with the Seliwanoff reaction. Furthermore, there are several analytical details that bear upon the interpretation of such low readings. The estimation of Seliwanoff colour cannot be accurate at these low values, for the variation in the strength of colour shown by the blanks approaches the maximum excess of colour developed by the blood filtrate over the blanks. Thus negative values for blood fructose are not uncommonly recorded. In calculating the mean concentrations, such negative values should, strictly, offset some of the positive values and by recording all negative values as zero there has been a slight inflation of the apparent concentration of fructose. Again, with such small foetuses foetal blood is relatively scarce, and often the final blood filtrate needs to be more dilute than the corresponding filtrates from ungulates. The basic variables of the method then become multiplied, and it has been observed that if two duplicate blood samples are not diluted equally, the weaker filtrate often yields a higher value for apparent fructose (either positive or negative). Examples of this effect can be seen with the puppies (blood filtrates from the first three were a 1:11 dilution of whole blood, that from the last was diluted 1:31), and the ferret (blood dilution for the first foetal group was 1:16; for the second, 1:23).

However, regardless of whether fructose is or is not present in trace amounts in the foetal blood of non-ungulates, it is clear that the pattern shown by the two mammalian groups is very different. If a problem exists concerning fructose in the foetus, it can do so in the first place only as a result of the pattern shown by the foetal ungulates, for Seliwanoff-positive material in

small amounts can be found in the blood and other fluids of adults. Whether non-ungulates possess the basic placental pathways to produce fructose from glucose so that the difference between the two mammalian groups, biochemically, is only one of degree, has been investigated by Hagerman & Villee (1952), although the limits of present analytical methods clearly handicap such studies.

From the grouping shown by these eleven species of common land mammals it has been proposed that among land mammals generally fructose occurs in high concentration in the foetus during late pregnancy only in ungulates (Goodwin, 1952). Such a view cannot be beyond doubt unless all species of foetal mammals are examined, but it can remain as a working proposal until fructose is demonstrated in high concentration in a non-ungulate foetus. There is no reason to wish that the position of ungulates in this respect should remain undisturbed and the discovery of a non-ungulate exhibiting the fructose mechanism during pregnancy might provide a useful experimental animal. The brief report of Ainsworth, Parr & Warren (1951) accords with the present findings, as these authors examined the mouse and hamster (in addition to some of the species contained in this study) and found that the foetal blood in these two mammals did not contain appreciable amounts of fructose.

While it is of interest to know that other ruminants such as the deer (Walker, 1954) conform to this pattern for ungulates, the real need now is to examine further species selectively. Thus it is important to know whether representatives of the Chiroptera or Insectivora can be classified with non-ungulates in this context, while there are some species, such as the American mole, that have important anatomical (placental) features common to ungulates but are, in zoological classification, removed from them. Unsuccessful attempts have been made by the writer to obtain foetal samples from these species and it would be simpler if they could be examined in their native lands. Possible affinities between ungulates and the whale are beyond the scope of this discussion.

Fructose occurs elsewhere in biological material in substantial concentration —in seminal plasma (Yamada, 1933*c*; Mann, 1946) and in the haemolymph of the third instar larva of *Gastrophilus intestinalis* (Levenbook, 1947)—and while it has been possible to indicate a similarity between the needs of the ungulate foetus and spermatozoa (Goodwin, 1954) further similarity is not apparent for *Gastrophilus*, if indeed it were justifiable to seek a comparison between the role of fructose in such widely differing media.

SUMMARY

1. Foetal blood from the horse, pig, sheep, ox, goat, whale, dog, cat, ferret, guinea-pig, rabbit and rat was examined for the presence of fructose.

2. Seliwanoff-positive material (taken as fructose) was present in high concentration in the samples from the horse, pig, sheep, ox, goat and whale. In the remaining species, Seliwanoff-positive material was present only in traces and at concentrations comparable to those found in the blood of adults.

3. It is suggested that, pending a much wider survey of the mammalian Orders, it may be assumed that the presence of fructose at high concentration in the foetal blood of land mammals at term is a peculiarity of ungulates.

Most of this work was carried out in the Department of Animal Pathology, Cambridge, and it has received the financial support of the Agricultural Research Council.

Obtaining samples from foals would have been extremely difficult but for the generous facilities provided by the Earl of Derby at his foaling stables. Mr M. Ryan, his stud groom, could not have been more helpful. The Equine Research Station, Animal Health Trust, besides arranging the access to this stud, provided nearby laboratory facilities for handling blood samples at night. The foetal whale samples were collected by Mr H. W. Symons in the 1952–3 whaling season. His efforts to obtain material as fresh as possible were particularly successful, while the filtrates were preserved with outstanding care until their safe delivery.

REFERENCES

AINSWORTH, I. B., PARR, C. W. & WARREN, F. L. (1951). Fructose formation in the placenta. *J. Endocrin.* **7**, lxiii–lxv.

ALEXANDER, D. P., ANDREWS, R. D., HUGGETT, A. ST G., NIXON, D. A. & WIDDAS, W. F. (1955). The placental transfer of sugars in the sheep: studies with radioactive sugar. *J. Physiol.* **129**, 352–366.

ALEXANDER, D. P., HUGGETT, A. ST G., NIXON, D. A. & WIDDAS, W. F. (1955). The placental transfer of sugars in the sheep: the influence of concentration gradient upon the rates of hexose formation as shown in umbilical perfusion of the placenta. *J. Physiol.* **129**, 367–383.

BACON, J. S. D. & BELL, D. J. (1946). The identification of fructose as a constituent of the foetal blood of the sheep. *Biochem. J.* **40**, xlii–xliii.

BACON, J. S. D. & BELL, D. J. (1948). Fructose and glucose in the blood of the foetal sheep. *Biochem. J.* **42**, 397–405.

BARKLAY, H., HAAS, P., HUGGETT, A. ST G., KING, G. & ROWLEY, D. (1949). The sugar of the foetal blood, the amniotic and allantoic fluids. *J. Physiol.* **109**, 98–102.

BERNARD, C. (1855). *Leçons de Physiologie Expérimentale*, vol. I, p. 405. Paris: Baillière.

CHINARD, F. P., DANESINO, V., HUGGETT, A. ST G., PAUL, W. M. & REYNOLDS, S. R. M. (1955). The passage of sugars across the monkey placenta. *J. Physiol.* **127**, 8–9*P*.

COLE, S. W. & HITCHCOCK, M. W. S. (1946). Sugars in the foetal and maternal bloods of sheep. *Biochem. J.* **40**, li–lii.

GOODWIN, R. F. W. (1952). Foetal fructose in various mammals. *Nature, Lond.*, **170**, 750.

GOODWIN, R. F. W. (1954). A comparative study of carbohydrate metabolism in the new-born mammal. Ph.D. Dissertation, University of Cambridge.

GÜRBER, A. & GRÜNBAUM, D. (1904). Ueber das Vorkommen von Lävulose im Fruchtwasser. *Münch. med. Wschr.* **51**, 377–378.

HAGERMAN, D. D. & VILLEE, C. A. (1952). The transport of fructose by human placenta. *J. clin. Invest.* **31**, 911–913.

HITCHCOCK, M. W. S. (1949). Fructose in the sheep foetus. *J. Physiol.* **108**, 117–126.

HUGGETT, A. ST G. (1950). Foetal physiology and child health. *Arch. Dis. Childh.* **25**, 101–109.

HUGGETT, A. ST G. & HAMMOND, J. (1952). Physiology of the placenta. In *Marshall's Physiology of Reproduction*, 3rd ed., vol. II, p. 350. Ed. Parkes, A. S. London: Longmans, Green.

HUGGETT, A. ST G., WARREN, F. L. & WARREN, N. V. (1951). The origin of the blood fructose of the foetal sheep. *J. Physiol.* **113**, 258–275.

ITIZYÔ, M. (1934). Über den Zuckergehalt des menschlichen Fruchtwassers und das Vorkommen der Lävulose in demselben. *Jap. J. med. Sci.* (*II. Biochem.*), **2**, 359–369.

KARVONEN, M. J. (1949*a*). The fructose of sheep foetal blood. *Ann. Med. exp. Fenn.* **27**, 197–214.

KARVONEN, M. J. (1949*b*). Absence of fructose from human cord blood. *Acta paediat., Stockh.*, **37**, 68–72.

KARVONEN, M. J. & RÄIHÄ, N. (1954). Permeability of placenta of the guinea pig to glucose and fructose. *Acta physiol. scand.* **31**, 194–202.

LEVENBOOK, L. (1947). Fructose and the reducing value of insects' blood. *Nature, Lond.*, **160**, 465.

MANN, T. (1946). Fructose, a constituent of semen. *Nature, Lond.*, **157**, 79.

MASUKO, C. (1940). On sugar element in human amniotic fluid. *Jap. J. Obstet. Gynec.* **23**, 102–103.

NEEDHAM, J. (1931). *Chemical Embryology*, vol. II, p. 1054. Cambridge University Press.

OKAMURA, H. (1938). Über die Blutlävulose bei Schwangerschaft. *Jap. J. med. Sci.* (*II. Biochem.*), **4**, 15–16.

ORR, A. P. (1924). Laevulose in the blood of the human foetus. *Biochem. J.* **18**, 171–172.

PATON, D. N., WATSON, B. P. & KERR, J. (1907). On the source of the amniotic and allantoic fluids in mammals. *Trans. Roy. Soc. Edinb.* **46**, 71–102.

SOMOGYI, M. (1945). A new reagent for the determination of sugars. *J. biol. Chem.* **160**, 61–68.

SUZUKI, M. (1925). Untersuchungen über Cetacea. XXIII. Über das Fruchtwasser des Pottwals. *Jap. J. med. Sci.* (*II. Biochem.*), **1**, 97–101.

TAKATA, M. (1922). Untersuchungen über Cetacea. VII. Über das Fruchtwasser des Seiwals. *Tohoku J. exp. Med.* **2**, 459–464.

WALKER, D. G. (1954). Fructose in the foetal fluids of deer. *Nature, Lond.*, **173**, 309–310.

WIDDAS, W. F. (1952). Inability of diffusion to account for placental glucose transfer in the sheep and consideration of the kinetics of a possible carrier transfer. *J. Physiol.* **118**, 23–39.

WINTERTON, N. V. (1949). On the origin of fructose in the foetal sheep. M.Sc. Thesis, University of London.

YAMADA, K. (1933*a*). Über den Zucker im Fruchtwasser. Beobachtungen am Fruchtwasser des Hühnerembryos. *Jap. J. med. Sci.* (*II. Biochem.*), **2**, 47–69.

YAMADA, K. (1933*b*). Über das Vorkommen von Lävulose im Fruchtwasser des Hühnerembryos. *Jap. J. med. Sci.* (*II. Biochem.*), **2**, 107–113.

YAMADA, K. (1933*c*). Über den Zuckergehalt des Samens. *Jap. J. med. Sci.* (*II. Biochem.*), **2**, 245.