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THE DISTRIBUTION OF SUGAR BETWEEN RED CELLS AND PLASMA: VARIATIONS ASSOCIATED WITH AGE AND SPECIES

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The distribution of the blood sugar between red cells and plasma has long been a subject of interest, but most investigators have had as their primary aim the study of various properties of the corpuscle and only a few have been more concerned with the concentration of sugar in the plasma. The latter workers have proceeded in the belief, stressed by Macleod in 1913, that it is the concentration of sugar in the plasma and tissue fluid to which the general body cells respond, but this concept has not been developed and it is the concentration of sugar in the whole blood which is now customarily estimated in metabolic studies.

During work that sought the role of fructose in foetal life, it was observed that only among ungulates and the whale could fructose be demonstrated at high concentration in foetal blood (Goodwin, 1952, 1956). The next stage in this comparative approach was to examine the maternal/foetal relationship of ungulates and non-ungulates (excluding the whale) to see whether there was a common obvious requirement for fructose in the first group of mammals that did not exist in the second. But before doing this it was important to ensure that any comparisons between the blood-sugar levels of different species at varying ages should have a common basis. If the blood sugar were distributed differently between cells and plasma in different blood samples, deductions made from analyses of whole blood could have been misleading. Furthermore, with blood containing both fructose and glucose it could not be assumed that each sugar would be distributed according to the partition that occurred in the same species with glucose alone.

The results described here are concerned essentially with the relationship between the concentration of sugar in whole blood and in plasma under normal conditions and while they have a bearing on permeability studies they are clearly no substitute for them. Conversely, the techniques used to show

the permeability of red cells to hexoses are not designed to demonstrate the concentrations of sugar as they occur *in vivo*.

A preliminary account of these findings has been given elsewhere (Goodwin, 1954).

METHODS

Sugar estimations. As plasma samples could not be satisfactorily deproteinized with sodium hydroxide and zinc sulphate, deproteinization was carried out with barium hydroxide and zinc sulphate throughout. The methods for estimating the concentration of fructose and glucose in protein-free filtrates were as outlined previously (Goodwin, 1956).

Technique for demonstrating the distribution of sugar. Blood samples were taken as rapidly as possible, generally under conditions that favoured normal sugar concentrations. The need thereafter was to separate the plasma quickly without allowing glycolysis or encouraging red-cell damage and a shift in the partition of sugar. Although glycolysis could be checked with sodium fluoride, better results in other respects were obtained by cooling heparinized samples until deproteinization was complete. This was done by first standing the blood in iced water immediately after collection and then by centrifuging it in glass tubes (internal diameter 1·4 cm) that were surrounded by packed ice chips or an ice mould in centrifuge cups (internal diameter 3 cm). The ice moulds, although requiring longer preparation, were preferable as they could be centrifuged at 3500 rev/min for about 15 min before melting completely. That this technique prevented glycolysis was shown by centrifuging rabbit's blood for 30 min, with one change of ice mould at 15 min. The concentration of sugar in the whole blood before centrifuging was 123·8 mg/100 ml. After centrifuging and then thoroughly remixing the cells and plasma, the equivalent concentration was 123·9 mg/100 ml. Both figures are the mean of three estimations.

Two methods were then available for determining the distribution of sugar: sampling the plasma and cells directly or sampling the whole blood and plasma and calculating the sugar concentration in the cells indirectly from a knowledge of the packed cell volume. The direct method was examined first but, although it was shown that packed cells could be kept cool for at least 6 hr without loss of sugar, there were technical difficulties with this procedure that made it unsuitable. First, the cellular sugar concentration, after centrifuging for 30 min at 3500 rev/min, varied with the depth of the sample within the packed cell mass, which argued a density gradient of cells similar to that described by Leeson & Reeve (1951): and secondly, the packed red cells could not easily be pipetted.

Thus the indirect method, using heparinized blood, became the standard procedure and all the sugar values given in the results were, unless otherwise stated, obtained with this technique. The advantage of this method is that there is minimal manipulation of the sample before deproteinization, while the red cells can be well packed without the need to inhibit glycolysis at the same time. For such packing the blood was centrifuged in 100×3 mm tubes for 1–1½ hr at 4000 or more rev/min. The maximal radius of centrifugation in all experiments has been 15 cm except for the heparinized cord samples of the foal for which the radius was about 22 cm. After sampling for the concentration of sugar in whole blood, the latter was centrifuged in ice for just so long as was necessary to expose the volume of plasma required (generally 5–10 min).

As many of these results were obtained during work in which the first aim was to determine the concentration of sugar in the plasma, haematocrit estimations were not always made concurrently. However, although the conclusion is less accurate, the ratio between the concentration of sugar in the plasma and in the whole blood can also be used as an indication of the extent to which different erythrocytes permit equalization of the sugar concentration across the cell membrane.

RESULTS

The pig

The findings for the pig are described first because in this species the changes in sugar partition are particularly well defined.

Adult blood. Many estimations were made of the concentration of sugar in the whole blood and plasma of sows at a time when sodium fluoride was used to prevent glycolysis. Invariably there was a great difference between the two concentrations of sugar, which indicated that the cellular sugar concentration was low. In fifteen instances simultaneous haematocrit estimations were made and the calculated mean concentration of glucose in the cells was 8·7 mg/100 ml. The mean sugar concentration in the plasma of these fifteen sows was 71·7 mg/100 ml. Although the use of fluoride appeared to give slightly less accurate results, it was clear that the cells of the adult pig contained very little reducing material. This was confirmed with five sows using the heparin-ice technique: the mean concentration of sugar in the cells was 7·6 mg/100 ml. while the mean concentration in the plasma was 81·1 mg/100 ml. For the latter results, the sugar estimations were made in triplicate and the haematocrit determinations in duplicate.

Foetal blood. Figures are available for fifteen near-term foetuses when the concentrations of sugar in the whole blood and plasma were estimated together. Fructose was present in high concentration in addition to glucose and in every case there was relatively little difference between the sugar concentrations of whole blood and plasma. This indicated that both fructose and glucose were present at high concentration within the red cells.

The methods employed here to estimate fructose and glucose are not completely reliable when the two hexoses are in the same solution. Consequently, in seeking the highest accuracy, standard solutions of fructose alone, glucose alone, and different mixtures of the two sugars together have been included when estimating unknowns. By these tests, the estimations for nine of the fifteen foetuses, together with the estimations for a further six pigs in which cord blood was analysed, were apparently satisfactory as all the standards read close to their theoretical values at the same time. The findings are summarized in Table 1. As the packed cell volume is obviously the same for both hexoses it follows, from the similarity of the plasma/whole-blood sugar-concentration ratios, that fructose and glucose were distributed across the erythrocyte membrane in approximately the same proportions.

Table 1 does not indicate the concentration of sugar within the cells but this has been calculated for seven of the same pigs where the packed cell volume was known (four foetal samples and three cord samples). These values are given in Table 2 in order to illustrate the variation of the corpuscular/plasma sugar-concentration ratio. From the fact that it is more difficult to estimate

glucose than fructose in a mixture of the two sugars and that the distribution ratio for fructose varies, with one exception, only between 68 and 85%, whereas the glucose distribution ratio is much more variable, it is felt that the variation is probably more a reflexion of weakness in the technique than of true *in vivo* differences. In this respect, it can be noted that if in pig 3 the plasma concentration of glucose had actually been 3 mg higher than estimated,

TABLE 1. Concentration of fructose and glucose in the whole blood and plasma of the foetal and new-born pig

	Samples from nine near-term foetuses			Samples from six pigs at birth		
	Mean whole-blood value	Mean plasma value	Mean of whole-blood/plasma sugar-ratios	Mean whole-blood value	Mean plasma value	Mean of whole-blood/plasma sugar-ratios
Fructose (mg/100 ml.)	66·1	73·3	1·11	62·1	68·8	1·11
Glucose (mg/100 ml.)	42·3	44·9	1·07	63·1	70·9	1·14

TABLE 2. Concentration of fructose and glucose in the whole blood, plasma and red cells of foetal and new-born pigs

		Concentration in whole blood (mg/100 ml.)	Concentration in plasma (mg/100 ml.)	Haematocrit reading	Calculated concentration in cells (mg/100 ml.)	Cell/plasma concentration ratio
Pig 1 (foetus)	Fructose	74	79	42½	67	85
	Glucose	43	44		42	95
Pig 2 (foetus)	Fructose	43	53	48¼	33	62
	Glucose	39	48		28	59
Pig 3 (foetus)	Fructose	64	68	41¼	58	84
	Glucose	42	42		42	101
Pig 4 (foetus)	Fructose	75	85	36¾	60	70
	Glucose	53	56		47	84
Pig 5 (new-born)	Fructose	58	68	47½	46	68
	Glucose	40	46		34	74
Pig 6 (new-born)	Fructose	79	85	43	70	83
	Glucose	46	56		32	58
Pig 7 (new-born)	Fructose	50	54	33	43	79
	Glucose	110	121		88	73

Note: But for haematocrits, the values shown are those of the nearest whole number.

the cellular/plasma distribution ratio for glucose would have been reduced from 101 to 84%. For the pigs listed in Table 2 the mean corpuscular/plasma sugar-concentration ratio was 76·1% for fructose and 77·8% for glucose. This again shows that the two hexoses are distributed similarly, but it demonstrates in addition that for each sugar there is, on the average, only a small concentration gradient across the cell membrane. In this section, the small blood samples did not allow more than about 60% of the sugar and haematocrit determinations to be made in duplicate.

Neonatal blood. The erythrocytes of the adult pig had been shown to contain very little sugar, but the concentration of sugar in the erythrocytes of the foetus closely approximated the concentration of sugar in the plasma; the blood of the growing pig was examined next, therefore, to find the period when the characteristics of the red cell were changing.

A preliminary experiment with a litter of seven pigs revealed that the difference between the sugar concentration in the whole blood and plasma increased rapidly after about the tenth day of life. This experiment was repeated on a second litter but with haematocrit determinations in addition. (On this occasion duplicate estimations were not made.) Fig. 1 shows the change in the mean corpuscular/plasma glucose ratio during the first month of

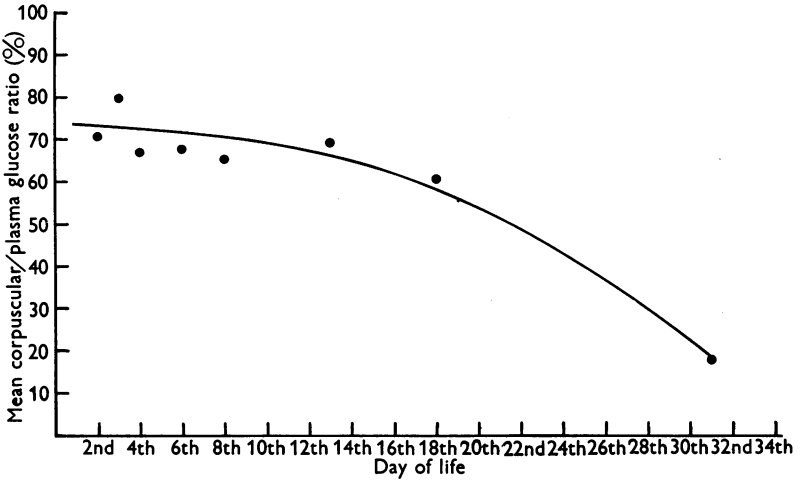

Fig. 1. Post-natal change in the distribution of blood glucose in the pig.

life. Each point is the mean value for either four or five litter-mates. The corpuscular sugar concentration remained high until the end of the second week; thereafter it decreased rapidly and by the thirty-first day the samples showed a distribution pattern for glucose that approximated the findings in the adult. Between the first week, when the mean cellular glucose concentration was 69·7 mg/100 ml., and the thirty-first day, when the concentration was 19·3 mg/100 ml., the glucose content of the cells had fallen by nearly 75%. The corresponding plasma glucose concentrations at these times showed a small increase from 98·6 to 110·3 mg/100 ml.

To confirm this early change in corpuscular behaviour, three pigs from another litter were bled on the twenty-sixth and forty-first day of life. Half of the sugar and haematocrit determinations were duplicated. At the younger age the mean cellular/plasma glucose ratio was 29·5%, the mean cell and plasma glucose concentrations being 36·9 and 124·5 mg/100 ml. respectively.

These values had changed to 21·9%, 27·6 and 126·7 mg/100 ml. by the second sampling.

The sheep, ox and horse

Three further species, the sheep, ox and horse, were then examined with particular reference to the characteristics of the erythrocytes during the neonatal period.

The sheep

Adult blood. Forty estimations have been made on the blood of eight ewes. The haematocrit value was the mean of two readings in each case. Two, three or, in most cases, four parallel estimations were made of the whole-blood and plasma glucose concentration in each sample. The mean corpuscular/plasma glucose-concentration ratio was 23·5% and in twenty-six of the forty samples the ratio was between 20 and 31%. The mean concentration of reducing material in the red cells was 13·3 mg/100 ml. Thus the red cells of the adult sheep contained reducing material at a much lower concentration than the plasma but at a higher concentration than the erythrocytes of the adult pig.

Neonatal blood. The new-born lamb, like the new-born pig, had a high concentration of sugar in the corpuscles, the mean cellular glucose concentration for three lambs up to the 6th day of life (thirteen estimations) being 73·3 mg/100 ml. The mean cellular/plasma glucose-concentration ratio for the same samples was 79·9%. By the eleventh day this ratio had dropped to 60·4% and it seemed that the characteristics of the cell were already changing. Although it was not possible to observe these particular lambs further, four other lambs were bled during their sixth week of life and they gave a mean ratio of 16·5% with a mean cellular glucose concentration of 15·4 mg/100 ml. All the estimations on lamb's blood were made in duplicate. Thus the lamb and the pig showed a common pattern for the change in the distribution of sugar after birth.

The ox

Adult blood. Samples from five cows showed a glucose distribution pattern that was almost identical with that found in adult sheep. The mean concentration of glucose in the cells was 15·0 mg/100 ml. and the mean corpuscular/plasma distribution-ratio was 24·2%.

Neonatal blood. Three calves were bled twice during the first week of life. The corpuscular/plasma glucose-concentration ratio was 57, 77 and 70% respectively, giving a mean ratio of 68%. The sugar estimations were usually made in quadruplicate; three or four parallel determinations were made of each packed cell volume. It seemed possible that the erythrocytes were already changing in type at birth, particularly in the first animal and this particular calf was bled repeatedly thereafter in the expectation that the

distribution of glucose would rapidly assume the pattern seen in the adult. Such an early change, however, did not occur (Table 3); instead, the distribution ratio showed a protracted and erratic decrease, and not until the fifth month were values of less than 30% obtained. It is probable, therefore, that in the calf the change in erythrocyte character after birth requires several months, rather than several weeks as in the pig and lamb.

TABLE 3. Post-natal change in the distribution of glucose between red cells and plasma in a calf

Age of calf when bled (days)	Corpuscular/plasma glucose-concentration ratio (%)	Age of calf when bled (days)	Corpuscular/plasma glucose-concentration ratio (%)
4	57	69	48
7	57	79	37
14	47	88	37
21	53	95	39
25	44	104	49
28	40	110	31
32	65	117	38
35	52	135	32
39	59	145	29
46	36	152	27

TABLE 4. Concentration of glucose in the red cells and plasma of a new-born foal

	Day of life								
	1	2	3	4	6	9	16	17	Mean
Plasma glucose (mg/100 ml.)	96·7	102·8	120·5	107·5	103·5	109	106·6	76·3	102·9
Corpuscular glucose (mg/100 ml.)	16·9	15·5	19·4	2·1	21·2	−0·3*	6	−2·3	9·8
Cell/plasma concentration ratio (%)	17·5	15·1	16·1	2·0	20·5	−0·3	5·6	−3·0	9·2

* Negative values have been recorded as such for calculating the means.

The horse

Adult blood. The concentration of glucose in the plasma of mares was found to be considerably greater than the concentration in the whole blood. It was probable, therefore, that glucose was at a low concentration in the corpuscle. Before substantiating this, the blood of foals was examined and the results obtained with neonatal blood allowed further work on adult blood to be postponed.

Neonatal blood. A foal was bled on 4 of the first 6 days of life: the mean whole-blood and plasma glucose concentrations were 80·1 and 120·8 mg/100 ml. respectively, which suggested that the erythrocytes contained little sugar and were thus unusual among neonatal corpuscles. A second foal was then examined in more detail, and Table 4 shows the concentration of reducing material in the cells and plasma between birth and the seventeenth day. All estimations, including the haematocrit determinations, were made in duplicate; half the glucose estimations were in triplicate.

It is difficult to interpret the changes in the cellular glucose concentration; they could be real, when they show that this concentration may fluctuate between zero and 21 mg/100 ml. with but little variation in the plasma sugar concentration, or they could be evidence of the extent of the variation allowed by the technique. However, the mean corpuscular glucose concentration of 9·8 mg/100 ml., at a mean plasma glucose concentration of 102·9 mg/100 ml., is almost as low as that shown by the adult pig. In the latter, the lower concentration within the cell might be favoured by the lower external concentration in the plasma.

Having found that the neonatal corpuscle contained little glucose it was of interest to observe the partition shown by fructose, for in the foal both fructose and glucose are present in the foetal circulation. Fresh cord blood was not readily obtained and, at first, analyses were made on four samples within 12 hr of their collection over sodium fluoride. The results proved unusual, for while the mean cellular/plasma glucose ratio of 35% showed that

TABLE 5. Concentration of fructose and glucose in the whole blood, plasma and red cells of two foals at birth

		Concentration in whole blood (mg/100 ml.)	Concentration in plasma (mg/100 ml.)	Haematocrit reading	Calculated concentration in cells (mg/100 ml.)	Cell/plasma concentration ratio
Foal 1	Fructose	108 (4)	129 (3)	45½ (4)	83	65
	Glucose	54 (4)	84 (2)		18	21
Foal 2	Fructose	103 (4)	125 (3)	42 (4)	72	57
	Glucose	51 (4)	79 (2)		12	15

Note. But for the haematocrits, the values shown are those of the nearest whole number. The figures in parentheses denote the number of parallel estimations.

the glucose was largely confined to the plasma, the corresponding ratio for fructose (79·5%) demonstrated that this sugar was at high concentration within the cell. More recently, two cord samples have been collected over heparin at birth and handled at once with the ice technique. The findings (Table 5) are not completely in accord with the earlier (fluoride) results but this might be expected. However, it may be seen that the mean concentration of glucose within the cell was 15 mg/100 ml. when, simultaneously, the mean cellular fructose concentration was 77·5 mg/100 ml.

It cannot be concluded from these few results that there is no interference between glucose and fructose, so that while glucose exists at very low concentration within the cell, the partition of fructose remains undisturbed and similar to that of both fructose and glucose in the new-born pig. Nevertheless, it does appear that the erythrocyte of the new-born foal not only contains little glucose but it permits fructose and glucose to be distributed quite differently across the cell surface.

The rabbit and guinea-pig

Some results are given now rather to illustrate the application of these general findings than to demonstrate again that the partition of sugar can vary with age. Table 6 shows the concentration differences for glucose across the placenta of the rabbit and guinea-pig when both whole-blood and plasma sugar concentrations are compared. It can be seen that, consequent upon the high concentration of sugar in the foetal corpuscle and the low concentration

TABLE 6. Glucose concentration gradients across the placenta of the rabbit and guinea-pig

	Glucose concentration in whole blood (mg/100 ml.)		Whole-blood concentration gradient (mg/100 ml.)	Glucose concentration in plasma (mg/100 ml.)		Plasma concentration gradient (mg/100 ml.)
	Maternal circulation	Foetal circulation		Maternal circulation	Foetal circulation	
Rabbit *						
1	79	60	19	123	66	57
2	99	54	45	126	63	63
3	97	61	36	126	74	52
4	86	55	31	122	62	60
5	92	76	16	128	91	37
6	94	76	18	122	79	43
Mean	—	—	27·5	—	—	52
Guinea-pig†						
1	75	52	23	105	67	38
2	92	92	nil	135	104	31
3	60	50	10	86	57	29
4	52	47	5	73	50	23
5	49	44	5	66	46	20
6	97	85	12	128	95	33
7	64	64	nil	85	75	10
8	88	87	1	115	97	18
9	93	80	13	128	87	41
10	81	72	9	118	79	39
11	81	77	4	118	87	31
Mean	—	—	7·4	—	—	28·4
Guinea-pig‡						
12	87	71	16	114	83	31
13	94	84	10	127	93	34
Mean	—	—	13	—	—	32·5
Guinea-pig§						
14	59	49	10	85	49	36
15	59	47	12	83	55	28
16	75	37	38	102	40	62
17	80	74	6	116	77	39
18	106	97	9	136	102	34
Mean	—	—	15	—	—	39·8

* All the rabbits were killed on the 29th day of gestation.

† Guinea-pigs 1 to 11 were very near to term, the foetuses being large, active and well-covered with hair.

‡ Guinea-pigs 12 and 13 were less heavily pregnant.

§ Guinea-pigs 14 to 18 had the youngest foetuses: well-developed but almost hairless and not active on exposure.

of sugar in the adult corpuscle, the concentration gradients as shown by the plasma glucose values greatly exceed the gradients derived from the whole-blood figures. It would seem possible that occasionally in these species the whole-blood figures might show a reverse gradient (the foetal circulation having the higher sugar concentration), but this has not yet been observed. However, in two of the eighteen guinea-pigs examined there was no difference between the whole-blood sugar concentrations on each side of the placenta.

The concentrations listed in Table 6 are probably close to those that occur in the physiological resting state. The pregnant animals were stunned with very little warning and the maternal blood samples taken within a few seconds. As many foetuses as were necessary to produce an adequate volume of pooled blood were then rapidly removed and bled by decapitation. The average time between stunning the mother and sampling the last foetus was just over $2\frac{1}{2}$ min for the rabbits and just under $3\frac{1}{2}$ min for the guinea-pigs. The maternal values are the result of two or more parallel determinations but only rarely did the volume of foetal blood allow an estimation to be repeated.

DISCUSSION

The permeability of red cells to sugar may change under non-physiological environmental conditions. In this investigation, however, the corpuscular behaviour was observed with the red cells in their normal complex medium. Furthermore, as the blood-sugar concentrations were within the narrow limits usual to each species, it is probable that the sugar was distributed according to the conditions that obtain in the steady state. If, as is believed, the brief cooling and centrifuging of the blood after sampling does not significantly redistribute the sugar across the cell surface, then the present results indicate the concentrations of reducing material as they occur in the plasma and whole blood of different species *in vivo*.

The proportion of this reducing material that is due to fructose and glucose cannot be known precisely, for the methods used to estimate both sugars are non-specific. It is generally held, however, that the estimation of glucose is more likely to include non-hexose material and hence the figures quoted for glucose concentration should, more accurately, refer to reducing substances. While the amount of non-hexose reducing material included in the glucose values is probably small (witness the low values of apparent glucose associated with the corpuscles of the adult pig) it could nevertheless be important in the interpretation of those results where the corpuscular concentration of total reducing substances approached zero.

The lack of specificity in estimating glucose does not affect the main conclusion of this work. The material recorded as hexose by routine analytical methods is distributed between the red cells and plasma in different species,

and also at different ages within a single species, in markedly different proportions. It follows that comparisons made between the sugar concentrations of whole-blood samples may not always be valid. Such is the case in several species when the sugar concentration gradient across the placenta is under consideration, for then the partition of sugar in the compared samples is most dissimilar. The period after birth could also invoke misunderstanding, for the decrease that commonly occurs in the whole-blood sugar concentration, largely as a result of the shift in the partition of sugar (from about 100 to 70 mg/100 ml. in the pig by the second month of life), might be attributed to some other concurrent but unrelated developmental change.

Previous observations on the partition of sugar in the blood of young animals appear to be few. Andreen-Svedberg (1933) obtained a mean corpuscular/plasma glucose-concentration ratio of 59% in some young calves and 42% in a 5-day-old pig. Hitchcock (1949) studied the distribution of fructose and glucose in the blood of the foetal lamb and found the cellular fructose concentration to be 73–84% of the plasma fructose concentration. The cellular glucose concentration, however, was always higher than the plasma glucose concentration. The latter type of partition has rarely been seen in this work and then only in blood containing fructose in addition to glucose (some of the foetal pig samples). In these few instances the corpuscular glucose concentration exceeded the plasma glucose concentration by but a few mg/100 ml., and as most samples from the foetal pig showed the plasma glucose concentration to be the higher, it has been assumed that the reverse condition is an artifact arising from the difficulty of estimating glucose in the presence of fructose. The results now presented show that in many new-born animals (the pig, lamb, calf, guinea-pig and rabbit) the concentration of glucose in the corpuscles is high. In the two species (foal and pig) where the distribution of fructose was examined, the cellular concentration of this sugar was likewise high. The difference between the cellular and plasma glucose concentrations is sufficiently small in the new-born pig and lamb to suggest that the rate of cellular glucose metabolism is not comparable with the rate of cell penetration, with the result that here the sugar may be in nearly equal concentration in the water phase of the cells and plasma, as seems to be the case with human blood (MacKay, 1932). In the absence of knowledge on the water content of neonatal corpuscles, however, this point cannot be pursued, nor can the slightly lower corpuscular/plasma glucose-ratio of the new-born calves be evaluated. The erythrocytes of the new-born foal are not of the type usually seen in young animals; on the contrary, they exhibit the cellular characteristics of the non-primate adult to a particularly marked degree.

The distribution of sugar in the blood of adults has been studied by many authors and the literature from 1877 until 1932 has been discussed in great detail by Andreen-Svedberg (1933). The work of others will be referred to here,

however, only to illustrate specific points. In this study, the corpuscular glucose concentration has been found to be low in all the adult species examined (the pig, ox, sheep, horse, guinea-pig and rabbit), but there are distinct species differences in the extent to which this concentration of glucose approaches zero. Thus the pig gives a consistently lower reading than the ox and sheep, a fact that can also be seen from the results of Andreen-Svedberg (1933) and Somogyi (1933). It might be concluded that the very low corpuscular glucose concentration in the pig is due to the erythrocytes being impermeable to glucose, and this has been the deduction of several authors, but it is more questionable whether this argument can be extended to the ruminants. Recent work on the extent to which the plasma is retained between the corpuscles when human blood is centrifuged (Chaplin & Mollison, 1952; Vazquez, Newerly, Yalow & Berson, 1954) gives, with the centrifugal force used here, a figure of about 2–3%. For the glucose of ruminant corpuscles to be due to retained plasma, the latter would need to constitute over 20% of the packed cell volume and it is difficult to agree with Somogyi (1933) that the corpuscular glucose of the sheep and ox is due solely to the retained plasma. Whether this apparent glucose is actually within the cells or adsorbed on to them, as Andreen-Svedberg (1933) believed, is another question and clearly the matter will not be resolved until techniques are employed that will both recognize glucose specifically and differentiate intracellular from adsorbed and extracellular sugar.

As there is no evidence that erythrocytes can effect a greater concentration of sugar inside the cell than is present in the environment, there are two possible extremes in the pattern of sugar partition. The first is when the sugar can attain equal concentration in the water phase of the cell and plasma, and the second is when the cell is completely impermeable to sugar. With regard to the partition of glucose, therefore, there appears to be a general tendency for the red cells of animals to change from a point near the first extreme to a point near the second. Such a change was reported by Hitchcock (1949) for the young lamb. It would be interesting to know whether the erythrocytes of the foetal foal contain a high concentration of glucose during early gestation or whether they never conform to the usual 'foetal' pattern. As it is well established that in primate corpuscles the intracellular sugar remains at high concentration in adult life, the change that occurs in the permeability of the human red cell with age (Widdas, 1955) is apparently insufficient to affect materially the corpuscular glucose concentration.

The simplest explanation of the slow change in sugar partition after birth is that there are two main cell types and that the 'foetal' type cell is progressively replaced by the 'adult' type cell (Goodwin, 1954). The blood of the young guinea-pig appeared to contain two cell populations of these types when examined by Widdas (1955), and the work of Goodwin & Coombs (1956)

also lends support to the idea of cell substitution. These authors found that the red cells of the new-born pig lack the group A antigen at birth but that during the first month of life it appears in increasing strength. When the antigen is first detected only small clumps of cells are agglutinated by anti-A serum and most of the cells appear unaffected. During the period when the cellular behaviour to glucose is progressively changing, the proportion of red cells that is agglutinated by anti-A serum also increases until, by the time the pig is 1–2 months old, almost the whole cell population behaves (both serologically and with respect to glucose distribution) as in the adult.

Studies on the permeability of red cells to hexoses differ in important respects from distribution studies, but it is nevertheless understandable that several authors have been concerned to reconcile the main conclusions of these two approaches. Foetal cells have been the subject of investigation only very recently, and hence the debate has centred on the permeability and distribution characteristics of the erythrocytes in adults. Here there is a superficial incompatability in the results. Permeability experiments have shown the erythrocytes of most non-primate adults to be impermeable to glucose *in vitro*. (The earlier literature has been reviewed by Andreen-Svedberg.) Distribution studies, however, show that glucose is associated with the cells, and this led Andreen-Svedberg (1933) to the conclusion that 'there must be glucose content without glucose permeability'. The same author pointed out, however, that the permeability investigations had not excluded the possibility that the cells were, in fact, very slowly permeable to glucose rather than completely impermeable, and the similar caution counselled by Widdas (1955) can now be considered in the light of the work of Morgan, Kalman, Post & Park (1955) who, by inhibiting utilization in the rabbit erythrocyte, have shown that glucose can enter the cell and accumulate there.

SUMMARY

1. Observations have been made on the distribution of glucose between the red cells and plasma in foetal, neonatal and adult blood from several species of mammals; horse, pig, ox, sheep, rabbit and guinea-pig. In the young of two species, the horse and pig, the distribution of fructose was also observed.

2. The material customarily estimated as glucose may be distributed differently in different species of adults and this variation in partition is often very marked in the blood of the same species at different ages.

3. Seliwanoff-positive material (taken as fructose) was at high concentration within the red cell, not only when the associated glucose showed a similar distribution ratio (as in the foetal pig) but also when the corpuscular glucose concentration was low (as in the new-born foal).

4. In general, new-born animals have a high corpuscular glucose concentration and this concentration decreases during the neonatal period to the

low levels that are characteristic of the adult. The horse is unusual, however, for the new-born foal has a very low corpuscular glucose concentration.

5. It is concluded that comparisons between the concentrations of sugar in whole-blood samples from different species or, particularly, from animals of different ages may not always be valid.

6. Some other implications of these findings are discussed.

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