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BLOOD SUGARS AND TISSUE CARBOHYDRATE IN FOETAL AND INFANT LAMBS AND RHESUS MONKEYS

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During a previous investigation (Dawes, Mott & Shelley, 1959), the carbohydrate content of various tissues from foetal lambs just over half-way through gestation was determined for comparison with that of foetuses near term. The carbohydrate concentrations in the heart, liver, lungs and skeletal muscle were very different in the two groups, and the work has now been extended to include lambs of earlier and intermediate ages so as to follow the changes in carbohydrate concentration throughout the last two-thirds of gestation. Large amounts of glycogen were laid down towards the end of gestation, not only in the liver but also in skeletal muscle, and measurements of tissue carbohydrate have been made in new-born and infant lambs of up to 17 days old so as to determine its fate after birth. The lamb is relatively mature at birth and exceptionally active, and the results suggest that both the liver and the skeletal muscle carbohydrate constitute carbohydrate reserves for use in the first 24 hr of extra-uterine life. The rhesus monkey is also fairly mature at birth, and estimates of tissue carbohydrate have been obtained in this species during the last third of gestation and in infant monkeys 8–15 days old. This paper compares the results obtained on lambs and monkeys with those in other species and discusses their possible significance. A preliminary account of this work has already been published (Shelley, 1960).

METHODS

Experimental procedure and animals

Foetal lambs. Observations have been made on forty-six foetal lambs (gestation age 58–146 days) from twenty-four cross-bred ewes; eight ewes carried single lambs, eleven carried twins, four carried triplets and one ewe carried quadruplets and developed signs of pregnancy toxaemia a few days before the experiment.

The lambs were delivered by Caesarean section under chloralose or pentobarbitone anaesthesia and were left attached to the ewe, care being taken to avoid stretching or compressing the umbilical cord. A foetal blood sample (0·8 ml.) was taken into a 1 ml. syringe, the dead space of which had been filled with 0·4% heparin in sodium fluoride

solution 8% (w/v), either from an arterial catheter or from the heart. A maternal blood sample had usually been taken a few minutes earlier by direct jugular puncture before anaesthesia or from a catheter in a forelimb artery after beginning anaesthesia. Foetal tissue samples (100–200 mg) were taken as soon as possible after delivery. In nine experiments the order of sampling was: skeletal muscle (lateral aspect of thigh), liver, lung (apex of left upper lobe), heart (apex of ventricles), cerebral cortex and kidney cortex. Brain and kidney samples were not taken from the remaining foetuses and in ten experiments the order of sampling was varied; the time interval between sampling different tissues did not exceed 2 min and the order of sampling did not appear to affect the results, though, since cardiac glycogen is very readily depleted in anoxia, it was considered advisable to sample the heart first. Samples of one or two tissues only were taken from each of another eleven lambs and blood samples only from the remaining sixteen since these were later asphyxiated by tying the umbilical cord and could not then be regarded as normal material (Dawes *et al.* 1959). In eight experiments blood and tissue samples were not obtained until at least one sibling had been asphyxiated, but during this period (1–2 hr) the lamb had remained *in utero*.

At the end of each experiment the foetus was weighed. Individual organs, including total skeletal muscle, were also weighed in nine experiments on lambs of 85–138 days gestation age; the results obtained, when expressed as percentage of body weight, were similar to those of Carlyle (1948) and Wallace (1948) and their data were therefore used to calculate the organ weights in the remaining foetuses.

Infant lambs. Venous blood and tissue samples were obtained from six lambs 2½–24 hr after normal delivery and from nine lambs 5–17 days old, all under pentobarbitone anaesthesia. Individual organs and total skeletal muscle were weighed in all experiments but one. Blood samples and skeletal muscle (usually semitendinosus) only were also taken from seven lambs under chloralose anaesthesia and from three lambs under local anaesthesia (xylocaine) at ages varying from 3 hr to 12 days after birth. In three other experiments muscle and blood samples were taken from the same lamb at different ages under local anaesthesia, final samples being obtained under chloralose anaesthesia. The lambs included four singletons, two from different sets of twins, seven complete sets of twins, two from one set of triplets and two complete sets of triplets. They had been housed in unheated premises with their dams up to the day of the last sample and were usually weighed on the day of the experiment.

Monkeys. Blood and tissue samples were obtained from twelve foetal rhesus monkeys (gestation age 115–158 days) and from three of their mothers, from seven infant monkeys (8–15 days old) hand-reared at 30° C and from two adolescent monkeys (1–2 years old), all under pentobarbitone anaesthesia. Blood samples were obtained from the foetal and six of the infant monkeys 1–2 hr after anaesthesia and tissue samples after 2–4 hr anaesthesia; these monkeys were being used for other studies (Dawes, Jacobson, Mott & Shelley, 1960), hence the delay. However, in all twelve foetal experiments the blood samples were taken while the foetus was still *in utero* (either from a leg withdrawn through a small uterine incision or from a carotid artery dissected *in utero*). In seven experiments the foetus was retained *in utero* throughout, and the tissues were sampled immediately after delivery by Caesarean section, while the foetus was still in good condition and attached to the mother by the umbilical cord; in one experiment the tissues were sampled 30 min after delivery but with the cord still intact, and in four others the foetuses (116–145 days gestation age) were deliberately asphyxiated by clamping and dividing the umbilical cord after delivery. Samples were also obtained, without anaesthetic, from a thirteenth foetus (gestation age 117 days) which had been born vaginally in a partially asphyxiated state. Two of the infant monkeys had been made anoxic by giving them 100% nitrogen to breathe and one infant was apnoeic for about 2 min shortly before the tissues were sampled. The adult monkey tissues were sampled after 4–5 hr of anaesthesia but samples were obtained from the adolescent monkeys and the

15-day-old infant within a few minutes of inducing anaesthesia. All the monkeys were singletons.

The sites of tissue sampling were similar to those described for lambs, except that biceps muscle was taken from the vaginally-born foetal monkey. Blood samples were taken from arterial catheters in the foetuses and two infants, from venous catheters in two infants, direct from the short saphenous vein in the adults, and direct from the heart in the adolescents and oldest infant. Total body weight and organ weights (liver, lungs and heart) were obtained for all the foetal monkeys, six infants and two adults.

Biochemical and histological methods

Blood. 0·2–0·5 ml. was deproteinized with NaOH and $ZnSO_4$ and the extract (dilution 1 in 10) was used for the determination of glucose (Huggett & Nixon, 1957), fructose (Bacon & Bell, 1948) and lactate (Barker & Summerson, 1941), except that in the sheep experiments lactate determinations were made on trichloroacetic acid extracts of blood.

Tissues. The tissue samples were divided into two equal portions. Within 10–20 sec of removal one portion was placed in McClung's picric acid-dioxane fix No. 2 (Cowdry, 1948), and was subsequently stained for glycogen by Best's carmine method, and the other was placed in 1·0 ml. deproteinizing solution (5 % trichloroacetic acid containing Ag_2SO_4 200 mg/100 ml.) in a weighed glass receptacle. This was reweighed immediately and the tissue was then chopped up finely with scissors and homogenized in a Potter–Elvehjem homogenizer. The homogenate was diluted to 5 ml. with deproteinizing solution, heated for 15 min in a boiling water-bath, cooled and centrifuged. The volume of the supernatant was adjusted to 5 ml. and was used for the determination of total carbohydrate, i.e. glycogen + glucose + fructose (Kemp & Kits van Heijningen, 1954; Mendel, Kemp & Myers, 1954), fructose and lactate.

RESULTS

Term occurs at about the 147th day of gestation in sheep and the 168th day in rhesus monkeys. Thus there is comparatively little difference in the length of the gestation period, and in Figs. 1 and 3–7 the results have been plotted against the number of days before term rather than against gestational age, since this made possible a closer comparison between the species. Lactate determinations were made on most of the blood and tissue samples as a check on the degree of hypoxia produced by the experimental conditions.

Blood sugars

Glucose. In Fig. 1 the blood glucose concentration has been plotted against age for lambs (○) from 58 to 138 days gestation age (89–9 days before term) and 2½ hr to 17 days after birth, and for monkeys (●) from 115 to 158 days gestation age (53–10 days before term) and 8–15 days after birth. Figure 1 includes not only results from the unasphyxiated foetal lambs which were used for the present tissue-carbohydrate study but also those obtained under similar conditions from their siblings before these were asphyxiated (see Methods); the values shown for foetal and infant monkeys were also obtained before any were made anoxic. It can be seen that in general the foetal blood glucose level was lower in the lambs than

in the monkeys and that there was no change with increasing gestational age in either species, though there was a wide scatter in both species, 3–32 mg/100 ml. in the lambs and 20–36 mg/100 ml. in the monkeys. In the lambs this scatter existed among litter-mates and was partly associated with the order of delivery and the duration of the experiment, since, in thirteen of fifteen experiments on sheep with multiple pregnancies, the foetal blood glucose was lower in the second or third members of sets of twins or triplets than in that delivered first. This difference was slight, less

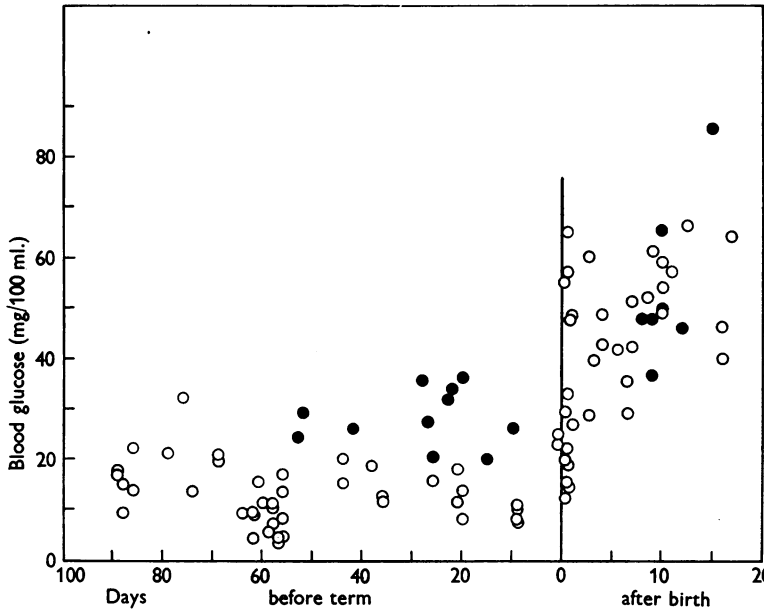

Fig. 1. Changes in blood glucose concentration with age in foetal and infant lambs (○) and monkeys (●). The results have been plotted against days before term for the foetuses and days after birth for the infants; the vertical line represents the time of birth.

than 2 mg/100 ml., when the foetuses were delivered within 20 min of each other but was sometimes considerable (5–10 mg/100 ml.) when the time interval was longer. The very low levels, less than 8 mg/100 ml., were all observed in foetuses from multiple pregnancies delivered after several hours anaesthesia.

Since it is known that anoxia affects the blood glucose level in foetal lambs and monkeys (Dawes *et al.* 1959; 1960), and since hypoxia is easy to produce unintentionally under these conditions, the lactate content of the foetal blood samples was considered in relation to their glucose content. Although, in the foetal monkeys, the highest blood glucose levels were associated with relatively high lactate levels which had decreased an hour

later, in all but one the lactate levels were less than 20 mg/100 ml., similar to those observed in the well-oxygenated infant monkeys, and were associated with O_2 saturations of 54–77 % (Dawes *et al.* 1960, Table 1). Thus the degree of hypoxia, when present, was slight, possibly because these blood samples had been taken while the foetus was still *in utero*. In the foetal lambs the blood samples had been taken after delivery, though with the umbilical cord intact, and the lactate levels were rather higher than in the monkeys. Nevertheless, in all but four of the younger lambs (up to 91 days gestation age), the blood lactate was less than 30 mg/100 ml. and in only two was there more than 40 mg/100 ml. In the older lambs (111–146 days

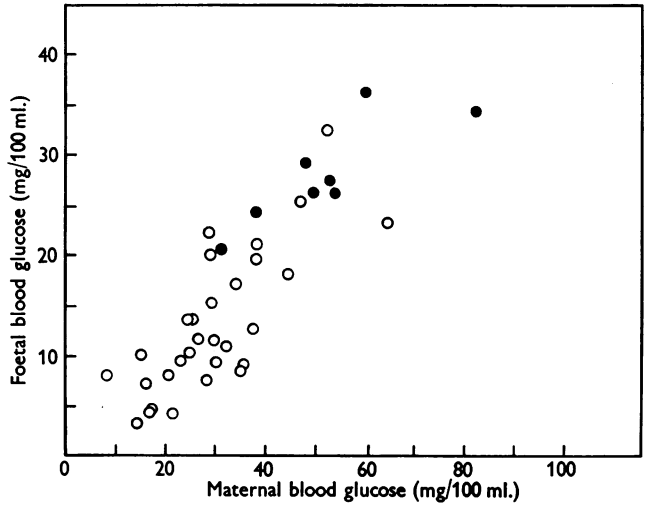

Fig. 2. Foetal blood glucose plotted against maternal blood glucose in lambs (○) and monkeys (●).

gestation age) evidence of hypoxia was present more often, and in all but three the blood lactate was more than 30 mg/100 ml. However, there was no correlation between blood lactate and glucose in the lambs, perhaps because in this species anoxia has a relatively slight effect on foetal blood glucose (Dawes *et al.* 1959). Only in two lambs (146 days gestation age) with blood lactates of 80–90 mg/100 ml. and blood glucose levels of 23–25 mg/100 ml. was there evidence of hyperglycaemia associated with a high blood lactate; a third foetus (127 days gestation age) with a blood lactate of 79 mg/100 ml. had a blood glucose of only 14 mg/100 ml. A much younger foetus (71 days gestation age) with the exceptionally high blood glucose of 32 mg/100 ml. had a blood lactate of only 27 mg/100 ml.

Since the wide scatter in foetal blood glucose levels could not be correlated with blood lactate, it was next examined in relation to maternal

blood glucose. Figure 2, in which foetal blood glucose has been plotted against the corresponding maternal level, shows that there is a close relationship, the foetal blood glucose being maintained at about half the maternal level in both species. The relatively high blood glucose levels of 23–32 mg/100 ml. observed in three lambs were associated with the three highest maternal blood glucose levels of 47–65 mg/100 ml. and were within the same range as the monkeys. Moreover, since in the sheep the maternal blood glucose nearly always fell by 4–17 mg/100 ml. during the

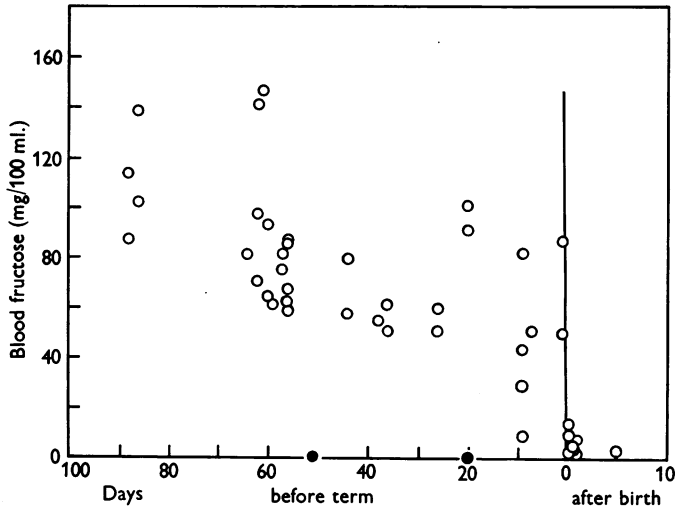

Fig. 3. Changes in blood fructose concentration with age in foetal and infant lambs (○) and monkeys (●). Conventions as for Fig. 1.

experiment, this would explain the low blood glucose levels observed in the second or third members of sets of twins or triplets and their inverse relationship to the period of anaesthesia.

Figure 1 also shows that the blood glucose in the infant lambs and monkeys was higher than in the foetuses. In five lambs the blood glucose was 48–65 mg/100 ml. within 24 hr of birth, but nine lambs less than 24 hr old had levels below 35 mg/100 ml., all but one lamb 2–7 days old had blood glucose levels of 29–49 mg/100 ml., and the older lambs had levels of 40–66 mg/100 ml. This suggests that the blood glucose was rising steadily over the first 10–15 days after birth and this was supported by observations on individual lambs. Thus in three experiments where samples were taken from the same lamb at different ages, the mean blood glucose levels on the first, third and twelfth days after birth were 21·5, 42·3 and 61·5 mg/100 ml. Although the blood glucose level in the foetal monkeys was about twice that in the foetal lambs, the levels in the infant monkeys 8–12 days old were usually lower than in lambs of the same age and were

similar to those observed in the adult pregnant monkeys (Fig. 2). Higher values, 85–90 mg/100 ml., were observed in the 15-day infant and the adolescent monkeys but these could have been due to excitement, as there was some delay between catching the monkeys and taking the samples.

Fructose. In confirmation of Hitchcock (1949), Barklay, Haas, Huggett, King & Rowley (1949) and Huggett, Warren & Warren (1951), the blood fructose level in the foetal lambs was very much higher than either the foetal or maternal blood glucose; it declined slowly with increasing gestational age and fell to very low values within a few hours of birth (○, Fig. 3). The wide range of blood fructose values at any given age could not be correlated with either foetal blood lactate or maternal blood glucose, though the lowest level recorded, 8 mg/100 ml. at 138 days gestation age, was associated with the exceptionally low maternal blood glucose of 8 mg/100 ml. Moreover, it was frequently observed that the blood fructose levels in the second and third members of sets of twins and triplets, sampled after a longer period of anaesthesia, were higher than in the first. Chinard, Danesino, Hartmann, Huggett, Paul & Reynolds (1956) have shown that there are negligible amounts of fructose in the blood of foetal rhesus monkeys; none was detected in the blood of two foetal monkeys in the present series (117 and 148 days gestation age, ●, Fig. 3).

Tissue carbohydrate

The method used to measure tissue carbohydrate (Kemp & Kits van Heijningen, 1954), gives an estimate of the total carbohydrate present, the chief constituents of which are glycogen, glucose and fructose (Mendel *et al.* 1954). The fructose content of the tissues from foetal lambs was determined separately but, as indicated earlier (Dawes *et al.* 1959), the amounts present, less than 2 mg/g, were usually negligible compared to the total carbohydrate; and since fructose was not detected in the blood of the foetal monkeys, it was assumed that the tissue fructose in this species would be even lower than in the lambs. Histological examination of the tissues suggested that their glycogen content was proportional to their total carbohydrate and it is therefore probable that the changes shown in Figs. 4–7 took place mainly in the glycogen fraction.

Liver. Figure 4 shows the changes in liver carbohydrate with age in foetal and infant lambs (○) and monkeys (●). Lambs of 58–90 days gestation age (89–57 days before term) had relatively little liver carbohydrate, less than 14 mg/g, but thereafter there was a rapid increase, levels of 60 and 98 mg/g being observed in two lambs at 138 days gestation age. The lowest values, less than 8 mg/g at 62–56 days before term, were all obtained from lambs with a low blood glucose after several hours anaesthesia; it was observed that their siblings, sampled earlier, usually had

slightly more liver carbohydrate even though they had been asphyxiated by tying the umbilical cord (Dawes *et al.* 1959). The results obtained from the foetal monkeys suggest that, as in the lambs, liver carbohydrate is high (more than 60 mg/g) by the 115th day of gestation, but since the gestation period is about 21 days longer than in the sheep the level is very much higher than in lambs at this time interval (53 days) before term. The data obtained from the infant lambs suggest that liver carbohydrate falls to less than 20 mg/g within a few hours of birth but is rising again towards the adult level by 5 days of age. The values obtained from the

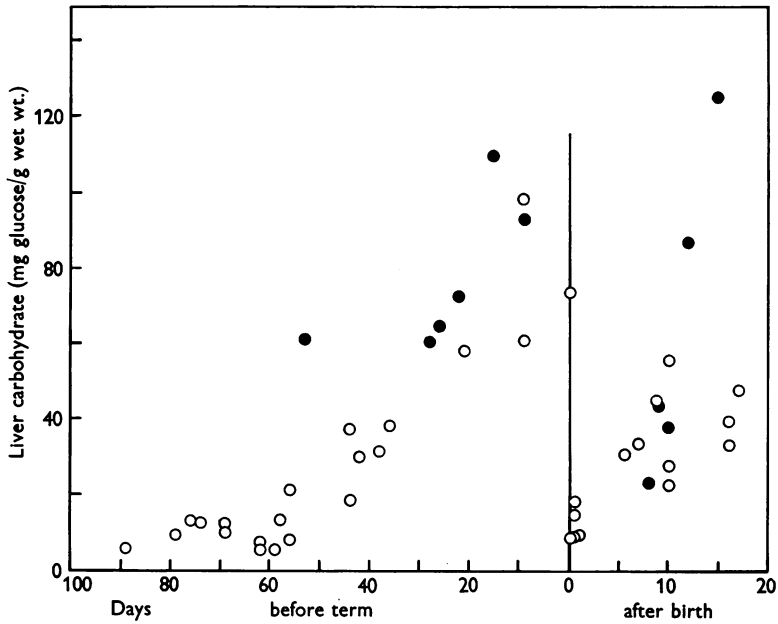

Fig. 4. Changes in liver carbohydrate with age in foetal and infant lambs (○) and monkeys (●). Conventions as for Fig. 1.

infant monkeys are probably low, since they were obtained after a 2–3 hr experiment during which a hyperglycaemia of +37–62 mg/100 ml. had occurred, but they suggest that, in this species, liver carbohydrate may rise very steeply at 8–15 days after birth and may regain the foetal level. Liver carbohydrate levels of 21 and 25 mg/g were observed in the adolescent monkeys and 34 and 46 mg/g in two of the adult pregnant monkeys; the third adult was discovered at autopsy to have a severe chronic respiratory infection, was in poor condition and had a liver carbohydrate of only 8·5 mg/g.

Data from lambs and monkeys where there was evidence of hypoxia (high blood lactates and liver lactates of more than 0·8 mg/g in lambs and

more than 0·4 mg/g in monkeys) have been excluded, since in these animals, all in the last third of gestation, the liver carbohydrate was usually low compared with the values in Fig. 4. Thus two lambs (127 and 146 days gestation age) with blood lactates of 80–90 mg/100 ml. had liver carbohydrates of only 13·0 and 16·3 mg/g, even lower than the values of 26–45 mg/g observed in lambs of this age asphyxiated by tying the umbilical cord (Dawes *et al.* 1959). Similarly three foetal monkeys (one from the adult with a respiratory infection, one where the mother had had an apnoeic episode and the one born vaginally at 117 days gestation), where there was evidence of hypoxia, had liver carbohydrates of 24–42 mg/g, whereas those asphyxiated deliberately by dividing the cord had liver carbohydrates of 41–73 mg/g; a fourth monkey (No. 110, Dawes *et al.* 1960), where the cord was divided after a long period of hypoxia, had a liver carbohydrate of only 15 mg/g. The two infant monkeys breathing 100 % nitrogen had terminal liver carbohydrate levels of 37 and 53 mg/g, similar to values observed in unasphyxiated infants of the same age.

Liver fructose fell slightly with increasing age; it was 1·6 mg/g in two lambs of 89 and 91 days gestation age and 0·5–1·3 mg/g in four lambs of 126–138 days gestation age.

Lungs. Dawes *et al.* (1959) found that the lung carbohydrate in lambs asphyxiated by tying the umbilical cord was of the same order as that in their unasphyxiated siblings, and a similar conclusion was reached in the work on foetal and infant monkeys (Dawes *et al.* 1960). Although the values shown in Fig. 5 for foetal lambs were all obtained from unasphyxiated animals, the number of unasphyxiated monkeys available was small and Fig. 5 shows results obtained on both asphyxiated and unasphyxiated monkeys. In the foetal lambs (○), lung carbohydrate rose from less than 15 mg/g at 58–71 days gestation age (89–76 days before term) to peak values of 16–27 mg/g at 85–109 days gestation age, after which it declined rapidly to less than 6 mg/g near term. High values, 14–19 mg/g, were observed in the foetal monkeys (●) at 115–117 days gestation age and there was a rapid decline to less than 8 mg/g at approximately the same time interval before term, as in the lambs, though at a slightly older gestational age. The lung carbohydrate concentration in the 5–17-day-old lambs and the infant monkeys was even lower, less than 4 mg/g, and values of 0·7–1·7 mg/g were observed in the adolescent and adult monkeys.

In the lambs lung fructose fell from 1–2 mg/g at 61–91 days gestation age to 0·5–1·1 mg/g at 126–138 days gestation age.

Heart. Figure 6 shows that the lambs (○) of less than 100 days gestation age (more than 47 days before term) had cardiac carbohydrates of 20–40 mg/g with a peak value at about 91 days gestation age; after this there was a decline to 14–22 mg/g nearer term, 11–21 mg/g in the new-born

lambs and 6–11 mg/g in the older lambs. A similar decline was observed in the monkeys (●) where the level was 23·5 mg/g at 115 days gestation age (53 days before term), 10–19 mg/g nearer term and 4–5 mg/g in the infants and adults. Since it is known that cardiac glycogen is very rapidly depleted in anoxia (Dawes *et al.* 1959, 1960), care has been taken to exclude from Fig. 6 all data where there was evidence of hypoxia. The lambs included in Fig. 6 had blood lactates of less than 42 mg/100 ml. (in all but two they were less than 30 mg/100 ml.) and all but one monkey had blood lactates of less than 20 mg/100 ml. The cardiac lactate concentration was

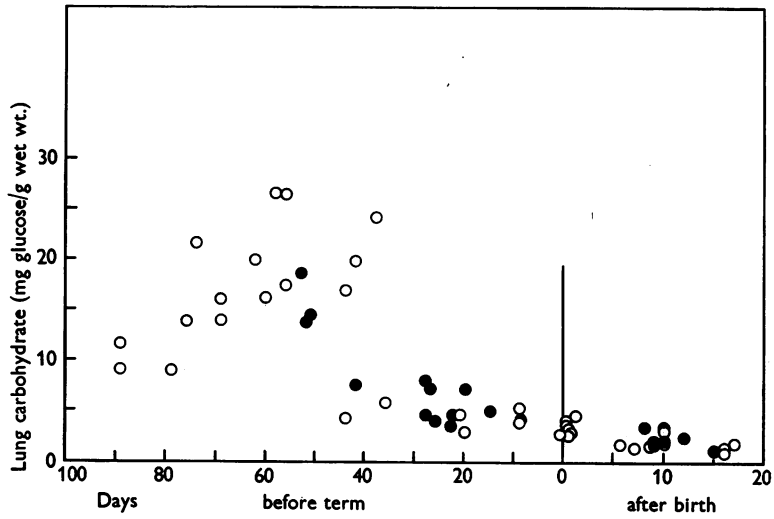

Fig. 5. Changes in lung carbohydrate with age in foetal and infant lambs (○) and monkeys (●). Conventions as for Fig. 1.

0·4–1·6 mg/g in the lambs (less than 1·0 mg/g in all but two) and 0·7–1·3 mg/g in the monkeys.

Cardiac fructose was 1·1–1·2 mg/g in the younger lambs and 0·3–0·8 mg/g in lambs nearer term (126–138 days gestation age).

Skeletal muscle. Like lung carbohydrate, skeletal muscle carbohydrate is relatively unaffected by acute asphyxia in either lambs or monkeys (Dawes *et al.* 1959, 1960) and therefore Fig. 7, like Fig. 5, includes results from both asphyxiated and unasphyxiated monkeys, though those shown for lambs (○) were all obtained from unasphyxiated animals. In lambs of less than 90 days gestation age (more than 57 days before term) the muscle carbohydrate was less than 14 mg/g, but thereafter it increased rapidly and lambs of more than 100 days gestation age had muscle carbohydrates of 20–47 mg/g. Similarly, the monkeys (●) had muscle carbohydrates below 20 mg/g at 51–53 days before term, but nearer term the level in-

creased to 19–34 mg/g; thus the increase in muscle carbohydrate occurred at approximately the same time interval before term as in the lambs, but at a slightly older gestational age. The wide scatter in the older lambs existed not only among individuals from different ewes but also between litter-mates. In general, comparing lambs or monkeys of the same age, those with a high muscle carbohydrate tended to have higher carbohydrate levels in the other tissues, though there was no correlation with blood glucose concentration. It is noteworthy that the four lambs (gestation

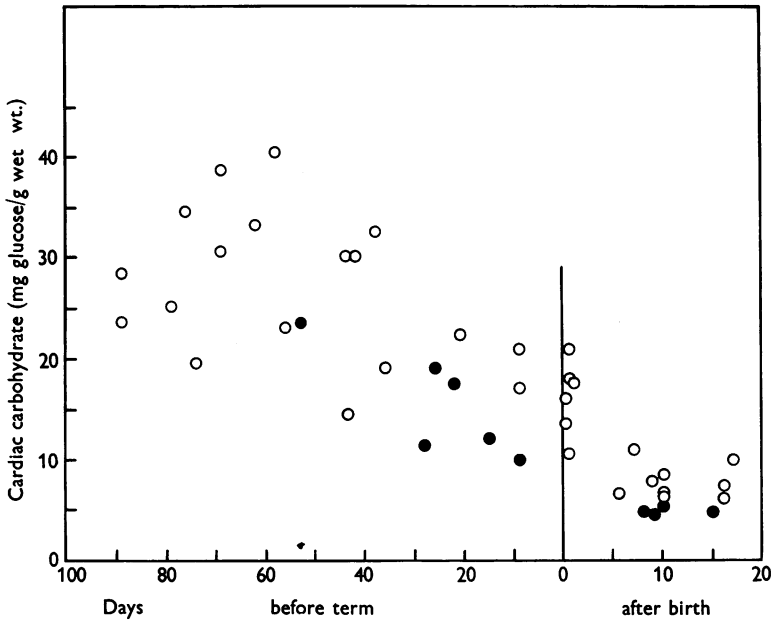

Fig. 6. Changes in cardiac carbohydrate with age in foetal and infant lambs (○) and monkeys (●). Conventions as for Fig. 1.

age 140 days) from the ewe with pregnancy toxaemia had muscle carbohydrates within the normal range.

Figure 7 also illustrates the dramatic fall in muscle carbohydrate which occurred in the lambs after birth. In lambs 3–12 hr old it had already fallen to 11–27 mg/g and lambs more than 24 hr old had muscle carbohydrates of only 7–15 mg/g, similar to values obtained by the same method in adult sheep (Shelley, unpublished). In the three experiments where muscle biopsy samples were taken from the same lamb at different ages, the mean values observed on the first, third, seventh and twelfth days after birth were 19·6, 9·7, 11·7 and 10·9 mg/g and one of these had a muscle carbohydrate of only 7·1 mg/g 41 days after birth. Whereas one lamb from a set of triplets had a muscle carbohydrate of 26·8 mg/g 5 hr after birth, its

litter-mates had less than 8 mg/g on the third and fourth days after birth. The extremely low value of 2·3 mg/g was obtained from one 9-day-old lamb and is of interest because this lamb was in poor physical condition with signs of muscular weakness. With one exception the 8–15-day-old monkeys had muscle carbohydrates within the same range as lambs of this age, slightly higher than the values of 5–9 mg/g observed in the adolescent and adult monkeys. The one exception (No. 101, Dawes *et al.* 1960), where the level was 18·6 mg/g 12 days after birth, is noteworthy in that this monkey had been born prematurely at 150 days gestation age and, though

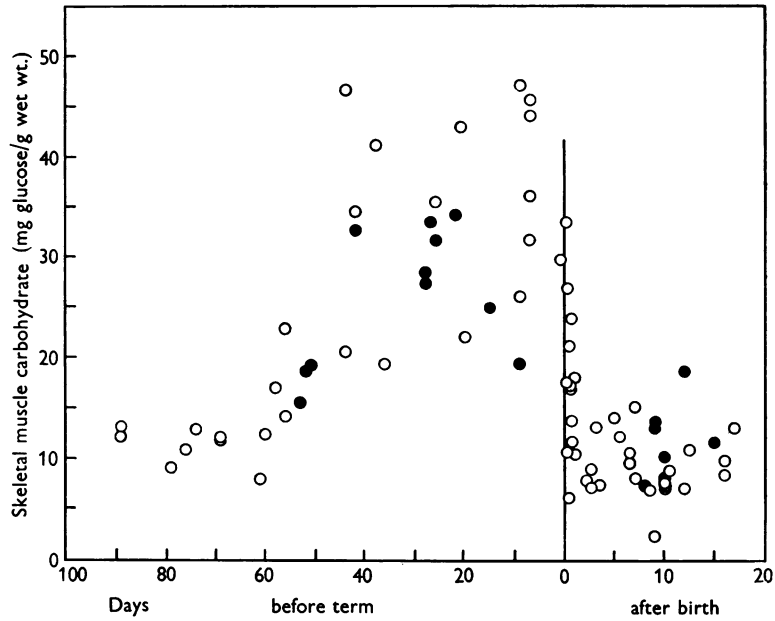

Fig. 7. Changes in skeletal muscle carbohydrate with age in foetal and infant lambs (○) and monkeys (●). Conventions as for Fig. 1.

in good condition, was smaller and possibly less active than the other infants. Thus in the monkeys, as in the lambs, muscle carbohydrate probably fell after birth.

Skeletal muscle fructose was 1·1–1·5 mg/g in the younger foetal lambs, 0·3–0·9 mg/g in lambs of 126–138 days gestation age and less than 0·3 mg/g in two lambs 3 and 10 hr after birth.

Other tissues. There was no change in either brain or kidney carbohydrate with increasing age in the foetal lambs. Thus the range for brain carbohydrate was 0·5–1·4 mg/g in lambs of 83–91 days gestation age and 0·5–1·6 mg/g at 111–146 days gestation age; the corresponding values for brain fructose were 0·4–0·6 mg/g and 0·3–0·6 mg/g in the two groups.

Slightly higher values were obtained for kidney carbohydrate, 2·8–4·6 mg/g in the younger lambs and 3·5–5·8 mg/g in the older lambs; the corresponding fructose concentrations were 0·9–1·7 mg/g and 0·6–2·0 mg/g.

Placental carbohydrate in the rhesus monkey probably falls in the last third of pregnancy, since there was 8·2 mg/g in the placenta from the one unasphyxiated foetus at 115 days gestation age and only 0·6–2·6 mg/g at 140–158 days gestation age. In one ewe at 144 days gestation age, where tissue samples were taken from various parts of the placenta, there was a mean carbohydrate concentration of 3 mg/g with 4·2 mg/g in the endometrium (H. B. Parry and H. J. Shelley, unpublished).

Total body carbohydrate. The total carbohydrate content of the liver, lungs, heart, skeletal muscle, brain and kidneys and the glucose content of the blood were calculated for each of nine foetal lambs from either known or calculated organ weights, taking the blood volume as 11–15 % of body weight depending on gestational age (Carlyle, 1948; Wallace, 1948). The sum of these values gave an approximate figure for total body carbohydrate, since it is probable that these tissues contain the greater part of the carbohydrate present. In practice, since skeletal muscle accounted for 22–52 % of the body weight as compared to 2–9 % for the liver and even smaller percentages for the other organs, it was found that skeletal muscle carbohydrate accounted for 67–92 % of the total body carbohydrate, liver carbohydrate for up to 30 % and the other tissues for only 2–3 %, the contribution of the brain and kidneys being negligible, less than 1 %. Comparable values for total body carbohydrate were therefore obtained for the other foetal lambs, the infant lambs, and the foetal and infant monkeys where brain and kidney carbohydrate had not been measured; in the monkeys total skeletal muscle mass was taken as 30 % of body weight and blood volume as 10 % for foetal monkeys and 6 % for infant monkeys (Gregersen, Sear, Rawson, Shu Chien & Saiger, 1959). However, it must be emphasized that because these calculations assume that the carbohydrate was distributed evenly in each tissue, the values obtained are only approximations. Since skeletal muscle carbohydrate formed such a large percentage of the total, its distribution was investigated in two foetal lambs (127 days gestation age) in each of which samples from 11 different muscles were analysed; the variation from muscle to muscle was considerable, 14–40 mg/g in one lamb, 21–42 mg/g in the other, but in both lambs the mean value was more than 30 mg/g and was similar to the concentration in the semitendinosus, the muscle usually taken for analysis. Muscle carbohydrate distribution was also investigated in fourteen infant lambs 2½ hr–17 days old, in each of which samples from at least five muscles were analysed; again, the concentration in the semitendinosus did not differ greatly from the mean.

These calculations revealed several points of interest. First, with the increase in weight of the foetal lambs from less than 100 g at 58 days gestation age to 549–1108 g at 85–91 days gestation age there was a proportional increase in total body carbohydrate from less than 0·5 g/foetus to 3–6 g/foetus. Thereafter, however, mainly owing to the large increase in liver and skeletal-muscle carbohydrate, the total body carbohydrate increased out of all proportion to body weight, to 29–38 g/foetus in lambs weighing 2·3–4·4 kg at 126–146 days gestation age. Calculated in terms of body weight, the total carbohydrate content of the foetal lambs increased from 5·7–8·8 g/kg (mean 7·5 g/kg) at 58–91 days gestation age to 7·1–14·1 g/kg (mean 10·6 g/kg) at 103–146 days gestation age.

Secondly, considering those tissues where there was a decrease in carbohydrate concentration in the last third of gestation, whereas in the lungs there was a decrease in the total amount present, from 559–1420 mg in lambs of 85–109 days gestation age to 162–530 mg at 111–146 days gestation age, in the heart the increase in weight more than made up for the decrease in cardiac carbohydrate concentration and the total cardiac carbohydrate increased from 133–161 mg in lambs of 85–91 days gestation age to 305–395 mg at 103–146 days gestation age.

Thirdly, although the foetal monkeys were only a tenth of the weight of the mature foetal lambs and their absolute carbohydrate content was therefore very much smaller, when expressed per kilogram body weight their total body carbohydrate was almost identical with that of the lambs. Thus the level was 7·7 g/kg in the unasphyxiated monkey at 115 days gestation age but had increased to 9·0–12·6 g/kg (mean 10·5 g/kg) at 140–158 days gestation age. As in the lambs, the total lung carbohydrate decreased with increasing gestational age but, in contrast, there was no change in total cardiac carbohydrate.

Finally, the changes occurring after birth were of particular interest. Table 1 gives the mean absolute values for skeletal muscle, liver and total body carbohydrate in the mature foetal and the younger infant lambs and monkeys and, by subtraction, the mean differences in tissue carbohydrate between the foetuses and infants of each species. The infants of both species were directly comparable with the mature foetuses since little gain in weight had occurred within the first 24 hr of birth in the lambs or 8–10 days in the monkeys (Dawes *et al.* 1960). The results from one $2\frac{1}{2}$-hr-old lamb, born prematurely at 142 days gestation age, have been omitted, since in this lamb the liver and muscle carbohydrate were still within the foetal range (Figs. 4 and 7). Since the skeletal muscle carbohydrate represented 80 % of the total carbohydrate in the mature foetal lambs and 76 % of that in the foetal monkeys, and since the amount present in the infants was only 48–58 % of that in the foetuses, the decrease

in muscle carbohydrate after birth represented a loss of 42% of the total foetal carbohydrate in the lambs and 32% in the monkeys and was considerably greater than the total carbohydrate content of the liver, 2·4 times the foetal liver carbohydrate in the lambs and 1·5 times that in the monkeys. This suggests that the foetal skeletal muscle carbohydrate in these two species constitutes a carbohydrate store about half of which is used up shortly after birth, and that quantitatively it is probably more important than liver glycogen. Table 1 also emphasizes even further the similarity between the lambs and monkeys, since the decrease in total

TABLE 1. Total body carbohydrate in mature foetal and infant lambs and rhesus monkeys

		Tissue carbohydrate (g/foetus or infant)			Total carbohydrate	
	No. of animals	Muscle	Liver	Other tissues	(g/foetus or infant)	(g/kg body wt.)
Lambs:						
Foetus, 126–146 days gestation age	5	28·7*	6·4	0·8	35·9	10·3
Infant, 3–24 hr after birth	5	13·6†	1·1	0·7	15·4	4·1
Difference		15·1	5·3	0·1	20·5	6·2
Monkeys:						
Foetus, 140–158 days gestation age	6	3·1	0·9	0·1	4·1	10·5
Infant, 8–10 days after birth	5	1·8	0·6	0·1	2·5	4·4
Difference		1·3	0·3	0	1·6	6·1

The mean total body carbohydrate has been calculated in two ways: (1) in g/foetus or infant as the sum of the mean values for each tissue, and (2) in g/kg body wt. from the total body carbohydrate for each animal. * Mean of 9 observations; † mean of 11 observations.

carbohydrate in g/kg body weight and the final levels reached in the infants were almost identical in the two species; the final levels (4·1 and 4·4 g/kg) were considerably lower than any observed in the foetuses.

However, the lambs and monkeys did differ considerably in their rates of growth after birth. Whereas the lambs had almost doubled their birth weight within 8–10 days of birth and had quadrupled it by the middle of the third week, the monkeys were only about 25% heavier by 8–10 days of age (Dawes *et al.* 1960) and would have been only 30–40% heavier by the middle of the third week (H. N. Jacobson, personal communication). Since in both species the various organ weights increased in roughly the same proportion as total body weight, it can be seen that in the lambs, although the carbohydrate concentration in the skeletal muscle and liver did not regain the foetal level, the total amount laid down very soon exceeded that present in the mature foetus. It is possible that the much

steeper rise in liver carbohydrate concentration and its restoration to the foetal level in the infant monkeys (●, Fig. 4) was associated with the much slower rate of growth in this species as compared with the lambs.

DISCUSSION

Foetal tissue glycogen in different species

Although it is just over one hundred years since Claude Bernard (1859*a*) identified glycogen in foetal tissues and described qualitatively the changes in content occurring in the different tissues during gestation, there have been no attempts to make as detailed a quantitative survey. Nevertheless, sufficient information is available to suggest that changes in tissue glycogen similar to those observed in the foetal lambs and monkeys also occur in other species, though their time relationships with respect to parturition may vary from species to species.

Liver. Bernard was unable to detect glycogen in the livers of foetal lambs during the first half of gestation, but with improved methods of extraction Pflüger (1903) showed that a small amount, 7·9 mg/g, was present in the liver of a 2-month-old foetal lamb, an estimate similar to those for total carbohydrate obtained in lambs of this age in the present work. In the older lambs, from 100 days gestation age onwards, i.e. in the last third of gestation, the liver carbohydrate increased steadily (Fig. 4) until near term it was considerably higher than the normal adult level of 40 mg/g (Parry & Shelley, 1958). A similar trend was observed by Aron (1922), but his values are much lower than those reported here, possibly because he estimated glycogen, not total carbohydrate, and had partially asphyxiated the foetuses by killing their mothers before delivery, a circumstance which would be expected to deplete liver glycogen near term (Dawes *et al.* 1959); one advantage of the present method of carbohydrate analysis is that the products of glycogenolysis as far as glucose 1-phosphate and free glucose are all included in the assay (Mendel *et al.* 1954). Liver glycogen levels of up to 90 mg/g have been observed in new-born lambs (Roderick, Harshfield & Hawn, 1937), in good agreement with the present results on mature foetuses. In the foetal monkeys liver carbohydrate was present in large amounts at an earlier stage of gestation than in the lambs and near term the concentration was 92–109 mg/g (Fig. 4), again much higher than in the adults. In man foetal liver glycogen starts to rise during the second third of gestation (Szendi, 1936; Villee, 1953*a*, 1954) and values of 40–50 mg/g were observed at 105–140 days gestation age (Villee, 1954); reliable information on older human foetuses is lacking, but Szendi's results suggest that the liver glycogen increases steadily up to term.

Similar rises in liver glycogen in the latter part of gestation have been observed in other species, though it is possible that some of the values given for foetuses near term are low, since the mother was usually stunned or killed before delivering the foetuses. In another ungulate, the pig, foetal liver glycogen also begins to rise at 105–120 days gestation age (Mendel & Leavenworth, 1907; Aron, 1922), though since the gestation period is only 120 days, the rise occurs nearer term than in the lambs; the livers of new-born piglets contain 85 mg/g (McCance & Widdowson, 1959). Liver glycogen also rises late in gestation in rodents; in rats the rise occurs in the last fifth of gestation to a maximum of 106 mg/g at term (Corey, 1935*a*; Stuart & Higgins, 1935; Martínek & Mikuláš, 1954; Jacquot, 1955), in rabbits in the last quarter to about 40 mg/g at term (Lochhead & Cramer, 1908; Snyder & Hoskins, 1928; Szendi, 1936; Jost & Jacquot, 1955) and in guinea-pigs during the last fifth to about 30 mg/g at term (Aron, 1922; Hard, Reynolds & Winbury, 1944). Foetal liver glycogen is still rising in the last fifth of gestation in dogs (Schlossmann, 1938) and this work is of particular interest in that the foetuses were delivered under general anaesthesia as in the present work and values for liver total carbohydrate of up to 117 mg/g were obtained near term, similar to those in the lambs and monkeys and to the liver glycogen of new-born puppies (Demant, 1887); values of up to 97 mg/g were also observed in mature foetal guinea-pigs delivered under general anaesthesia (Shelley, unpublished), in contrast to the much lower values obtained by earlier workers (see above). Schlossmann also showed that the total carbohydrate content of the foetal livers exceeded their glycogen content by less than 1%, suggesting that the results obtained in the present work give a reliable estimate of liver glycogen.

Lungs. Lung carbohydrate is not depleted by acute asphyxia in foetal lambs (Dawes *et al.* 1959) and this may explain why previous observations on foetal lung glycogen are in better agreement with the present results than were those on liver glycogen. Calculation from the results of Fauré-Fremiet & Dragoiu (1923) on foetal lambs show that, as in the present work, peak values of up to 28·5 mg/g were observed just over half way through gestation, after which the level dropped abruptly. They confirmed Bernard's observations that the glycogen was concentrated in epithelial tissue and estimated that this must contain 75–78 mg/g; the fall in glycogen concentration was associated with structural alterations in the lungs during which the epithelial tissue disappeared. In man, also, lung glycogen is concentrated in the alveolar epithelium and reaches a peak value about half way through gestation (Szendi, 1936); the values of 27 mg/g at 19 weeks and 3 mg/g near term observed by Villee (1953*a*, 1954) are in excellent agreement with the peak and full-term values in the lambs (Fig. 5). Monkeys half way through gestation were not available but Fig. 5 shows

that lung carbohydrate was falling in the last third of gestation and, again, the fall was associated with the disappearance of the alveolar epithelium. In rabbits the peak lung glycogen concentration occurs rather late, in the last fifth of gestation (Szendi, 1936), but, as in the other species, it decreases to 3–4 mg/g near term.

Heart. Whereas all the species so far investigated have high liver glycogen and low lung glycogen concentrations at term, regardless of their relative maturity, cardiac glycogen appears to vary inversely with maturity. Thus in lambs and monkeys, which are able to stand or crawl within an hour of birth and are born covered with wool or fur and with their eyes open, the cardiac carbohydrate fell during the last third of gestation to 10–20 mg/g near term (Fig. 6): values of 30–40 mg/g had been observed in lambs just over half way through gestation, comparable to the cardiac glycogen in human foetuses in the first half of gestation (Villee, 1954). There are no quantitative estimates of cardiac carbohydrate in foetuses of other species, but new-born rats, which are relatively immature at birth, have 22–31 mg/g, new-born rabbits have 5–13 mg/g, and the exceptionally mature new-born guinea-pig has only 4–5 mg/g (Dawes *et al.* 1959), similar to the level in the infant and adult monkeys and in other adult mammals (Cruickshank, 1936); new-born piglets have 15 mg/g (McCance & Widdowson, 1959) and new-born kittens have 11–14 mg/g (Shelley, unpublished).

Skeletal muscle. Foetal skeletal muscle carbohydrate also appears to be related to maturity and it is tempting to argue that species which are capable of vigorous muscular activity soon after birth need larger amounts of muscle glycogen than those born in a less mature condition. Thus in the lambs and monkeys the muscle carbohydrate increased during the last third of gestation from less than 20 mg/g to 20–47 mg/g near term (Fig. 7). Similarly, carcass glycogen in foetal piglets increases to 11 mg/g near term (Mendel & Leavenworth, 1907), a value in excellent agreement with the calculated whole-body carbohydrate in the full-term lambs and monkeys; muscle from new-born piglets may contain more than 70 mg/g (McCance & Widdowson, 1959). Foetal guinea-pigs have a muscle carbohydrate concentration of 19–25 mg/g near term, but the less mature full-term foetal rabbit has only 13–17 mg/g (Shelley, unpublished) and the full-term foetal rat has a muscle glycogen of only 12 mg/g (Martínek & Mikuláš, 1954); foetal puppies near term have 14–21 mg/g (Schlossmann, 1938). Nevertheless, in all these species the foetal muscle carbohydrate concentration near term was at least twice the corresponding adult level.

Other tissues. The suggested decline in placental glycogen during the latter part of gestation in the monkeys would be in agreement with observations on man (Szendi, 1934), rats (Corey, 1935*a*) and rabbits (Lochhead & Cramer, 1908; Huggett, 1929; Loveland, Maurer & Snyder,

1931; Szendi, 1934) where the level begins to fall at about the time that foetal liver glycogen begins to rise. In sheep and cows the placenta is relatively poor in glycogen throughout gestation but glycogen-rich 'plaques' on the inner surface of the amnion and the umbilical cord disappear in the latter part of pregnancy, again at the stage at which foetal liver glycogen accumulates (Bernard, 1859*b*). In the foetal lambs' kidneys, the glycogen was concentrated in the tubular epithelium and, as in man (Villee, 1953*a*), remained relatively constant throughout the last two-thirds of gestation. Although in the lambs the carbohydrate content of the cerebral cortex did not appear to change with increasing gestational age, the amounts present were so small as to be near the limit of the method; Villee (1954) has observed a decrease in the glycogen content of human foetal brains in the first half of gestation.

Blood sugars. In the lambs and monkeys the foetal blood glucose was closely related to the maternal level (Fig. 2) and did not change with increasing gestational age (Fig. 1), but in other species, although the foetal level was always lower than the maternal level, a rise in foetal blood glucose was usually observed towards the end of gestation (Aron, 1923; Snyder & Hoskins, 1928; Britton, 1930; Schlossmann, 1931, 1938; Corey, 1932; Hard *et al.* 1944). It is possible that this rise was associated with accidental asphyxia at a time when large amounts of glycogen were present in the liver, a factor which was excluded as far as possible in the present work. Values of about 30 mg/100 ml., similar to those in the foetal monkeys, were observed in several species earlier in gestation, and it is probable that the higher level in these species, as compared with lambs, is due to the higher level in the adults as compared with adult sheep, rather than to differences in foetal metabolism. The similarity in the ratio of foetal to maternal blood glucose in the lambs and monkeys was surprising, because whereas the sheep placenta converts part of the foetal blood glucose into fructose (Huggett *et al.* 1951; Alexander, Andrews, Huggett, Nixon & Widdas, 1955), the monkey placenta at this stage of gestation does not (Chinard *et al.* 1956). The fall in blood fructose with increasing gestational age in the lambs (Fig. 3) was reflected in the tissues, which near term usually contained less than 1 mg/g.

Factors affecting foetal tissue glycogen

Little is known about the factors responsible for the appearance of glycogen in foetal tissues, but the present work makes it clear that exogenous factors alone cannot be responsible. Although there is a correlation between the appearance of islets of Langerhans in the foetal pancreas and the increase in foetal liver glycogen (Aron, 1922, 1931) and although the latter is dependent on foetal pituitary and adrenal function (Jacquot,

1955; Jost & Jacquot, 1955), the appearance of insulin and adrenocorticoids in the foetal circulation at this late stage in gestation would not explain the much earlier deposition of glycogen in the heart, lungs and skeletal muscle. It is more likely that the appearance of glycogen in a given tissue coincides with the development in that tissue of the enzymes necessary for its synthesis, of which the 'branching enzyme' needed for the formation of glycogen 'primer' may be among the last to appear (Nemeth, Insull & Flexner, 1954). Similarly, changes in enzyme activity may well be responsible for the decrease in lung and cardiac carbohydrate concentration in the latter part of gestation and for the difference between foetal and adult skeletal muscle.

Although differences in enzyme activity could account for changes in the general pattern of foetal tissue glycogen distribution, they are unlikely to account for the variations observed between different individuals of the same age. Such variation is more likely to be associated with differences in the availability of substrates for glycogen synthesis, particularly in those tissues where glycogen is not depleted by asphyxia. The injection of insulin into pregnant rats produced foetal hypoglycaemia and a fall in foetal liver glycogen (Corey, 1932, 1935*b*) and starving pregnant rats for 22 hr (Stuart & Higgins, 1935) or treating pregnant rabbits with phlorrhizin (Lochhead & Cramer, 1908) also depleted foetal liver glycogen. Conversely, treating pregnant rats with glucose increased the foetal liver and muscle glycogen (Corey, 1935*b*), though maintaining pregnant rabbits on a supposedly carbohydrate-rich diet (carrots and cabbage) had little effect (Lochhead & Cramer, 1908). Some long-term experiments on the effects of maternal nutrition on foetal tissue glycogen are clearly needed.

The importance of foetal tissue carbohydrate

Importance to the foetus. Even less is known about the importance to the foetus of its glycogen reserves since, although observations have been made on the glycogen content of various tissues, few physiological experiments have been made. It has been assumed that foetal liver glycogen behaves in the same way as adult liver glycogen and this hypothesis is supported by the fall in liver glycogen and rise in blood glucose observed in foetal lambs and monkeys asphyxiated by tying the umbilical cord (Dawes *et al.* 1959; 1960) and by its depletion in the presence of foetal hypoglycaemia (see above). Thus foetal liver glycogen is highly labile and Goldwater & Stetten (1947) estimated its rate of turnover in the foetal rat to be 5 mg/g/hr. Since large amounts of glycogen are present in the placenta and lungs at a time when very little glycogen is present in the liver, it has been suggested that these act as temporary glycogen stores until the liver is able to take over (Bernard, 1859*b*; Lochhead & Cramer, 1908; Szendi, 1934,

1936; Corey, 1935*a*; Villee, 1953*b*), but there is no evidence for its liberation from these organs under conditions of foetal stress. The placental glycogen of the rabbit is unaffected by adrenaline or hypoglycaemia (Huggett, 1929) and there is no change in lung carbohydrate in acutely asphyxiated foetal lambs and monkeys (Dawes *et al.* 1959, 1960). In contrast, there is a well-established correlation between cardiac carbohydrate concentration and the ability to survive anoxia (Dawes *et al.* 1959, 1960; Stafford & Weatherall, 1960), and since the danger of hypoxia is probably one of the chief hazards of intra-uterine life, the possession of a high cardiac glycogen concentration may be highly beneficial to the foetus and should help it to survive the trauma of birth.

Importance to the new-born animal. The changes in tissue glycogen in new-born animals suggest that both liver glycogen and skeletal muscle glycogen are important stores for use immediately after birth. A profound fall in liver glycogen occurs within a few hours of birth in dogs (Demant, 1887), guinea-pigs (Hard *et al.* 1944), rats (Martínek & Mikuláš, 1954; Stafford & Weatherall, personal communication), lambs and possibly monkeys (Fig. 4) and may be responsible for the initial rise in blood glucose observed in the lambs (Fig. 1) and guinea-pigs (Hard *et al.* 1944) soon after birth. The liver glycogen remained low, below 20 mg/g, for about a fortnight after birth in the dogs, rats and guinea-pigs but was rising again within a week of birth in the lambs and monkeys. The blood glucose level also rises in human babies after birth (Desmond, Hild & Gast, 1950; Cornblath, Levin & Marquetti, 1958) and liver glycogen also falls in chickens after hatching (Needham, 1942). A fall in skeletal muscle carbohydrate after birth has been observed not only in kittens (Bernard, 1859*a*) and in lambs and monkeys (Fig. 7), but also in the much less active new-born rat (Martínek & Mikuláš, 1954). In the last three species the level fell to less than half that in the full-term foetus; whereas in the lambs this occurred within 24 hr of birth, the fall was slower in the rat and, since the initial value was low (12 mg/g), the decrease in muscle glycogen was less. The rate of fall in the infant monkey is not known, since all those available were at least 8 days old, but in the kitten the fall occurs in the first 24 hr after birth. The rough calculations of total body carbohydrate in Table 1 suggest that in the lambs and monkeys the total fall in muscle carbohydrate was several times greater than the fall in liver carbohydrate, and similar calculations suggest that even in the rat the amount of glycogen lost from the muscles was at least half that disappearing from the liver. Whereas in all the species studied there was a rise in liver glycogen 10–16 days after birth, there was no such rise in muscle glycogen.

These observations suggest that the metabolic requirements of new-

born animals are so great that their carbohydrate reserves are utilized in the first few hours after birth, and that they are unable to obtain sufficient food both to satisfy these needs and to restore their liver glycogen until more than a week later, and can never restore their muscle glycogen. It is known that in foetal rats near term the turnover rates of several substances, including glycogen, are several times greater than in the adult (Goldwater & Stetten, 1947) and there is no reason to suppose that they are any lower in the new-born animal; indeed, the increase in oxygen consumption observed after birth in many species, including lambs, monkeys and rats, suggests that the turnover rates may actually increase in new-born animals (Gelineo, 1954; Dawes & Mott, 1959; Mount, 1959; Dawes *et al.* 1960; Taylor, 1960). The drastic effects of starving new-born animals are particularly well illustrated in the pig, where in the absence of food the rise in blood glucose occurring after birth is soon succeeded by hypoglycaemia and death (Goodwin, 1957). In some species, notably man, lactation is not fully established until 24–48 hr after parturition and during this period the new-born animal must be largely dependent on its own reserves, of which the liver and muscle carbohydrate evidently form an important part; for instance, McCance & Widdowson (1959) estimated that carbohydrate accounted for 84% of the solids metabolized by starved new-born piglets, 67–69% being contributed by muscle and liver glycogen. It is therefore possible that factors which reduce the glycogen content of the foetus *in utero* may well jeopardize the infant's chances of survival after birth. Although acute anoxia only partially depletes liver carbohydrate and does not affect skeletal muscle carbohydrate in foetal lambs and monkeys (Dawes *et al.* 1959, 1960), the results obtained on one particular monkey (No. 110, 145 days gestation age, Dawes *et al.* 1960), where placental infarcts were present and there was evidence of prolonged hypoxia, suggest that a prolonged period of placental insufficiency may not only deplete liver carbohydrate to a greater extent than does acute anoxia, but may also affect muscle carbohydrate; the latter was only 15 mg/g in this foetus, less than half the normal value. Such an infant would probably have a poor chance of survival after birth, and further work is needed to determine to what extent the deleterious effects of maternal malnutrition, placental insufficiency and birth asphyxia may be due to the presence of inadequate carbohydrate reserves in the new-born animal.

SUMMARY

1. Blood glucose and fructose and the total carbohydrate concentration in several tissues have been measured in foetal lambs in the last two-thirds of gestation. Similar observations were also made on lambs from 2½ hr to 17 days after birth and monkeys 8–15 days after birth.

2. The blood glucose did not change with increasing gestational age and was equal to about half the maternal level in both foetal lambs and monkeys. After birth it rose rapidly to near-adult levels.

3. In the lambs blood fructose fell with increasing gestational age and reached very low levels soon after birth; none was detected in the blood of two foetal monkeys.

4. Liver carbohydrate rose during the last third of gestation in the foetal lambs, from less than 14 mg/g to 60–98 mg/g near term; monkeys in the last third of gestation had 60–104 mg/g. Observations on infant lambs and monkeys suggested that it fell soon after birth and rose again 5–10 days later.

5. In the lambs lung carbohydrate rose to up to 27 mg/g just over half way through gestation, after which it fell rapidly to 3–4 mg/g near term; a similar fall occurred in the foetal monkeys and even lower levels were observed in the infant animals.

6. The cardiac carbohydrate rose to up to 40 mg/g in lambs just over half way through gestation, after which it fell in both species; the fall continued after birth.

7. Skeletal muscle carbohydrate rose during the last third of gestation in both species to a maximum of 47 mg/g in lambs and 34 mg/g in monkeys. In the lambs it fell to less than half the foetal level within 24 hr of birth; similar low levels were observed in the infant monkeys.

8. The fructose concentration in these tissues was usually negligible compared with the total carbohydrate, less than 2 mg/g in the foetal lambs.

9. Histological examination of the tissues suggested that their glycogen content was proportional to the total carbohydrate concentration.

10. It was concluded that the results were consistent with observations on other species and that these carbohydrate stores may be of great importance, particularly to the new-born animal.

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