

**THE IMPORTANCE OF CARDIAC GLYCOGEN FOR THE
MAINTENANCE OF LIFE IN FOETAL LAMBS
AND NEW-BORN ANIMALS DURING ANOXIA**

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It is a matter of common knowledge that foetuses or new-born animals of many species are able to survive in the absence of oxygen for a much longer period of time than adults of the same species. Thus it is difficult to drown new-born kittens and puppies and a foetus may be safely delivered some time after the death of its mother. These facts have been repeatedly confirmed (Reiss & Haurowitz, 1929; Kabat, 1940; Fazekas, Alexander & Himwich, 1941; Glass, Snyder & Webster, 1944) and it has also been established that the tolerance of new-born animals of different species to anoxia is inversely related to their maturity at birth. It is believed that these very young animals survive without oxygen by deriving energy from glycolysis. The difference between the new-born and the adult has been attributed by Himwich and his colleagues to the greater energy requirement of the brain in the adult (Fazekas *et al.* 1941; Himwich, 1953). These conclusions were based on experiments with small animals such as new-born rats, kittens, puppies, rabbits and guinea-pigs.

It seemed worth while to explore the possibility of using the foetal lamb to study these phenomena, in the hope that it would possess the ability to withstand prolonged anoxia at an age and size such that a number of blood samples could be withdrawn for biochemical analysis, a procedure which had not so far proved practicable with smaller species. There was an additional advantage to be expected from using the foetal lamb, in that such studies would not be complicated by the various circulatory and metabolic changes which take place at birth, and which are not complete for some while thereafter. In the event, lambs of 83-91 days gestation age (i.e. just over halfway through gestation; term is about 147 days) proved suitable for this purpose, since they mostly weighed 0.5 kg or more, and survived anoxia for 40-60 min. This paper describes some of the cardiovascular and biochemical changes which ensue when the umbilical cord is tied in foetal lambs which are either too

young to expand their lungs or which are prevented from breathing. The results suggest that it is the maintenance of the circulation which is of predominant importance in survival and that this is related to the carbohydrate concentration in the heart at the onset of anoxia. This conclusion is also supported by observations upon new-born rats, rabbits and guinea-pigs.

METHODS

Experimental procedure and animals

Lambs. Observations were made on four lambs of 59–61 days gestation age, delivered from three ewes; on twenty-seven lambs of 71–91 days gestation age, from fourteen ewes; on two lambs of 111 days gestation age, from one ewe; and on thirteen lambs of 121–146 days gestation age, from seven ewes.

The ewe was laid on her right side and anaesthetized with chloralose 50 mg/kg. The trachea was cannulated and a foetus was delivered by Caesarean section through a mid-abdominal incision. The foetus was kept warm and was left attached to the ewe by the umbilical cord, which was so arranged that it was under no tension. A catheter was introduced into a carotid artery for the withdrawal of blood samples. Blood pressure was measured by a condenser manometer either from this same catheter, or, in large lambs, from one inserted into a femoral artery. Heart rate was measured by using the pressure pulse to actuate a heart-rate meter (Wyatt, 1956, 1957). Mean blood pressure and heart rate were recorded, alternately, at intervals of 6 sec, by a Cambridge Instrument Co. Slow Recorder.

Blood pressure and heart rate were recorded for 10–20 min until we were sure that the foetus was in a steady state. Blood samples were then taken into 1 ml. syringes, the dead space of which was filled with a solution containing 0.4% heparin (Boots Pure Drug Co.) and NaF solution 8% (w/v). The number of blood samples was limited by the size of the lamb. Thus in very small lambs weighing less than 100 g only a single sample was taken initially, and a second one after a prolonged period of asphyxia. In mature lambs, weighing 3–4 kg, eight to ten 1 ml. samples were withdrawn during an experiment. After taking one or two control blood samples, the umbilical cord was tied and further samples were taken at intervals. Finally samples of tissue (100–200 mg) were removed as rapidly as possible in the following order: muscle (lateral aspect of thigh), liver, lung (apex of left upper lobe), heart (apex of ventricles), cerebral cortex and kidney cortex. Twins were used as controls; in these an identical sampling procedure was followed without tying the umbilical cord. In some experiments foetal urine flow was measured by tying a catheter into the bladder, and ligating the urachus; urine was collected into measuring cylinders for 10 min periods. Blood samples were also withdrawn from the ewe through a catheter inserted into a forelimb artery and passed some distance centrally.

Sixteen of the ewes were asphyxiated after the foetuses had been removed and the abdominal incision sutured. Blood pressure and heart rate were recorded, and arterial blood samples were withdrawn at intervals. The ewe was asphyxiated by attaching to its tracheal cannula a collapsible rubber bag filled with nitrogen. In some of these experiments an attempt was made to revive the ewe, after a period of asphyxia, by artificial ventilation with 100% oxygen.

Other animals. Observations on other animals were confined to estimating the total carbohydrate content of the ventricles of the heart in young rats, rabbits and guinea-pigs which had been born naturally, the time of birth having been recorded. The animals were decapitated and the ventricles were removed immediately for biochemical analysis or histological examination.

Biochemical and histological methods

Blood. 0.1 ml. was used for lactate determination (Barker & Summerson, 1941) and 0.25–0.5 ml. was deproteinized with NaOH and ZnSO₄ and the filtrate (dilution 1 in 10) used for determination of glucose (Huggett & Nixon, 1957), fructose (Bacon & Bell, 1948) and total ketones (Greenberg

& Lester, 1944). Blood pH was determined directly on 0.8 ml. blood at 37° C by means of the Stadie electrode system using an E.I.L. pH meter. For the sake of economy, whenever pH was measured the same blood sample was used subsequently for the other determinations.

Urine. Urine glucose, fructose and lactate were all determined on a 1 in 20 dilution of urine which had been treated with NaOH and ZnSO₄ as for blood.

Tissues. Tissue samples from the foetal lambs 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 AgSO₄ 200 mg/100 ml. in a weighed glass receptacle. This was re-weighed 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 suitable portions were used for the determination of total carbohydrate, i.e. glycogen + glucose + fructose (Mendel, Kemp & Myers, 1954; Kemp & Kits van Heijningen, 1954), fructose and lactate.

The ventricles from the other animals were used either for histological examination or for estimation of total carbohydrate, since the amount of tissue available from a single heart was often insufficient for both: the heart of a new-born rat weighs less than 20 mg. For some of the older animals (e.g. 31-day-old rabbits whose hearts weighed more than 1 g) the total volume of deproteinizing reagent used was increased to 10 ml.

RESULTS

Cardiovascular changes after tying the umbilical cord

Lambs of 83–91 days gestation age. When the umbilical cord is firmly tied with a piece of string, the O₂ saturation of the arterial blood falls very rapidly to less than 10% and remains at this level for the rest of the experiment. Fig. 1 shows the cardiovascular changes in a typical experiment in a lamb of 91 days gestation age. When the umbilical cord is tied there is an immediate rise of blood pressure. After half a minute the blood pressure usually falls slightly, and there is then a secondary further rise which reaches a peak 2–3 min after the cord has been tied. This sequence of events is particularly well seen in Fig. 2. The pressure then falls quite rapidly to the pre-asphyxial level 8–10 min after tying the cord, by which time the pulse pressure is much decreased. For the next 40–50 min the blood pressure shows a further steady decline, usually interrupted, as Figs. 1 and 2 show, by minor fluctuations. In contrast, when the cord is tied the heart rate falls almost at once, from an initial rate of 200 or more beats per minute to less than 100 beats per minute. Thereafter it declines more slowly, and the beat is almost always quite regular for 30–40 min. When the blood pressure has fallen below 20 mm Hg the heart rate may become irregular, and short periods of tachycardia and occasional extrasystoles may be seen.

Although the umbilical vessels at the end of gestation are very sensitive to manipulation, they are less so earlier on. The cord may be untied, as Fig. 2 shows, after a period of total asphyxia as long as 40 min. In this experiment the blood pressure, which had fallen below 20 mm Hg, then immediately

returned towards the initial level, the heart rate rose to exceed the initial rate and the O_2 saturation of the carotid blood increased. About half an hour later the ewe, which had been anaesthetized for $2\frac{1}{2}$ hr, became restless. The foetus began to make regular respiratory efforts and moved its limbs. Thus it had survived a period of 40 min total asphyxia without impairment of the mechanisms involved in these activities. Similar observations have been made on

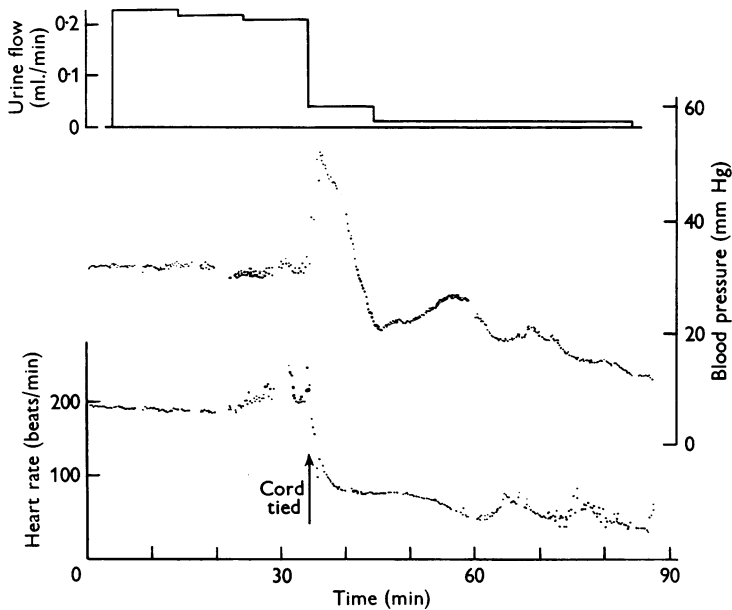


Fig. 1. Foetal lamb, 91 days gestation age, 639 g. Records of urine flow (measured over 10 min intervals), mean blood pressure and heart rate. The umbilical cord was tied at the arrow.

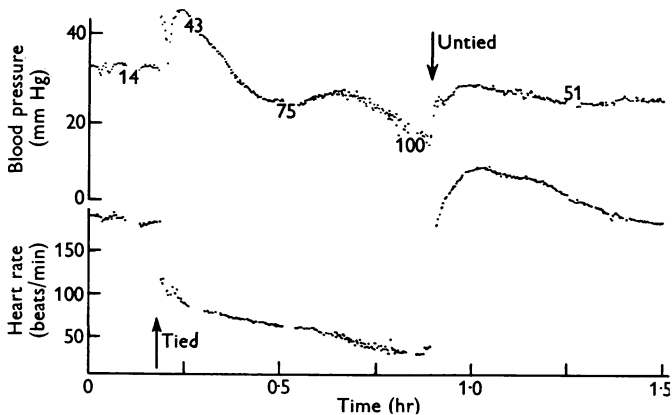


Fig. 2. Foetal lamb, 88 days gestation age, 350 g. Records of mean blood pressure and heart rate. The umbilical cord was tied at the first arrow and untied at the second arrow. The figures interpolated in the blood pressure record indicate the blood lactate (mg/100 ml.)

three other lambs of 86–90 days gestation age. In one of these recovery was not maintained, but in the other two the umbilical cord was tied for 20–40 min and then untied with successful recovery on two separate occasions in each lamb.

Lambs of other gestational ages. In three lambs of 59–61 days gestation age the cardiovascular changes after tying the umbilical cord were followed for 45–60 min. The initial mean blood pressure before asphyxia was lower than that of the previous group of lambs (Fig. 3). On tying the cord the rise of pressure was less pronounced and less well maintained. However, half an

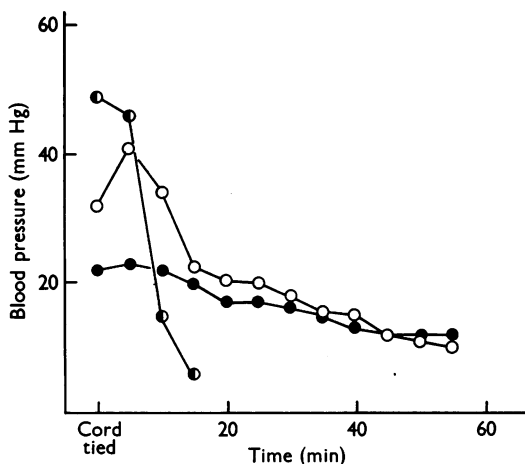


Fig. 3. Mean blood pressure before and at 5 min intervals after tying the umbilical cord, in ten lambs of 85–91 days gestation age (○), three lambs of 59–61 days gestation age (●) and seven lambs of 121–146 days gestation age (◐).

hour later the mean blood pressure of the two groups was very similar. This might suggest that the younger lambs were better able to endure asphyxia, but as they only weighed 60–80 g the number of blood samples that could be taken was very limited and no further experiments on lambs of this gestation age were undertaken.

Observations on lambs of 121–127 and of 138–146 days gestation age (term is about 147 days) gave results that were very similar, and these results have therefore been combined for purposes of description. The initial blood pressure was higher than that of younger lambs (Fig. 3). When the cord was tied there was an immediate rise of blood pressure sometimes exceeding 20 mm Hg. This, however, was of very short duration, so that at the end of 5 min the pressure had, on the average, returned to its initial level, and the rise is therefore not shown in Fig. 3. Ten minutes after tying the cord the mean pressure had fallen considerably and by 15 min it was less than 10 mm Hg in every lamb.

The changes in heart rate were similar to those in younger lambs, that is to say, the rate fell very abruptly when the cord was tied, and thereafter declined more slowly. However, in lambs of 121–146 days gestation age the rate fell below 50 beats per minute within a mean 13 min (range 8–18) of tying the cord, as compared with 39 min (range 20–60) in lambs of 83–91 days gestation age. In one lamb of 140 days gestation age the cord was untied after 6½ min, by which time the blood pressure had fallen from 70 to 22 mm Hg; the blood pressure and heart rate returned rapidly to normal values, showing that it is possible to perform this manoeuvre satisfactorily in a lamb near term.

Adult ewes. Adult ewes were given nitrogen to breathe from a collapsible bag attached to the tracheal cannula. The blood pressure rose during the next 2 min from an initial level of just over 100 mm Hg up to 200 mm Hg or more. The heart rate also increased from 120–150 beats/min to about 300 beats/min. After an interval, which varied in different sheep from 3½ to 6 min, the blood pressure started to fall very rapidly. Cardiac pulsations, recorded from a catheter introduced into a central artery, were no longer seen after 6–8 min. The initial hyperpnoea was very great; the last gasping movement was seen at about the time when the blood pressure took its final plunge.

In eight sheep artificial ventilation with 100% O₂ was begun after respiratory movements had ceased and after the mean blood pressure had fallen below the initial level. In five of these the circulation recovered and spontaneous breathing began. They had been subjected to anoxia of from 4·8 to 6·8 min duration; in two of them the blood pressure had fallen as low as 75 and 60 mm Hg before artificial respiration was begun. The other three sheep were subjected to 7–7·5 min anoxia, and did not recover, in spite of subsequent artificial ventilation with oxygen.

Changes in the blood, urine and tissues of foetal lambs after tying the umbilical cord

Blood. In each of sixteen lambs of 83–146 days gestation age there was a rapid fall in blood glucose after tying the umbilical cord. Within 1 min it had decreased by 1·4–4·2 mg/100 ml. and by 2·7–13·0 mg/100 ml. at the end of 5 min. This fall occurred in all the lambs in spite of a wide variation in the initial concentration (3·2–14·0 mg/100 ml. at age 83–91 days, 10·0–11·5 mg/100 ml. at 126–138 days and 25·2–27·0 mg/100 ml. at 140–146 days) which could be partly accounted for by a similar wide variation in maternal blood glucose (8·0–47·0 mg/100 ml.); low foetal blood glucose levels usually coincided with low maternal levels and high foetal levels with high maternal levels. Figure 4 shows the mean foetal blood glucose levels at different intervals of time after tying the umbilical cord. In the very young foetuses (83–91 days gestation age) the blood glucose remained below 5 mg/100 ml., at 25–50% of the initial level, for the rest of the experiment. In the older lambs (126–138

days gestation age) there was an increase in blood glucose 10 min after tying the cord and after 15 min the mean level was up to 8.2 mg/100 ml., 75% of the initial value. The two oldest lambs (140 and 146 days gestation age) were moribund within 10 min of tying the cord and the initial fall in blood glucose was not succeeded by a rise. In spite of every precaution these two lambs were blue on delivery; the cord was not tied until over half an hour later, by which time their colour had greatly improved, but it is possible that their high initial blood-glucose level was a consequence of asphyxia during delivery.

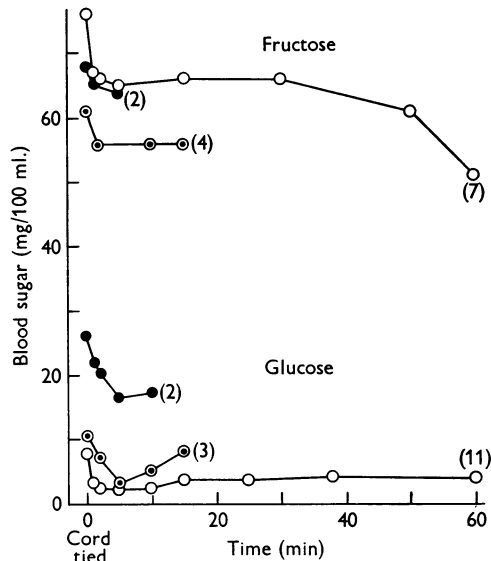


Fig. 4. Mean blood fructose and glucose concentrations before and at intervals after tying the umbilical cord in lambs of 83-91 (○), 126-138 (⊙) and 140-146 (●) days gestation age. The figures in brackets indicate the number of lambs in each age group.

In eight out of thirteen lambs (83-146 days gestation age) there was also a fall in the blood-fructose level during the first 5 min after tying the cord. The initial foetal fructose level (30-90 mg/100 ml.) also varied with the maternal blood glucose but it was always very much higher than either the foetal or maternal blood glucose (Fig. 4). Although the blood-fructose level was so high, the maximum initial decrease was only 14 mg/100 ml. and in the older lambs the blood fructose never fell below 80% of the initial level. In one of the younger lambs, aged 91 days, there was a further fall in blood fructose 30-60 min after tying the cord but the level did not fall below 70% of the initial value. In three out of four other lambs aged 85-91 days, which had initial blood fructose levels of 97-140 mg/100 ml. and are not included in Fig. 4, there was a similar late fall in blood fructose to 70-90% of the initial value.

The pH of the blood began to fall within 1 min of tying the umbilical cord in

12 lambs. The magnitude of the fall was independent of both the age of the foetus and the initial pH of the blood, which was 7.40–7.60 at age 83–91 days, 7.40–7.54 at 126–138 days and 7.30–7.32 at 140–146 days. This is illustrated in Fig. 5, which gives the mean pH values in the three age groups at different intervals of time after tying the cord. Fig. 5 also shows that the pH fell exponentially throughout the experiment.

The fall in pH after tying the umbilical cord was accompanied by a rise in blood lactate. Although the initial blood lactate concentration tended to be higher in the older lambs (15.0–49.6 mg/100 ml. at age 83–91 days, 27.4–91.5 mg/100 ml. at 121–138 days and 83.5–91.5 mg/100 ml. at 140–146 days),

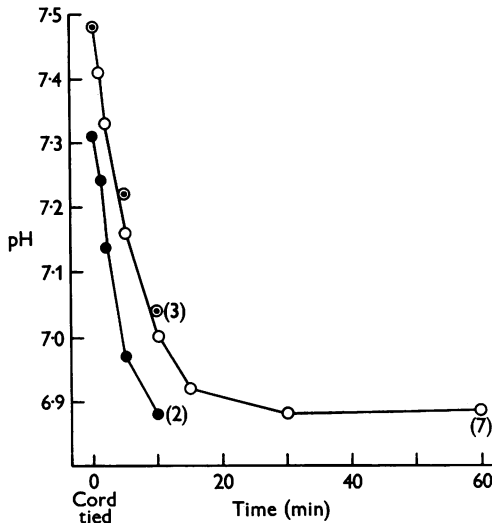


Fig. 5. Mean blood pH before and at intervals after tying the umbilical cord in lambs of 83–91 (○), 126–138 (⊙) and 140–146 (●) days gestation age. The figures in brackets indicate the number of lambs in each age group.

the initial rate of lactate accumulation was similar in all three age groups, as can be seen from the mean values in Fig. 6. It is probable that all the lambs which had an initial lactate level of more than 40 mg/100 ml. (one of twelve at age 83–91 days, three out of five at 121–138 days and both of two at 140–146 days) had been partially asphyxiated on delivery; the likelihood of this occurring increases with age, and temporary spasm of the cord or contraction of the uterus was sometimes observed. In seven adult ewes, where there was less risk of accidental asphyxia, the initial blood lactate level (6.8–22.0 mg/100 ml.) was rather lower. When they were given nitrogen to breathe, the blood lactate rose in five of these ewes and the rate of accumulation was similar to that in the foetal lambs (Fig. 6). In general there was a close inverse relationship between the changes in blood lactate concentration and pH and

Fig. 6 summarizes the results in the younger lambs in each of which the rate of lactate accumulation also became slower towards the end of the experiment.

The blood total-ketones level in three lambs was less than 2.6 mg acetone/100 ml. and did not change after tying the umbilical cord.

The changes in blood sugar, pH and lactate were reversed in those experiments where the lambs recovered on untying the umbilical cord. Thus in one lamb, gestation age 140 days, the blood glucose rose from 14.0 to 22.3 mg/100 ml. and the blood fructose from 44 to 49 mg/100 ml. within 3 min of untying the cord; in the same lamb, the pH of the blood rose from 6.95 to 7.03 within 3 min and to 7.20 within 13 min. In another lamb the blood

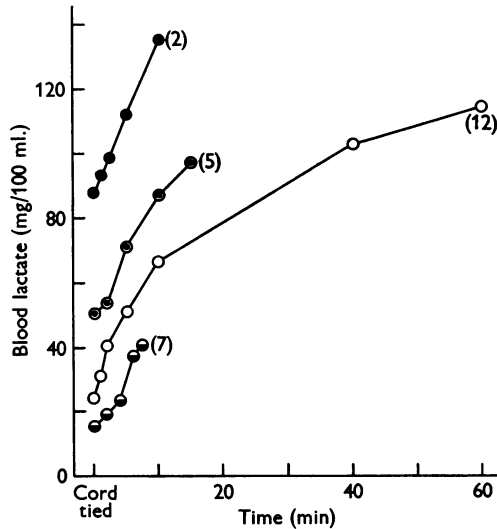


Fig. 6. Mean blood lactate before and at intervals after tying the umbilical cord in lambs of 83-91 (○), 126-138 (◐) and 140-146 (●) days gestation age; and also before and after giving adult sheep nitrogen to breathe (◑). The figures in brackets indicate the number of animals in each age group.

lactate had fallen from 100 mg/100 ml. 40 min after tying the cord to 51 mg/100 ml. 25 min after untying the cord (Fig. 2).

Urine. In two lambs of gestation age 91 days, the rate of urine flow fell from a resting level of 2-3 ml./10 min to 0.4 and 0.8 ml. in the first 10 min after tying the cord and then to less than 0.1 ml./10 min (Fig. 1). No glucose was detected in these urine samples, but the fructose levels were very high, 776 and 552 mg/100 ml. in the two lambs after tying the cord. Although the total volume of urine excreted after tying the cord was very small, it represented a loss of 5.4 and 5.5 mg fructose, respectively, and assuming that at this age the total blood volume is 50-70 ml., this would represent a fall in blood fructose of 11.0-7.7 mg/100 ml. Since in other fetuses urine fructose levels of up to 2000 mg/100 ml. were observed, it is possible that excretion of

fructose in the urine may account for the fall in blood fructose observed immediately after tying the cord in the majority of lambs. The urine lactate level did not rise above 80 mg/100 ml., so loss of lactate in the urine was negligible.

Tissues. Tissue samples for histological and biochemical examination were taken from twenty-three lambs of 83–91 days gestation age and eleven lambs of 121–146 days gestation age. In fourteen of the younger lambs and ten of the older lambs, samples of muscle, liver, lung, heart ventricle, brain cortex and kidney cortex were taken at the same time, either after tying the cord or from control lambs where the cord had not been tied. Thus tissue samples from control lambs were compared with samples from other lambs after varying periods of asphyxia. In four lambs muscle or lung samples were taken from the same lamb before and after tying the cord; single observations on two or three tissues only were made in the other three lambs. Since the results were surprisingly consistent, it has been possible to illustrate them by the mean values shown in Figs. 7–10.

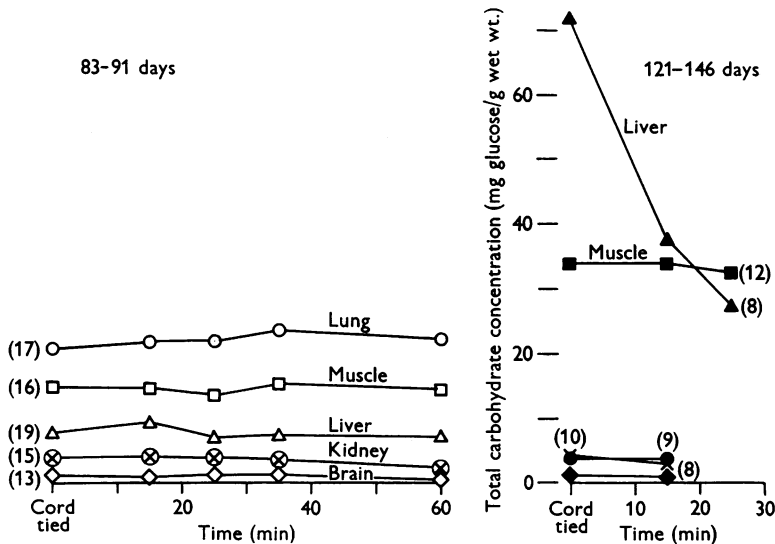


Fig. 7. Mean tissue total carbohydrate concentration in unasphyxiated lambs and at intervals after tying the umbilical cord; lung (○, ●), muscle (□, ■), liver (△, ▲), kidney (⊗, ×) and brain (◇, ◆) in lambs of 83–91 and of 121–146 days gestation age. The figures in brackets indicate the total number of lambs used in each age group.

It can be seen from Fig. 7 that in the unasphyxiated controls (time = 0), although the brain and kidney carbohydrate levels were similar in all the lambs, there were considerable differences in carbohydrate concentration in the liver, lungs and skeletal muscle in the two age groups. These differences will be discussed in detail elsewhere. Histological examination showed that

whenever the carbohydrate concentration exceeded 5 mg/g, deposits of glycogen could be observed; there was good agreement between the amount of glycogen and the total carbohydrate concentration up to the highest level recorded, 98 mg/g in the liver of a 138 day lamb. The tissue fructose concentrations never exceeded 2 mg/g (Fig. 8).

Figure 7 shows that after tying the umbilical cord there were negligible changes in the carbohydrate concentration of the lungs, muscle, kidney and brain in the two age groups. In the lambs of gestation age 83–91 days the liver carbohydrate was also unaffected, but in the older lambs in which the initial liver carbohydrate level was almost 10 times that of the younger lambs, there was a rapid and significant fall in liver carbohydrate. This had fallen by 16–50 mg/g to less than 50% of the control value within 15–25 min of tying the cord; histological examination showed corresponding changes in liver glycogen. Figure 8 shows small decreases in tissue fructose after tying the cord, but since these did not exceed 0.5 mg/g, they are of doubtful significance.

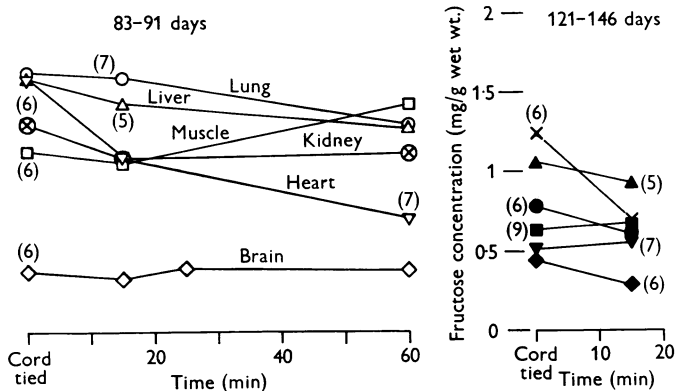


Fig. 8. Mean tissue fructose concentrations in unasphyxiated lambs and at intervals after tying the umbilical cord; lung (○, ●), muscle (□, ■), liver (△, ▲), kidney (⊗, ×), brain (◇, ◆) and heart (▽, ▼) in lambs of 83–91 and of 121–146 days gestation age. The figures in brackets indicate the total number of lambs used in each age group.

The decrease in liver glycogen and rise in blood glucose after tying the umbilical cord of the older lambs suggested that lambs of 121–146 days gestation age should be better able to survive asphyxia than lambs aged 83–91 days which did not mobilize their glycogen reserves. In fact, the reverse was true and analysis of samples of cardiac muscle supplied a possible explanation. Figure 9 shows that there were considerable carbohydrate reserves in the hearts of both groups of lambs but that in control lambs of 83–91 days gestation age the cardiac carbohydrate concentration was nearly twice that in the control lambs of 126–146 days gestation age. Moreover, whereas 15 min after tying the umbilical cord the cardiac carbohydrate level was less than

5 mg/g in lambs of the older age group, in the younger lambs it was still 19–22 mg/g and did not reach 5 mg/g until 60 min after tying the cord. These results were confirmed histologically. The results also suggested that the initial rate of glycolysis might have been higher in the hearts of the older lambs. This suggestion was supported by the rate of lactate accumulation in the heart (Fig. 10); the initial rate of lactate accumulation was slightly higher in the hearts of lambs of 126–146 days gestation age than in those of the younger lambs.

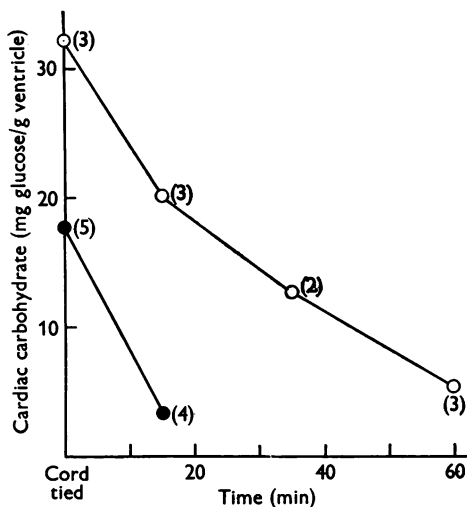


Fig. 9. Mean total carbohydrate concentration in the hearts of unasphyxiated lambs and at intervals after tying the umbilical cord at 83–91 (○) and 121–146 (●) days gestation age. The figures in brackets indicate the total number of lambs used in each age group.

Figure 10 also shows that the slow rate of lactate accumulation in skeletal muscle and the more rapid rates in liver, lungs and kidney cortex were similar in the two groups of lambs, but that in the older lambs the rate of accumulation in the brain cortex was rather faster than in the younger ones. However, the most interesting feature of Fig. 10 is that it shows clearly that the rate of lactate accumulation in the heart is much greater than that of any other tissue, suggesting that the rate of glycolysis in the heart during asphyxia is much greater than that of other tissues. The cardiac lactate concentrations in 83–91 day lambs after 35 and 60 min anoxia were all higher than simultaneous values in other tissues.

Cardiac carbohydrate in other young animals

The experiments on foetal lambs suggested that the maintenance of life after tying the umbilical cord might be related to the carbohydrate content of the heart at the onset of anoxia. However, since the carbohydrate content of

the heart decreases during anoxia and since there was reason to believe that several of the older lambs had been accidentally asphyxiated during delivery, it might have been merely fortuitous that the cardiac carbohydrate level in the control lambs was lower in the older age group; the length of the survival period might have been related to some other factor which had not been investigated. For this reason the carbohydrate content of the heart was determined in neonatal rats, rabbits and guinea-pigs of different ages. Since these animals were breathing spontaneously, the risk of accidental asphyxia was

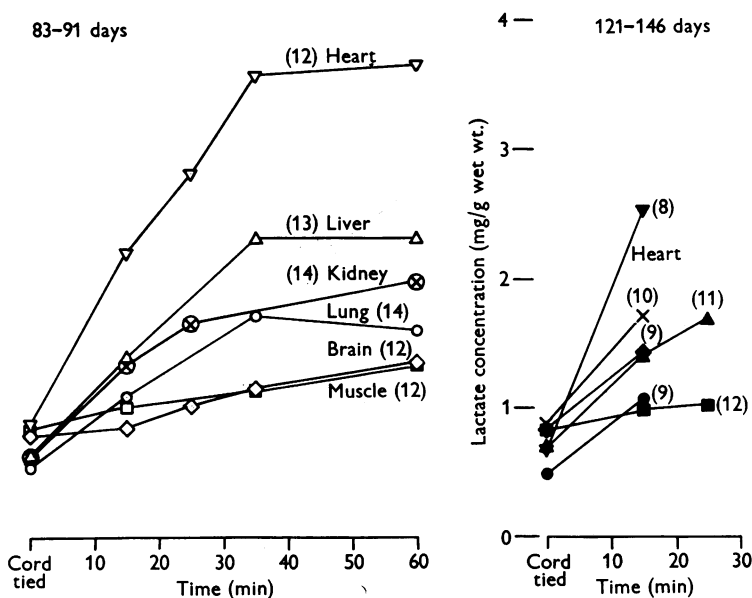


Fig. 10. Mean tissue lactate concentration in unasphyxiated lambs and at intervals after tying the umbilical cord; lung (○, ●), muscle (□, ■), liver (△, ▲), kidney (⊗, ×) brain (◇, ◆) and heart (▽, ▼) in lambs of 83-91 and 121-146 days gestation age. The figures in brackets indicate the total number of lambs used in each age group.

small and Fazekas *et al.* (1941) had shown that there are considerable differences in the anoxic survival time of the new-born of these species. Thus, at 24° C, new-born rats continue gasping in nitrogen for 50 min, new-born rabbits survive for only 17 min and new-born guinea-pigs for only 7 min. As the animals get older there is a progressive decrease in survival time so that at 21 days of age rats survive for only 1½ min in nitrogen; this survival time approximates to that of the adult rat.

In the present work, analysis of the ventricles showed considerable differences in cardiac carbohydrate levels in new-born rats, rabbits and guinea-pigs. Thus rats less than 24 hr old (animals from four litters) had a cardiac

carbohydrate concentration of 22–31 mg/g (mean 25.2 mg/g), rabbits of this age (four litters) had 5–13 mg/g (mean 8.5 mg/g) and new-born guinea-pigs (one litter) had only 4–5 mg/g (mean 4.6 mg/g). Moreover, there was a progressive decrease in cardiac carbohydrate concentration with age; rats from single litters of 2, 3, 6 and 10 days of age had mean cardiac carbohydrate

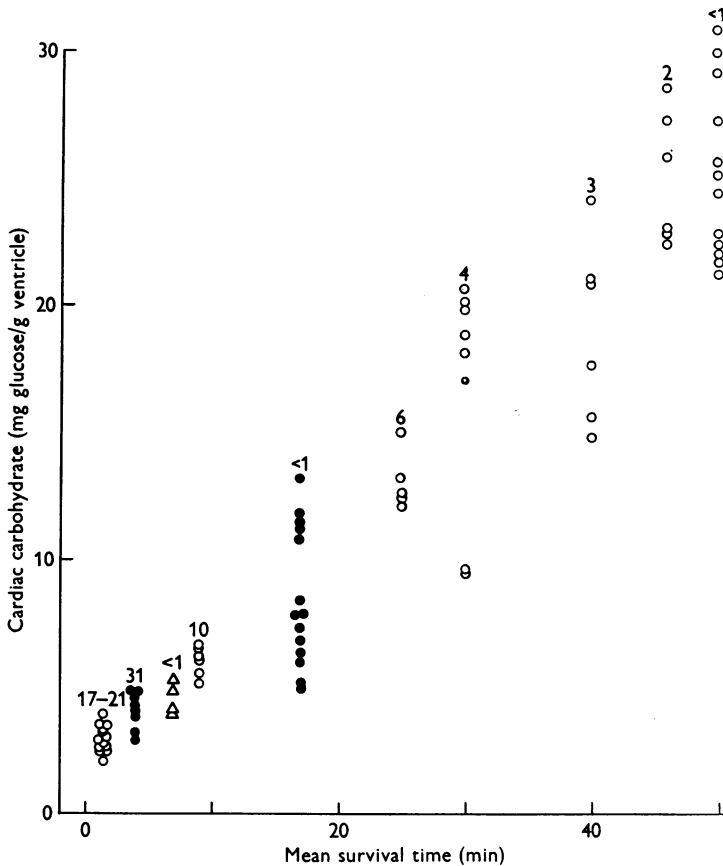


Fig. 11. Total carbohydrate concentration in the cardiac ventricles of rats (○), rabbits (●) and guinea-pigs (△) plotted against mean survival time in nitrogen at 24° C (from Fazekas *et al.* 1941). The figures above each group of observations indicate the age from birth in days.

levels of 25, 21, 13 and 6 mg/g, rats from two litters at 4 days of age and three litters at 17–21 days had mean values of 17 and 3 mg/g and rabbits 31 days of age (three litters) had a mean value of 4 mg/g. In one experiment the hearts from six of a litter of rats were analysed on the day of birth and those from their litter mates (four rats) when they were 21 days old; the mean cardiac carbohydrate concentrations were 25.6 and 3.0 mg/g respectively. These differences in carbohydrate concentration at different ages were confirmed by

histological examination; the younger the animal, the more glycogen was visible in the cardiac muscle fibres.

In Fig. 11 our data for the carbohydrate concentration in heart ventricles have been plotted against the corresponding mean survival time in nitrogen at 24° C observed by Fazekas *et al.* (1941). In spite of the wide variation in cardiac carbohydrate level in each species at a given age (this variation occurred within the litter, not merely between individuals from different litters), there is an astonishingly close, almost linear, relationship between cardiac carbohydrate concentration and survival time. It is possible that the relationship may be even closer than is suggested by Fig. 11, where there is some overlap, since Fazekas *et al.* (1941) give mean survival times only for rats, rabbits and guinea-pigs, with no indication of the variation between individuals; they observed considerable variation in the survival times of new-born puppies. Moreover, Kabat (1940) observed survival times of up to 70½ min for new-born rats in nitrogen, whereas Fazekas *et al.* (1941) give a mean value of 50 min. Preliminary observations in nitrogen at 30° C (Shelley and Weatherall, unpublished) suggest that there is indeed some variation in survival time between litter mates of the same age and that the relationship between cardiac carbohydrate and survival time is also observed at this temperature.

DISCUSSION

The ability of foetal lambs to survive anoxia

The results show that foetal lambs of 83–91 days gestation age can survive in the absence of oxygen for at least 40 min after tying the umbilical cord. When the umbilical cord was untied the heart rate and blood pressure rose towards their original pre-anoxic values and half an hour later the lamb could make co-ordinated muscular and respiratory movements. Although this does not mean that no permanent damage had been done, it suggests that the vital organs were still intact. Older lambs of 121–146 days gestation age did not survive so well, as judged by changes in blood pressure and heart rate, and it was not possible to resuscitate adult ewes after anoxia of 7 min duration. These observations confirm, in yet another species, the generalization that very young animals are better adapted to withstand anoxia than are adults. They also show, in an animal which is relatively mature at birth, that this adaptability begins to be lost during the latter half of gestation. Therefore its loss is not related to the changes which ensue at birth, such as the large increase in the oxygen tension of the arterial blood, the alteration in the course of the circulation or the metabolic consequences of an independent life. Nor is it to be attributed to a change in the resting oxygen consumption per kilogram of the lamb, since this is constant during the latter half of gestation (Acheson, Dawes & Mott, 1957; Dawes & Mott, 1959).

The importance of glycolysis for survival in anoxia

It is well known that under anaerobic conditions vertebrate tissues obtain energy by converting glucose or glycogen to lactic acid. The injection of sodium iodoacetate or fluoride, both of which inhibit enzymes involved in glycolysis, reduces the survival time of new-born rats in nitrogen to that of the adult (Himwich, Bernstein, Herrlich, Chesler & Fazekas, 1942) and reduces the tolerance to low oxygen tensions of adult dogs (Fazekas & Himwich, 1943). The survival time of new-born rats in nitrogen is also reduced in insulin hypoglycaemia (Himwich *et al.* 1942; Himwich, Fazekas & Homburger, 1943; Hicks, 1953); administration of glucose will protect young rats from this effect of insulin and will increase the tolerance to anoxia of normal 8 day old rats.

It had been suggested earlier that the ability of young animals to withstand long periods of anoxia was associated with an unusual ability to glycolyse anaerobically, which was lost with increasing maturity (Reiss, 1931). This conclusion was based on experiments showing that young rats and mice in the terminal stages of anoxia contained more lactate per unit weight than anoxic adult rats and mice. It is probable that this difference was merely due to the younger animals being able to glycolyse anaerobically for a longer period of time than the adults since *in vitro* experiments on tissue slices and brei show that the maximum rate of glycolysis of most tissues increases rather than decreases with age (Chesler & Himwich, 1944*a, b*; Villee, 1953, 1954) and the present work on foetal lambs is consistent with these observations. In spite of the differences in anoxic survival time, the initial rate of accumulation of lactic acid and the rate of fall of pH in the blood were similar in both groups of lambs and in the adult sheep (Figs. 5 and 6). The tissue lactate levels were similar in both groups of control lambs and, where there were differences in the rates of carbohydrate loss and lactate accumulation in the tissues during asphyxia, the rates were higher in the shorter-lived, older lambs (Figs. 7, 8, 10). Thus there was no suggestion that the younger lambs could glycolyse more rapidly than the older ones.

Chesler & Himwich (1944*a*) attempted to account for the decrease in survival time with increasing age in terms of the energy deficit in the brain under anaerobic conditions. They argued that since the *in vitro* rate of oxidative metabolism in rat brain increases with age more rapidly than the rate of anaerobic glycolysis, the energy deficit sustained in anoxia will also increase with age, resulting in a decreased tolerance to anoxia. However, this explanation is not satisfactory; no increase in aerobic metabolism was observed until the rats were 10 days old, whereas the survival time in nitrogen begins to decrease within 24 hr of birth (Fazekas *et al.* 1941) and (at 24° C) has already declined from 50 min at birth to less than 10 min at 10 days of age.

The availability of carbohydrate in anoxia. The obvious importance of glycolysis in anoxia, and the observation that older foetal lambs could glycolyse faster than younger ones, suggested that the carbohydrate content of the tissues should be measured, since the amount of substrate available might determine survival time. Such measurements have been made in the past, but no correlation with survival was established (Chesler & Himwich, 1943; Ferris & Himwich, 1946; Vilee, 1953, 1954). In the present work the total carbohydrate content of the tissues was determined, rather than their glycogen content, because this gave a truer estimate of the substrate available and reduced errors due to glycogenolysis during sampling (the method used includes glucose-1-phosphate in the determination). Estimation of tissue fructose showed that the control levels in all tissues except brain were rather lower in the older lambs, but that the amount present (less than 2 mg/g) and the decrease during anoxia (less than 0.5 mg/g) were so small, by comparison with changes in total carbohydrate, that fructose is of doubtful importance as a source of energy in anoxia when glucose and glycogen are available (Figs. 7, 8, 9).

Although substantial amounts of glycogen were present in the lungs of the younger lambs and in the skeletal muscle of both groups of lambs, these did not decrease during anoxia, suggesting that the glycolytic activity of these tissues *in vivo* was small and that the carbohydrate could not be mobilized for use by other tissues. Similarly, there was no decrease in liver carbohydrate during anoxia in the younger lambs, but in the older lambs, where the initial liver carbohydrate level was much higher, there was a rapid decrease which was more than sufficient to account for the rise in blood glucose in this age group (Fig. 4). The decrease in brain and kidney carbohydrate during anoxia was small, but there was a rapid decrease in cardiac carbohydrate and in all these tissues the level decreased rather more rapidly in the older lambs. Only in the brain and heart was there any suggestion that the carbohydrate reserves had been exhausted in the terminal stages of anoxia, and it is therefore significant that the cardiac carbohydrate concentration in the younger control lambs was almost twice that in the older ones. Observations on the rate of lactate accumulation (Fig. 10) confirmed these impressions of the relative rates of glycolysis in the different tissues.

Cardiac carbohydrate and anoxia. Rough calculations of total carbohydrate loss and total lactate production, based either on actual organ weights or on weights calculated from total body weight (Carlyle, 1948; Wallace, 1948), showed not only that all the lactate produced could be accounted for in terms of carbohydrate loss, but that a large part of the lactate must have been formed from carbohydrate originating in the heart. Thus during the 60 min period after tying the umbilical cord of lambs of 85–91 days gestation age the mean total lactate production was 181 mg and the mean total carbohydrate loss was

179 mg; the mean loss of carbohydrate from the heart was 162 mg, 90% of the total loss. During 15 min asphyxia the mean total lactate production in lambs of 126–146 days gestation age was 642 mg and the mean total carbohydrate loss was 878 mg; the mean loss of cardiac carbohydrate was 375 mg, 43% of the total loss. In spite of the relatively small size of the heart, these results are not surprising when it is remembered that the heart is the only tissue doing regular, active, mechanical work in the foetal lamb during asphyxia. It is reasonable to assume that the energy requirements of the heart is higher per unit weight than that of any other tissue, that during anoxia the heart converts its carbohydrate reserves to lactic acid and, since the lactate accumulating in the heart is equivalent to only one-tenth of the carbohydrate lost from the heart, the lactate diffuses rapidly from the heart into the blood, so accounting for a large part of the rise in blood lactate during asphyxia.

The importance of glycolysis for the maintenance of the heart beat under anaerobic conditions has been shown in work on isolated heart and atrial preparations (Clark, Eggleton, Eggleton, Gaddie & Stewart, 1938; C. L. Evans, 1939). Abnormally low cardiac glycogen levels have been observed in various species exposed to anoxic or severely hypoxic conditions (G. Evans, 1934; Cordier & Dessaux, 1951; Hicks, 1953; Merrick, 1954; Merrick & Meyer, 1954), and, since circumstances which deplete skeletal muscle glycogen, e.g. physical exercise or injection of adrenaline, have no effect on cardiac glycogen (Evans, 1934) it has already been suggested that it provides an emergency reserve for use by the heart in anoxia.

Analysis of cardiac tissue from new-born rats, rabbits and guinea-pigs suggested that in these species, also, the concentration of carbohydrate in the heart may be important for survival in anoxia. A linear relationship was observed between the cardiac carbohydrate concentration in non-asphyxiated individuals and their survival time in nitrogen at 24° C predicted from the data of Fazekas *et al.* 1941 (Fig. 11). It would appear that not only can the survival times of individuals of the same species be predicted from knowledge of their cardiac carbohydrate reserves, but that the same linear relationship may hold for many species. The data from foetal lambs do not fit, but those experiments were executed at a higher temperature, under anaesthesia, and only the tip of the ventricle was taken for analysis. An increase in environmental temperature is known to reduce survival time (Reiss, 1931; Adolph, 1948; Miller & Miller, 1954), and the method of sampling under anaesthesia would lead to a high estimate of cardiac carbohydrate (Evans, 1934; Russell & Bloom, 1955; Timiras, Hill, Krum & Lis, 1958; Weisberg & Rodbard, 1958). The relationship between cardiac carbohydrate and resistance to anoxia at different ages may also apply to man. Villee observed glycogen levels of over 20 mg/g in human foetal hearts, and new-born infants have been known to survive periods of complete anoxia of up to 30 min (Bullough, 1958);

adult man is notoriously susceptible to asphyxia and probably has a much lower cardiac glycogen content (Berblinger, 1912).

Perusal of the literature suggests that many of the differences in resistance to anoxia in adult animals may also be related to differences in cardiac carbohydrate concentration. Thus, although intravenous glucose administered just before exposure to anoxia did not increase the survival time of adult rats (Himwich *et al.* 1943), circumstances which tend to increase the cardiac glycogen content, e.g. prolonged pre-treatment with glucose or maintenance on a high carbohydrate diet, do increase their resistance to oxygen lack (Evans, 1934; Britton & Kline, 1945). Evans (1934) has shown that profound insulin hypoglycaemia, which is known to reduce survival time in anoxia or hypoxia, tends to lower the cardiac glycogen level in adult rats, and Britton & Kline (1945) observed not only a reduced tolerance to hypoxia but also abnormally low cardiac glycogen levels in rats asphyxiated after adrenalectomy. Moreover, very high cardiac glycogen levels have been observed in many lower vertebrates, which also have a high resistance to anoxia (Clark *et al.* 1938; Davies & Francis, 1941; Wu, Yeh & Chang, 1941; Wu & Chang, 1947; Cordier & Dessaux, 1951; Merrick, 1954) as compared with adult mammals (Cruickshank, 1936; Evans, 1939). Arguing teleologically, it may be significant that extensive cardiac glycogen reserves have so far been observed only in foetuses, fish, amphibia and reptiles. Many such animals live under conditions where there is a greater risk of temporary oxygen lack than is usually encountered by adult mammals. In this connexion it is of interest that rats born and maintained at high altitude had a higher cardiac glycogen content than the parent generation born at sea level (Timiras *et al.* 1958).

Factors determining survival in anoxia. It remains to establish whether the relationship between cardiac carbohydrate concentration and the ability to survive anoxia is causal. It seems reasonable to postulate that the differing abilities of foetal lambs of different ages to maintain their blood pressure and heart rate in the absence of oxygen might be related to their cardiac carbohydrate reserves, but in the other species the relationship demonstrated was between cardiac carbohydrate concentration and the duration of gasping. This could be due to a relatively short interval of time between respiratory failure and failure of the circulation, as has been shown in dogs and puppies (Swann & Brucer, 1951; Swann, Christian & Hamilton, 1954). It is possible that the cardiac carbohydrate concentration might be more directly related to the time interval between the onset of anoxia and the point of circulatory failure.

We now have to consider whether the maintenance of the circulation is of paramount importance in anoxia, or whether there is some other limiting factor determining survival, such as the resistance of the brain to lack of oxygen. Swann & Brucer (1951) showed that adult dogs resuscitated within 1 min

after circulatory failure by cardiac massage in addition to artificial respiration, recovered completely. But if cardiac massage and artificial respiration were delayed for 2-3 min the dogs recovered only partially; although breathing and the circulation were restored, the animals were usually ataxic and blind, frequently failed to recover consciousness and usually died within a few days. These dogs had evidently sustained permanent damage to the central nervous system. This suggested that failure of the circulation occurred in adult dogs before the onset of permanent cerebral damage. There is evidence in the literature suggesting that in young animals, also, the same is true (Ford, 1928; Windle & Becker, 1943; Hicks, 1953; Hurder & Sanders, 1953; Cassin & Fregley, 1957). In addition Kabat (1940) showed, from experiments in which he arrested cerebral blood flow in dogs of different ages, that the ability of the brain to survive such treatment was greater than that of the whole animal to survive anoxia. He concluded that the circulation failed before the brain was permanently damaged.

Finally, it must be recognized that the differences between the very young and the adult animals are not confined to the carbohydrate reserves of the heart. For instance, Kabat (1940) showed conclusively that the brains of young puppies were much better able to withstand cerebral stasis than those of adult dogs, and this is probably a consequence of the low rates of aerobic metabolism and anaerobic glycolysis in puppies' brains compared with those of adult dogs (Chesler & Himwich, 1944*b*). In rats, also, there are definite changes in brain composition and metabolism with age but, as emphasized by Adolph (1957), these are all too gradual to account for the rapid decrease in tolerance to anoxia observed in the first few days after birth. The decrease in cardiac carbohydrate with age described in the present work is, to date, the only known change which exactly parallels the decrease in tolerance to anoxia. This suggests that the maintenance of the circulation is of paramount importance in anoxia. Future work must determine whether controlling tissue pH and ensuring an adequate supply of glucose will increase both the ability of the heart to maintain the circulation and also the ability of the brain to withstand anoxia.

SUMMARY

1. The ability of foetal lambs to survive anoxia has been investigated by recording the foetal blood pressure and heart rate continuously before and after tying the umbilical cords of lambs delivered by Caesarean section under chloralose anaesthesia.

2. Lambs of 83-91 days gestation age maintained their blood pressure above 16 mm Hg and their heart rate above 50 per minute for up to 60 minutes after tying the umbilical cord; if the cord was untied within 40 minutes, the blood pressure and heart rate returned to their pre-anoxic levels. Older lambs, 126-146 days gestation age, maintained their blood pressure and heart

rate for only 10–15 minutes after tying the cord. Adult sheep rebreathing nitrogen did not survive for more than 7 minutes.

3. The rates of anaerobic glycolysis in foetal lambs were compared by measuring the blood and tissue lactate and carbohydrate levels in unasphyxiated control lambs and in other lambs at various intervals of time after tying the umbilical cord. The initial rates of glycolysis in the tissues of the older lambs were either equal to or greater than those in the younger lambs. There was a correlation between survival time and the initial cardiac carbohydrate concentration in the lambs of each age group.

4. The cardiac carbohydrate concentration has been measured in unasphyxiated new-born rats, rabbits and guinea-pigs of different ages; there was a linear relationship between cardiac carbohydrate concentration and the predicted time of survival in 100% nitrogen.

5. These results have been discussed with reference to previous hypotheses as to the ability of very young animals to survive anoxia longer than adults. It was concluded that the maintenance of the circulation is of primary importance in anoxia.

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