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THE SURVIVAL OF YOUNG RATS IN NITROGEN

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Young animals that are born blind and relatively immobile can survive for a considerable period in the absence of oxygen. This resistance to anoxia decreases rapidly with increasing age, and reaches adult values within a few days to three weeks after birth. It has been established that glycolysis provides energy for anaerobic survival in young (Himwich, Bernstein, Herrlich, Chesler & Fazekas, 1942; Hicks, 1953) and foetal animals (Dawes, Mott & Shelley, 1959). The investigations of Himwich and his colleagues have been concerned mainly with the resistance of the brains of young animals to oxygen lack (Chesler & Himwich, 1944). More recently, Dawes *et al.* (1959) showed that the survival of foetal lambs after tying the umbilical cord is related to the amount of glycogen stored in the heart, and suggested that one of the limiting factors in anaerobic survival is the ability of the heart to maintain the circulation. There was also a direct correlation between the initial levels of carbohydrate in the hearts of new-born rats, rabbits and guinea-pigs and the times for which gasping movements persisted in nitrogen (Dawes *et al.* 1959). The experiments reported in this paper were designed to investigate the importance of the glycogen in the hearts of new-born rats for their survival in nitrogen, and to attempt to define more clearly the factors determining the anaerobic survival of new-born animals.

METHODS

Animals. Parturition in a rat usually lasts about 2 hr. During this time the first-born rats become cold. After labour is complete, the mother gathers all the rats into a nest and they start to feed. The temperature of the nest is 31–33° C. The number of animals in a litter varies from about eight to fifteen. The weight of a new-born rat is 4–5·5 g; the animals in the larger litters usually weigh less than the animals in the smaller litters. Before experiments the young rats were removed from the nest and were kept in a box maintained at 33° C. Except in fasting experiments, rats were used within 2 hr of removal from the nest Rats of either sex and from 0 to 18 days of age were used.

Exposure to nitrogen. Rats were placed in a double-walled glass tube. Water from a thermostatically controlled bath was pumped between the walls of the tube so that the temperature within the tube was constant to within ±0·2° C. Air or nitrogen passed through the tube was humidified by being bubbled through water and warmed by being passed through coils in a water-bath. Nitrogen, as supplied commercially, was passed through at about 6 l./min

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initially, and at about 2 l./min after 2 min. Samples of nitrogen from the cylinder and of the effluent gas, collected after passing nitrogen through the tube for 2 min, were analysed by Haldane's method, and contained less than 0·1 % oxygen. During exposure to nitrogen the rats were watched through the walls of the tube. The time between starting nitrogen and the last breath was recorded. The heart rate was recorded electrically from two fine wires looped round the thorax and lower abdomen, making contact through electrode jelly. The rat was placed in the tube and the electrode wires were led through the gas outlet to an amplifier which drove a pen writer and a loudspeaker. The heart rate was counted at intervals before and during exposure to nitrogen.

Biochemical estimations. Tissues were extracted in 5 % trichloroacetic acid containing 0·1 % Ag_2SO_4 (Kemp & Kits van Heijningen, 1954) and total carbohydrate in the deproteinized extracts was measured by the method of Mendel, Kemp & Myers (1954). As this method measures glycogen, glucose-1-phosphate, glucose and fructose, losses arising from glycogenolysis during sampling were reduced. Hearts were removed from the rats within 15 sec after decapitation. If other tissues were taken from the same animals, the sampling was completed within 60 sec. For estimation of total lactic acid production rats were decapitated and the head and body homogenized in deproteinizing fluid in a M.S.E. high-speed blender. Lactic acid was measured in the deproteinized extract by the method of Barker & Summerson (1941). Blood was collected on a siliconed watch-glass. The volume was measured in a graduated 0·1 ml. pipette which had been rinsed with heparin-fluoride solution (0·4 % heparin, 8 % sodium fluoride). Volumes between 0·04 and 0·10 ml. could be obtained from new-born rats. Blood was deproteinized with 5 % $ZnSO_4$ $7H_2O$ and 0·3 N-NaOH. Glucose was measured by the glucose oxidase method of Huggett & Nixon (1957) and lactic acid by the method of Barker & Summerson (1941).

The figures quoted for survival times and for biochemical estimations are expressed as mean ± standard error throughout.

RESULTS

The behaviour of new-born rats in nitrogen

Shortly after new-born rats (< 1 day old) were placed in nitrogen their respiration became rapid and shallow. They struggled a little, but did not have convulsions; there was a pronounced fall in heart rate (Fig. 8). From 2 to 20 min after exposure to nitrogen breathing became irregular and sometimes intervals as long as 4 min were seen between gasps. From about 20 min onwards, gasps became more frequent (1–2/min) and reached a maximum rate of about 12/min which persisted for only 30–60 sec. They stopped abruptly. After this there were no further respiratory movements. Rats taken out of nitrogen during this final phase of rapid gasping recovered, apparently completely. Rats left in nitrogen until 15 sec after breathing had stopped did not breathe again. The survival time of a new-born rat in nitrogen was about 28 min at an environmental temperature of 36° C. Electrical activity in the heart was measurable for 5–10 min after the last breath. As the last breath could be timed with reasonable precision, this was used as an end point for survival. At lower environmental temperatures (20° C) the terminal acceleration of breathing was not so clear, but in experiments at 36° C it was seen in all rats.

Rats 16 days old had convulsions from about 15 to 30 sec after exposure to nitrogen. There was then a period of apnoea lasting 30 sec or more, which

was followed by gasps which increased in rate and decreased in depth for the last 15 sec. The mean survival time (to the last gasp) was 2·5 min at 36° C. Rats of 2, 4 and 8 days of age behaved like new-born rats, but the duration of each phase became shorter with increasing age.

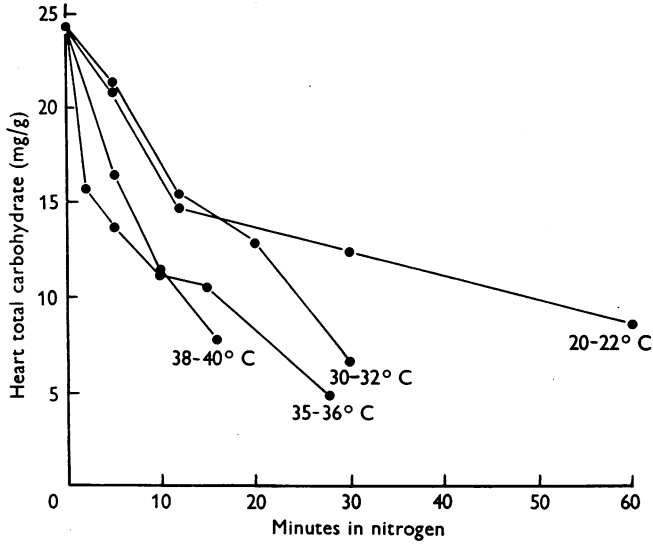

Fig. 1. Rates of depletion of total carbohydrate in the hearts of new-born rats exposed to nitrogen at different environmental temperatures. Each point is the mean of two to four observations. The last point is at the mean time to last breath.

Changes in blood and tissue carbohydrate and lactic acid in new-born rats exposed to nitrogen

Figure 1 shows the decrease of heart carbohydrate in rats less than 24 hr old which were placed in nitrogen at different environmental temperatures. The time to last breath decreased as the temperature of the chamber was increased; heart carbohydrate fell more rapidly at higher temperatures. In all the following experiments in this series rats were exposed to nitrogen at 35–36° C.

Changes in blood glucose and the carbohydrate concentrations in various tissues were measured in rats less than 24 hr old exposed to nitrogen. Preliminary experiments indicated a wide variation in the initial concentration of liver carbohydrate, which ranged from 4 to 100 mg/g in rats less than 24 hr old. On further investigation it became apparent that the concentration of carbohydrate in the liver was related to the time after birth. Figure 2 shows that the concentration of liver carbohydrate in two litters of unasphyxiated rats at birth ranged from 50 to 90 mg/g, but fell rapidly during the first 18 hr of life. This fall occurred although the young rats were suckled, and milk was visible in their stomachs through the

body wall. On exposure to nitrogen a rise in blood glucose was found in rats which had initially a high liver carbohydrate, but only a progressive fall in blood glucose in rats with a low liver carbohydrate. For instance, the mean liver carbohydrate in two litters of rats less than 4 hr old was $58 \pm 7{\cdot}7$ mg/g and during exposure of litter-mates to nitrogen this fell to $36 \pm 3{\cdot}4$ mg/g. The blood glucose concentration, which was initially $73 \pm 8{\cdot}5$ mg/100 ml., rose during anoxia to $116 \pm 7{\cdot}2$ mg/100 ml. and remained at about this level until the last breath.

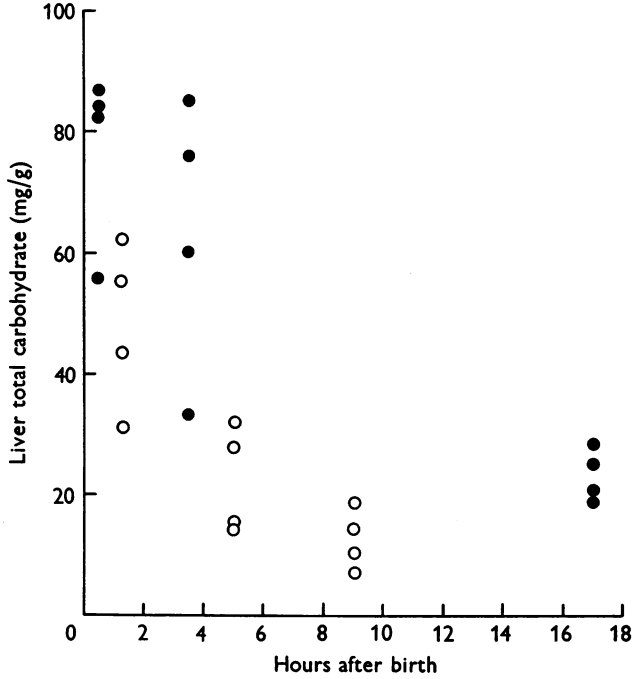

Fig. 2. The concentration of carbohydrate in the livers of new-born rats from two litters (○, litter A; ●, litter B) killed at different times during 18 hr after birth.

In three litters of rats 18–24 hr old the concentration of liver carbohydrate, which was initially much lower than in the younger rats, fell during exposure to nitrogen by only about 4 mg/g (Fig. 3). The blood glucose, initially $63 \pm 6{\cdot}9$ mg/100 ml., fell during exposure to nitrogen to $23 \pm 5{\cdot}2$ mg/100 ml. The total carbohydrate in the heart was almost depleted but in skeletal muscle, lung and skin fell only slightly during anoxia (Fig. 3). As the mean survival time (28·6 min) of litter-mates of ten rats with a low initial liver carbohydrate (< 10 mg/g) was not significantly different ($P > 0{\cdot}9$) from that (28·4 min) of litter-mates of fifteen rats with a high initial liver carbohydrate (mean 51 mg/g), it appears that the ability to mobilize liver glycogen was not of great importance for survival in nitrogen.

The changes in concentrations of lactic acid and of heart and liver carbohydrate in new-born rats before and after exposure to nitrogen are summarized in Table 1. From this it will be seen that during anoxia lactic acid in the bodies of rats less than 12 hr old increased from 0·24 to 1·78 mg/g; this amounted to a change of +7·3 mg in a rat weighing 4·7 g. Concentrations of carbohydrate in the heart and liver fell by about the same amount (21–22 mg/g) but the liver weighed about eight times as

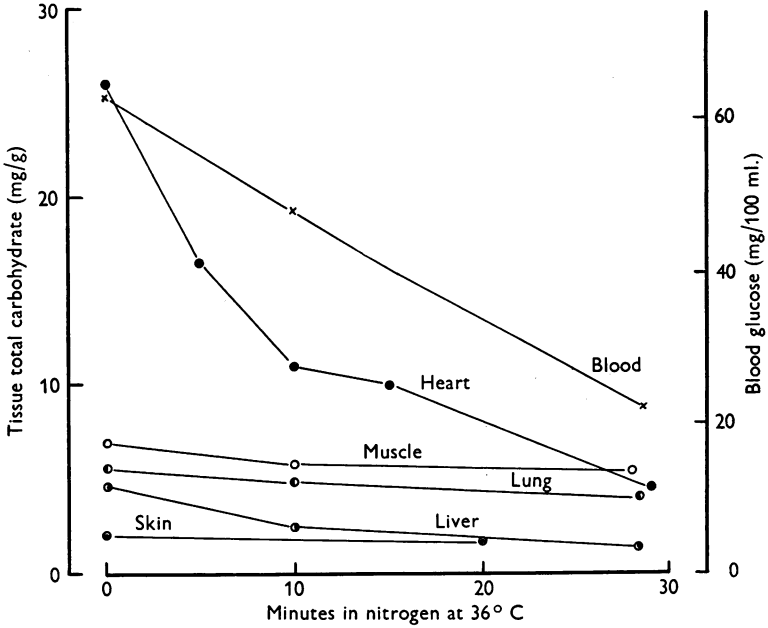

Fig. 3. Concentrations of blood glucose and tissue carbohydrate in new-born rats (18–24 hr old) after exposure to nitrogen for 10 or 20 min or until the last breath. Each point is the mean of four to ten observations (× blood glucose; ● heart; ○ skeletal muscle; ◐ lung; ◑ liver; ⊙ skin).

much as the heart. The loss of carbohydrate from the liver and heart was estimated as 5·2 mg and could account for 72 % of the increase in lactic acid. These results suggested that the carbohydrate mobilized from the livers of young rats during anoxia was being metabolized to lactic acid, but further experiments seemed necessary to determine the relative significance of heart and liver carbohydrate as energy reserves for anoxic survival.

Changes in survival time in nitrogen and in heart and liver carbohydrate with age

The survival times in nitrogen and the initial concentrations of carbohydrate in the hearts and livers of rats from 0 to 16 days of age are shown

in Fig. 4. It will be noticed that the large fall in liver carbohydrate during the first 24 hr after birth was accompanied by only small changes in the concentration of heart carbohydrate and in the survival time. After the

TABLE 1. Mean concentrations of lactic acid in whole rats, and of carbohydrate in hearts and livers of rats less than 12 hr old before anoxia, and of litter-mates exposed to nitrogen until last breath

	Before anoxia (mg/g)	After exposure to nitrogen (mg/g)	Change in concentration (mg/g)	Mean tissue weight (g)	Total change (mg)	
Whole body lactic acid	0·24 (7)	1·78 (9)	+1·56	4·7	+7·3	
Heart carbohydrate	26 (7)	4·7 (13)	−21·3	0·03	−0·64	−5·2
Liver carbohydrate	58 (8)	36 (8)	−22	0·21	−4·6	

The figures in brackets indicate the number of rats.

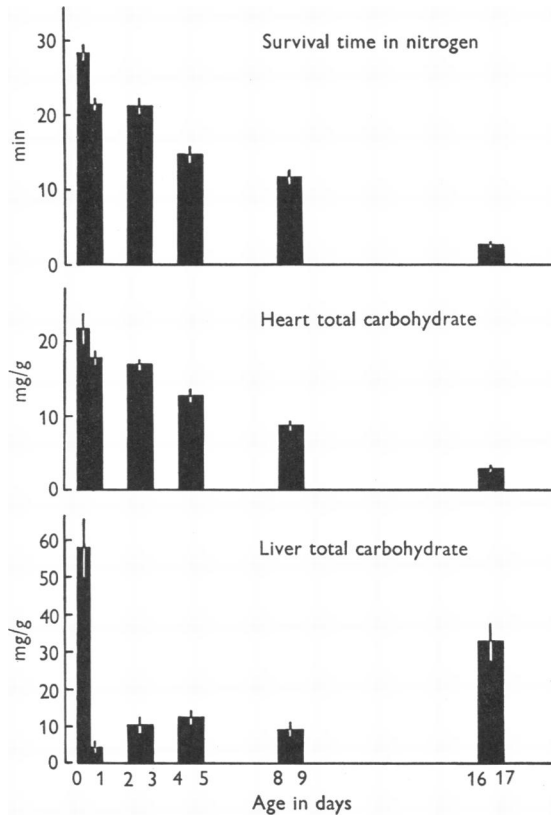

Fig. 4. Concentrations of carbohydrate in the hearts and livers of unasphyxiated new-born rats and the survival times in nitrogen of their litter-mates during 17 days after birth (each block shows the mean and S.E. of ten observations).

first day of life, the concentration of carbohydrate in the liver began to rise, until in 16-day-old rats it had risen to more than half the concentration at birth. At this age the concentration of carbohydrate in the heart and the survival time in nitrogen had fallen to the lowest levels observed in these experiments.

The effect of fasting on heart and liver carbohydrate and on survival time in nitrogen

Rats up to 6 hr old were removed from their nests and kept in a humidified box maintained at 33 ± 1° C. Preliminary experiments showed that control of temperature was essential, as the body temperature (measured with a rectal thermocouple) followed the environmental temperature closely and the carbohydrate in the tissues disappeared more slowly at lower temperatures. The concentrations of carbohydrate in the heart and liver and the survival times in nitrogen of litter-mates were measured before fasting and after 3, 6 and 12 hr fasting.

After 12 hr fasting rats had a lower concentration of carbohydrate in their livers than fed rats of the same age. During fasting the concentration of carbohydrate in the liver decreased rapidly, while the concentration of carbohydrate in the heart fell more slowly. After 3, 6 and 12 hr fasting, the percentage reductions in survival time and in heart carbohydrate (CHO) were closely similar; the percentage fall in liver carbohydrate was much greater (Fig. 5).

The terminal concentration of carbohydrate in the hearts (samples 15 sec after the last breath in nitrogen) of fasting and fed rats were not significantly different (Table 2), although terminal concentrations of lactic acid in the hearts and blood were lower in the fasting than in the fed rats. These results suggest that the high terminal concentration of lactic acid in the heart and blood do not prevent the utilization of the 5–6 mg/g of carbohydrate which remained in the heart when breathing stopped.

Recovery of carbohydrate stores in heart and liver and of survival times in nitrogen after a non-fatal period of anoxia

Rats from six litters (16–20 hr old) were placed in nitrogen at 36° C for about 25 min, and were then placed in air at 33° C. All the rats were breathing normally within a few minutes and appeared to recover completely. After 1 hr in air, five–six rats were decapitated, and the concentrations of blood lactic acid and of carbohydrate in the hearts and livers were measured; five–six of their litter-mates were again placed in nitrogen and the time to last breath measured. Similar observations were made after 2, 3 and 4 hr recovery in air. The results are shown in Fig. 6. After depletion by anoxia the concentration of heart carbohydrate returned almost

to its initial level in 3 hr. The time to last breath of animals exposed for a second time to nitrogen increased in the same proportion as the increase in concentration of the heart carbohydrate. The concentration of carbohydrate in the liver remained low during the 4 hr after anoxia, although the blood lactic acid fell from a mean of 148 mg/100 ml. to normal levels within 2 hr (Table 3).

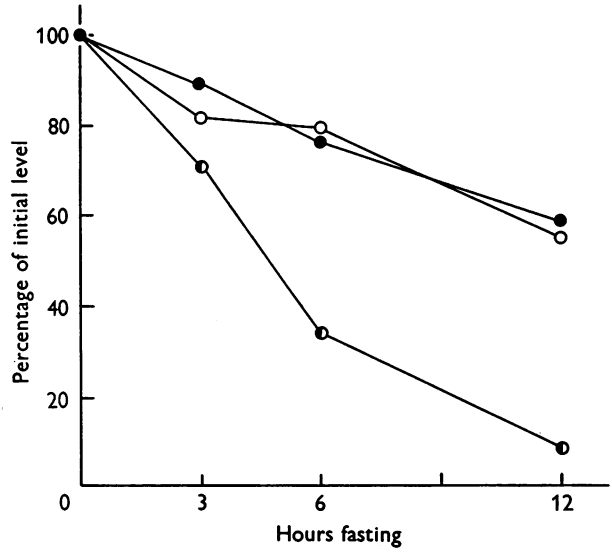

Fig. 5. Percentage changes in the concentrations of carbohydrate in the livers and hearts of new-born rats fasting for 3, 6 and 12 hr and percentage change in survival time in nitrogen of their litter-mates. Each point is the mean of fifteen observations (◑, liver; ●, heart; ○, survival time).

TABLE 2. Terminal concentrations of carbohydrate in the heart and of lactic acid in the heart and blood after exposure of new-born rats and their fasting litter-mates to nitrogen. (Each figure is the mean of 13 observations).

	Unfasted	Fasted	Significance of difference
Heart total carbohydrate (mg/g)	4·8 ± 0·5	6·3 ± 1·2	$P > 0·1$
Heart lactic acid (mg/g)	4·2 ± 0·1	2·7 ± 0·3	$P < 0·001$
Blood lactic acid (mg/100 ml.)	240 ± 12	152 ± 13	$P < 0·001$

The effects of insulin on blood glucose, heart carbohydrate concentration and time to last breath in nitrogen

Figure 7 shows that insulin (1 or 2 u./g subcutaneously) caused a fall in blood glucose in new-born rats which was maximal within 2 hr. At this time after injection there was a small but significant decrease in the mean concentration of carbohydrate of the hearts from 26·4 ± 0·67 mg/g to 23·3 ± 1·1 mg/g (Table 4).

In order to test the effect of insulin on the time to last breath in nitrogen, rats from two litters less than 24 hr old were divided into two groups. Rats of one group were injected subcutaneously with 1 u./g of insulin; the others were injected with the same volume of 0·9 % NaCl. Between 1 and 2 hr after injection, all the rats were placed in nitrogen.

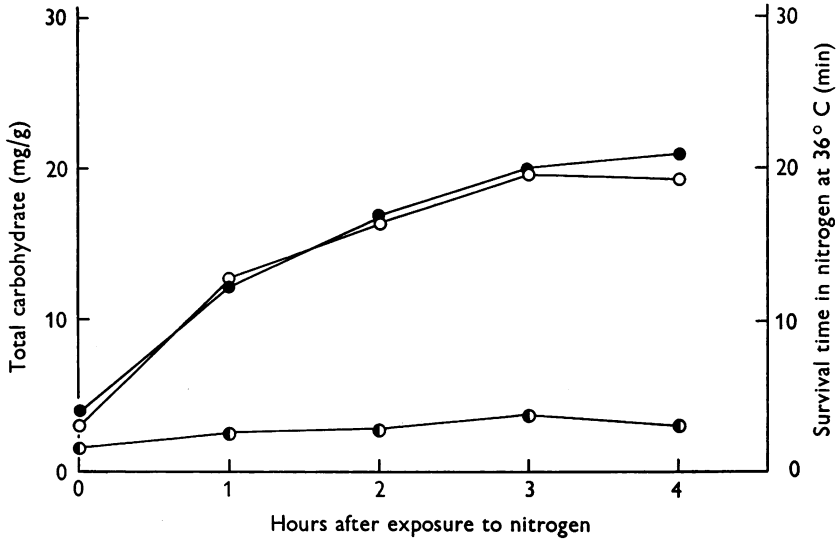

Fig. 6. Concentrations of carbohydrate in the heart and liver of new-born rats and survival times in nitrogen of litter-mates during recovery from exposure to nitrogen (each point is the mean of five or six observations; ●, heart; ◑, liver; ○, survival time).

TABLE 3. Decrease in blood lactic acid in new-born rats after a non-fatal period of anoxia. (Each estimation from 1 rat)

Hours after exposure to nitrogen	0	1	2	3
Blood lactic acid (mg/100 ml.)	137	40	12	12
	146	17	16	3
	160	68	7	9

Heart rates were recorded and the times to last breath and to last heart beat were observed. The time to last breath was decreased in insulin-treated animals. Insulin-treated animals stopped breathing after 14·2 ± 1·0 min in nitrogen, whereas litter-mate controls continued to breathe for 24·2 ± 1·1 min in nitrogen (Table 4). The mean heart rate in the control rats at 36° C before exposure to nitrogen was 316 beats/min and in the insulin-treated rats was 292 beats/min, and changes in the heart rate during exposure to nitrogen were similar in insulin-treated and control animals. The heart rate of both groups decreased rapidly during the first 4 min and then more slowly (Fig 8) and the times for which the heart con-

tinued to beat were not significantly different in the two groups of rats. From this it appears that the heart rate during exposure to nitrogen is not affected by low blood glucose levels. However, rats treated with insulin stopped breathing while the heart rate was 95 beats/min, whereas control rats stopped breathing when the heart rate was 62 beats/min.

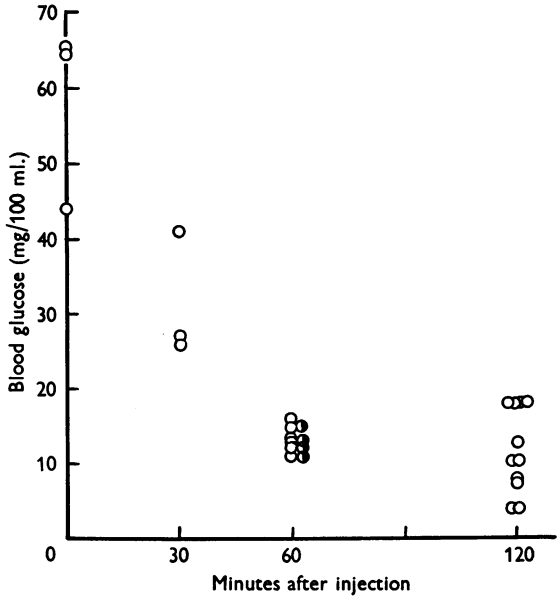

Fig. 7. Concentrations of blood glucose in new-born rats after subcutaneous injection of 1 u./g (○) or 2 u./g (◑) of insulin.

TABLE 4. The effect of insulin on carbohydrate levels in new-born rats and on their survival in nitrogen

	Initial heart carbohydrate (mg/g)	Initial blood glucose (mg/100 ml.)	Time to last heart beat in nitrogen (min)	Time to last breath in nitrogen (min)
Untreated rats	26·4 ± 0·67 (7)	60 ± 3·0 (10)	33 ± 2·6 (7)	24·2 ± 1·1 (7)
Insulin-treated rats (1 u./g)	23·3 ± 1·1 (8)	12 ± 1·4 (11)	31 ± 1·8 (6)	14·2 ± 1·0 (6)
Significance of difference between control and treated rats	$0·05 > P > 0·02$	$P < 0·001$	$0·7 < P < 0·8$	$P < 0·001$

(Figures in brackets indicate the number of observations).

The effects of glucose on the survival times of young rats

In the following experiments litter-mates were paired for size. Ten minutes before exposing them to nitrogen one rat of each pair was injected intraperitoneally with 0·01 ml./g of 20% glucose and the other with 0·9% NaCl.

Rats less than 24 hr old. In one litter (A) in which the blood glucose of the control rats at the time of last breath in nitrogen was greater than 97 mg/100 ml., the survival time was not increased by injection of glucose, although the blood glucose concentration was significantly increased (Table 5). In another litter (B) in which the control rats died in nitrogen

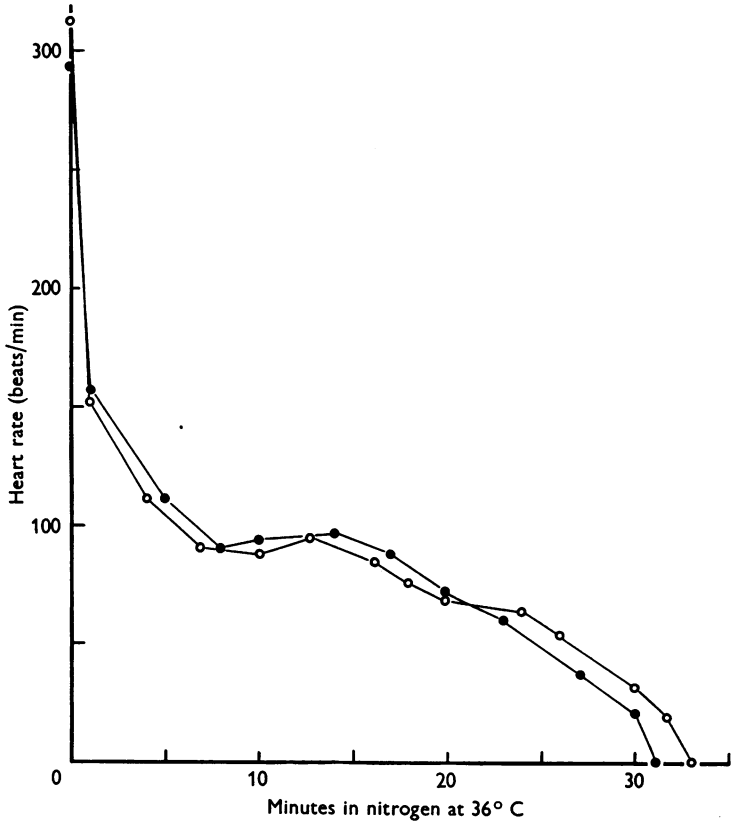

Fig. 8. The heart rates of untreated and insulin treated new-born rats during exposure to nitrogen (each point is the mean of six to seven observations; ●, insulin-treated rats, 1 u./g s.c.; ○, control rats 0·9 % NaCl.)

with a low blood glucose (< 25 mg/100 ml.) injection of glucose before exposure to nitrogen increased the survival time (Table 5).

However, in each litter there was no significant difference between the final concentrations of carbohydrate in the hearts of glucose-treated and of control rats (Table 5).

These results suggested that intraperitoneal glucose prolongs the survival time of new-born rats if the blood glucose cannot be maintained. Other conditions in which injection of glucose may be expected to prolong

the anoxic survival time were then investigated. Rats from three litters were exposed to nitrogen for 22 min and then allowed to recover in air. After 1 hr litter-mates were treated with intraperitoneal glucose or saline and 10 min later were re-exposed to nitrogen. Survival times and terminal blood glucose were measured, and under these conditions a significant prolongation of the time to last breath was found (Table 6). However, part of this increase in survival time is attributable to the increase in total

TABLE 5. Effect of glucose injection on the survival of new-born rats in nitrogen. (Each figure is the mean of 4 observations)

		Survival time in nitrogen (min)	Terminal heart carbohydrate (mg/g)	Terminal blood glucose (mg/100 ml.)
Litter A	Control	24·4	8·5	131
	Glucose-treated	24·8	9·1	223
	Significance of difference	$0{\cdot}6 < P < 0{\cdot}7$	$0{\cdot}8 < P < 0{\cdot}9$	$0{\cdot}05 < P < 0{\cdot}1$
Litter B	Control	22·8	1·5	14
	Glucose-treated	31·8	2·0	95
	Significance of difference	$P < 0{\cdot}001$	$0{\cdot}5 < P < 0{\cdot}6$	$P = 0{\cdot}02$

TABLE 6. Effect of glucose injection on the survival time in nitrogen and terminal blood glucose of new-born rats in which carbohydrate reserves were partially depleted by a previous period of anoxia. (Each figure is the mean of 11 observations)

	Mean survival time in nitrogen (min)	Terminal blood glucose (mg/100 ml.)
Controls	$17{\cdot}1 \pm 0{\cdot}9$	$10 \pm 1{\cdot}0$
Glucose-treated (2 mg/g I.P.)	$28{\cdot}4 \pm 0{\cdot}9$	95 ± 14

heart carbohydrate which occurs during the 10 min after glucose injection. For example, control rats 1 hr after the first exposure to nitrogen had a heart carbohydrate of $15{\cdot}8 \pm 1{\cdot}54$ mg/g, and their litter-mates 10 min after the injection of glucose had a heart carbohydrate of $18{\cdot}4 \pm 0{\cdot}39$ mg/g.

Rats of 4, 8 *and* 18 *days of age.* Glucose (2 mg/g in solution) was given intraperitoneally to one of a pair of litter-mates and saline intraperitoneally to the other. Ten minutes later both rats were exposed to nitrogen. The results are shown in Table 7. In rats of all ages the terminal blood glucose concentration was significantly increased by injection of glucose before exposure to nitrogen. In rats of 8 and 18 days of age, the survival time in nitrogen of glucose-injected rats was significantly increased ($P < 0{\cdot}01$), but there was no significant increase of survival time in rats 4 days old. Terminal concentrations of heart carbohydrate were not significantly different in control and glucose-treated rats.

TABLE 7. Effect of glucose injection on the survival of young rats in nitrogen: paired litter-mates were used

Age in days	Control			Glucose-treated		
	Survival time (min)	Terminal heart carbohydrate (mg/g)	Terminal blood glucose (mg/100 ml.)	Survival time (min)	Terminal heart carbohydrate (mg/g)	Terminal blood glucose (mg/100 ml.)
4	18·6 (7)	3·6 (7)	78* (7)	21·8 (7)	4·3 (6)	185* (7)
8	8·3 (8)*	0·8 (4)	134* (4)	11·1* (8)	0·9 (4)	316* (4)
18	1·6 (6)*	0·8 (6)	71* (6)	2·4* (6)	0·9 (5)	151* (6)

* Differences between control and glucose-treated groups are significant ($P < 0·01$). Figures in brackets indicate the number of observations.

DISCUSSION

During the exposure of new-born rats to nitrogen, the concentrations of carbohydrate in the heart and liver were reduced much more than those in the lung, muscle or skin. Hicks (1953) showed by histological methods that the heart and liver glycogen stores were depleted under these conditions, but the glycogen contents of the skeletal muscle and skin were not appreciably reduced. He showed that brain glycogen was low and was unable to measure histologically any change during anoxia. Shapiro & Wertheimer (1943) found that there was no phosphorylase in the skeletal muscles of the new-born rat. They also found that extracts of brains of new-born rats, because of the absence of phosphoglucomutase, did not break down glycogen. It seems therefore unlikely that endogenous glycogen can be metabolized in either the skeletal muscle or brain of new-born rats, and that blood glucose must provide most of the energy for both muscle and brain metabolism. When the rat is placed in nitrogen the availability of blood glucose to the brain and muscle must become an important factor in determining the activity of these tissues. When a new-born rat is placed in nitrogen there is a rise in blood glucose, but in a rat about 24 hr old there is no rise in blood glucose during anoxia, probably because by this age the initial liver carbohydrate has fallen too low to maintain the blood glucose level. However, as there is only a small decrease in survival time during the first 24 hr of life, it appears that blood glucose is not an important limiting factor in determining the anoxic survival. Also, injections of glucose before exposure to nitrogen produced only slight, if any, prolongation of the survival time. These results immediately cast doubt on the importance of liver glycogen reserves in the anoxic new-born rat, as liver glycogen can be made available to other tissues only after mobilization to blood glucose. We find no relation between the liver carbohydrate concentration and the time for which young rats continue to breathe in nitrogen. The most striking fall in liver carbohydrate which

was found occurred in the first 18 hr after birth and was not accompanied by a proportionate decrease in the survival time in nitrogen. Britton & Kline (1945) appear to have over-emphasized the part played by liver glycogen in determining anaerobic survival; they state: 'the relatively large size of the liver and its high concentration of glycogen (6–8 %) in young rats (Corey, 1935) would, for example, appear to be involved in the striking degree of tolerance (to anoxia) observed...when liver glycogen levels have declined to approximately adult values, at 3–4 weeks of age (Britton & Silvette, 1932), anoxic resistance significantly reaches adult or even subadult levels'. We have found in normal new-born rats that neither liver glycogen nor blood glucose determines the survival in nitrogen. However, under some experimental conditions blood glucose may become a limiting factor. For example, when insulin-treated rats in which the initial blood glucose is less than 18 mg/100 ml. are exposed to nitrogen, gasping movements persisted only 14 min as opposed to 24 min in their litter-mate controls. Glucose has been found to restore to normal the survival time in nitrogen of insulin-treated rats (Himwich, Fazekas & Homburger, 1943). Glucose injection also prolongs the survival time in nitrogen of rats which have been allowed to recover partially from a previous period of anoxia.

The importance of carbohydrate in the heart. Under all the experimental conditions tested, with the single exception of the insulin-treated rats, the time to last gasp in nitrogen was proportional to the initial level of carbohydrate in the heart. First, during fasting, the survival time decreased in the same proportion as heart carbohydrate; liver carbohydrate decreased more rapidly. Secondly, during recovery from non-fatal exposure to nitrogen, heart carbohydrate and survival time increased together while liver carbohydrate remained low. Thirdly, with increasing age, survival time followed closely changes in the heart carbohydrate, but not changes in liver carbohydrate. This supports the suggestion of Dawes *et al.* (1959) that the heart carbohydrate concentration and the survival in anoxia may be causally connected. In this case, cessation of respiration would be due to failure of the circulation to the brain and respiratory muscles and their nerves. Even in insulin treated animals, there was a correlation between the initial level of carbohydrate in the heart and the time for which the heart continued to beat in nitrogen. Only after insulin did the respiration of an anoxic new-born rat appear to fail from lack of blood glucose while there was still a relatively high heart rate.

It should, however, be emphasized that these investigations, while they support the view that the initial heart carbohydrate concentration is one factor in determining survival during anoxia, do not exclude the participation of other variables. The importance of environmental temperature

has already been referred to (Fig. 1) and has been investigated by other workers. The fall in blood pH which occurs during anoxia is another determining factor under certain experimental conditions (Dawes, Mott & Stafford, unpublished). There is a further variable related to changes in the tissues with increasing age, irrespective of the integrity of the circulation. Thus it is well known, and we have confirmed the observation, that the decapitated head of a new-born rat will continue to make respiratory efforts for many minutes, far longer than that of an adult yet less than that of an intact anoxic new-born rat. In addition, Whittam (1960) has shown that kidney slices from immature animals retain potassium during anoxia for longer than those from mature animals of the same species. In the future we shall have to study not only the decreasing ability of individual tissues to withstand anoxia with increasing age, and the reason for the fall in the carbohydrate reserves of the heart, but also the way in which these, and other factors, contribute to the astonishing ability of immature animals to withstand anoxia.

SUMMARY

1. The times to last breath of new-born rats in nitrogen increased with decreasing environmental temperature. Heart carbohydrate was depleted more rapidly at higher temperatures.

2. During exposure of new-born rats to nitrogen, the heart carbohydrate was almost depleted, the liver carbohydrate was depleted or reduced, and skin, muscle and lung carbohydrate concentrations were only slightly decreased. Increase in blood glucose was related to the concentration of carbohydrate in the liver. Loss in liver and heart carbohydrate during exposure of new-born rats to nitrogen accounted for 72% of the lactic acid production, but during the first day of life while the liver carbohydrate was rapidly falling, the time to last breath in nitrogen decreased only slightly.

3. Heart carbohydrate and survival time in nitrogen decreased steadily between 0 and 16 days of age; liver carbohydrate fell from a mean of 58 mg/g to a minimum (< 10 mg/g) during the first 24 hr of life and increased gradually to adult levels (33 mg/g) at about 16 days of age.

4. During depletion of liver and heart carbohydrate by fasting, survival time decreased in the same proportion as heart carbohydrate. When the tissue carbohydrate was reduced by exposure to nitrogen, heart carbohydrate and survival time in rats re-exposed to nitrogen recovered in the same proportion.

5. Insulin (1–2 u./g) reduced the blood glucose of new-born rats to less than 20 mg/100 ml. and reduced the mean time to last breath in nitrogen

to 14·2 min; initial heart carbohydrate and the time for which heart beats persisted in nitrogen were only slightly reduced by insulin.

6. The survival times of new-born rats in nitrogen were prolonged by intraperitoneal injection of glucose only under the following conditions: (*a*) In litters in which untreated rats died with a very low blood glucose or (*b*) in rats whose carbohydrate levels were depleted by previous exposure to nitrogen. The survival times of 8- and 18-day-old rats were slightly but significantly increased by glucose injection 10 min before exposure to nitrogen.

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