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THE EFFECTS OF INSULIN ON THE NEW-BORN CALF

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SUMMARY

1. The normal variations in the concentrations of glucose, fructose and lactic acid in the blood of the calf which occur during the first few weeks after birth have been examined.

2. The responses of calves of different ages to intravenous injections of insulin have been examined by recording both the incidence of convulsions and the changes in the concentration of glucose, fructose and lactic acid in the blood.

3. New-born calves rarely convulsed during prolonged and severe hypoglycaemia and, if convulsions occurred, the onset was delayed by 6–8 hr. At 7 days of age convulsions usually followed the injection of insulin within $1\frac{1}{2}$–2 hr.

4. No relationship could be found between the duration of hypoglycaemia and the incidence of convulsions at different ages. Hypoglycaemia was most prolonged in new-born calves which rarely convulsed.

5. Insulin hypoglycaemia during the first 24 hr after birth was associated with a rise in the concentration of lactate in the blood. Similar changes did not occur in calves at 7 days of age, in which the incidence of convulsions was much higher, or in weaned animals.

6. After both splanchnic nerves had been cut, insulin always caused convulsions in 24-hr-old calves. There was no rise in the blood lactate concentration in these animals.

7. Intravenous infusions of adrenaline but not noradrenaline in amounts similar to those known to be released from the adrenal medulla of the calf of this age prevented convulsions in 24-hr-old calves after section of the splanchnic nerves. These infusions had little effect on the blood glucose concentration but caused a similar rise in the lactic acid concentration to that found in normal animals at this age during hypoglycaemia.

8. At 7 days of age convulsions could only be prevented during hypoglycaemia by infusing larger doses of adrenaline which significantly raised the blood glucose concentration. The increase in the blood lactate concentration was less than that in the new-born animals.

9. The resistance to insulin hypoglycaemia which occurs immediately after birth is transient; it depends upon the release of adrenaline from the adrenal medulla and is associated with high concentrations of lactate in the blood during hypoglycaemia.

INTRODUCTION

Marked changes in the distribution of carbohydrate occur immediately before and just after birth. The concentration of glycogen in the liver, which is extremely high at the end of gestation, falls abruptly and the blood glucose concentration rises progressively during the next 24 hr (Shelley, 1960, 1961). These changes presumably depend upon the mechanisms which regulate carbohydrate metabolism and may be accentuated in ruminants, in which the concentration of glucose in foetal blood at term (20–30 mg/100 ml.) is lower than in other species.

Alterations in the activity of various enzyme systems which are implicated in the metabolism of carbohydrate are known to occur at the end of gestation (Dawkins, 1961, 1963, 1966; Burch, Lowry, Kuhlman, Skerjance, Diamant, Lowry & Von Dippe, 1963), but little is known about the hormonal or other mechanisms which influence the concentration of glucose in the blood immediately after birth. In the present experiments, the variations in the concentration of glucose, fructose and lactic acid in the blood of normal new-born calves have been established and the response of these animals to insulin hypoglycaemia has been examined.

Some of these results have been published previously as preliminary communications (Comline & Edwards, 1962; Edwards, 1964).

METHODS

The experiments were carried out on Jersey calves (18–25 kg) which were removed to the laboratory immediately after birth. Those animals which were used within 24 hr were not fed, others received colostrum, and thereafter were given milk (6 pints/day, i.e. 3·4 l./day) until they were introduced to solid food and weaned completely between 60 and 90 days of age.

Animals which were tested before weaning were not fed on the day of the experiment and food but not water was withheld for 48 hr before experiments on weaned animals. Only one experiment was carried out on any individual calf since preliminary experiments showed that injections of insulin may sensitize animals to hypoglycaemia for prolonged periods.

Experimental procedures. All blood samples (3–5 ml.) were withdrawn from the jugular vein and transferred immediately to tubes containing heparin and sodium fluoride. Samples were collected from several calves within 1 min of birth following normal pregnancy and unassisted parturition. Blood was also obtained from four foetuses at the end of gestation immediately after they were removed from the uterus. The cows were anaesthetized with sodium pentobarbitone and the technique used in these experiments is described elsewhere (Comline & Silver, 1966).

Normal aseptic precautions were taken in cutting the splanchnic nerves and the operations were always carried out 20 hr before the injection of insulin. The majority of animals were anaesthetized with halothane B.P. In the remainder, anaesthesia was induced with ethyl chloride and maintained with ether. The nerves were identified through a paravertebral incision in the abdominal wall and cut immediately behind the diaphragm. The operation site was checked at the end of each experiment.

A single intravenous injection of insulin (Burroughs Wellcome) was given in all experiments. Blood samples were collected 15 min before the injection of insulin and at timed intervals thereafter; the animals were observed continuously throughout the experiments. Rectal temperature, respiratory rate and heart rate were recorded with the minimum of disturbance to the animal.

Infusions of adrenaline and noradrenaline were administered through a polyethylene catheter previously inserted into a branch of the right femoral vein at doses ranging from 0·165 to 0·5 μg/kg/min catecholamine base by means of a slow injection pump (Palmer). Adrenaline hydrogen tartrate (B.D.H.) and noradrenaline bitartrate (Bayer) were dissolved in NaCl solution (0·9 g/100 ml.), to provide an appropriate dilution of the base for infusion.

Chemical estimations. Glucose was estimated by the glucose oxidase method (Huggett & Nixon, 1957); lactic acid with lactic dehydrogenase according to the method of Barker & Britton (1957). The concentration of fructose in blood was estimated by Cole's modification of Roe's method (Roe, 1934) as described by Bacon & Bell (1948). No evidence could be found for the presence of significant amounts of other substances which would give this reaction in the blood of the calf. Blood pH was measured with an EEL capillary electrode.

RESULTS

The resting concentrations of metabolites in the blood

Glucose. Figure 1 shows that there are wide variations in the concentration of glucose in the blood of calves over the first 30 hr after birth. The values which were obtained at birth (10–40 mg/100 ml. blood) were comparable with those in late-term foetuses and the blood glucose concentration rose steadily during the first 24 hr of life, although these animals were not fed. The mean blood glucose concentration of unsuckled calves between 17 and 27 hr of age was $69 \pm 3{\cdot}28$ (S.E. of mean) mg/100 ml.; this was not significantly different from the post-absorptive blood glucose concentration of 2 to 5-day-old calves. Older animals, 12–24 weeks of age, had a lower mean post-absorptive blood glucose concentration ($55{\cdot}6 \pm 2{\cdot}5$ mg/100 ml.).

When calves were fed 4 pints (2·3 l.) of colostrum within 2 hr of birth the blood glucose concentration rose more rapidly. The mean value of ten calves which were treated in this way was $87{\cdot}9 \pm 5{\cdot}23$ mg/100 ml. at 20–27 hr of age which was significantly different from that found in unsuckled calves of the same age ($P < 0{\cdot}01$).

Fructose. The concentration of fructose in the foetal blood at the end of gestation was considerably higher (120–140 mg/100 ml. blood) than the values found at birth (49–62 mg/100 ml. blood, Fig. 1). The blood fructose concentration fell steadily thereafter to below 10 mg/100 ml. within 14 hr of birth in all animals (Fig. 1).

Lactic acid. Wide individual variations in the blood lactate concentration were found shortly after birth (Fig. 2). The values tended to be higher over the first 20 hr of life and fell to about 10 mg/100 ml. blood by 30 hr.

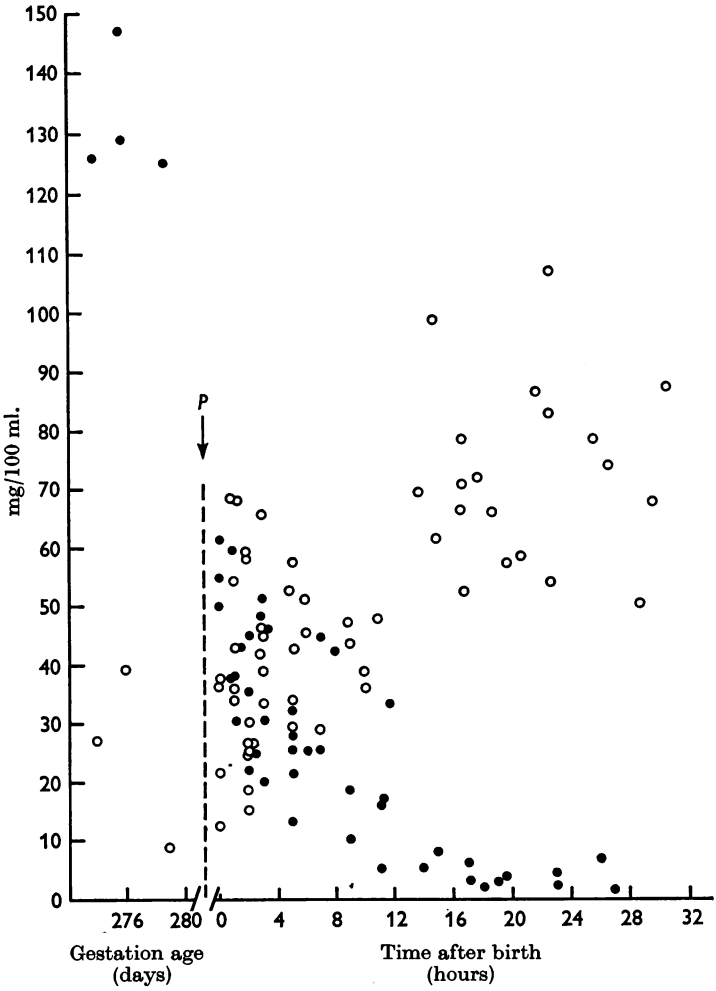

Fig. 1. Concentration of glucose (○) and fructose (●) in the jugular blood of foetuses at the end of gestation and in calves which had not been fed over the first 30 hr after birth. Ordinate: glucose mg/100 ml. blood. Abscissa: gestation age in days; hours after birth. *P*: parturition.

One animal which had a blood lactate concentration in excess of 150 mg/100 ml. immediately after birth showed signs which suggested that parturition had been prolonged. This calf was not viable and the results have not been included in this series.

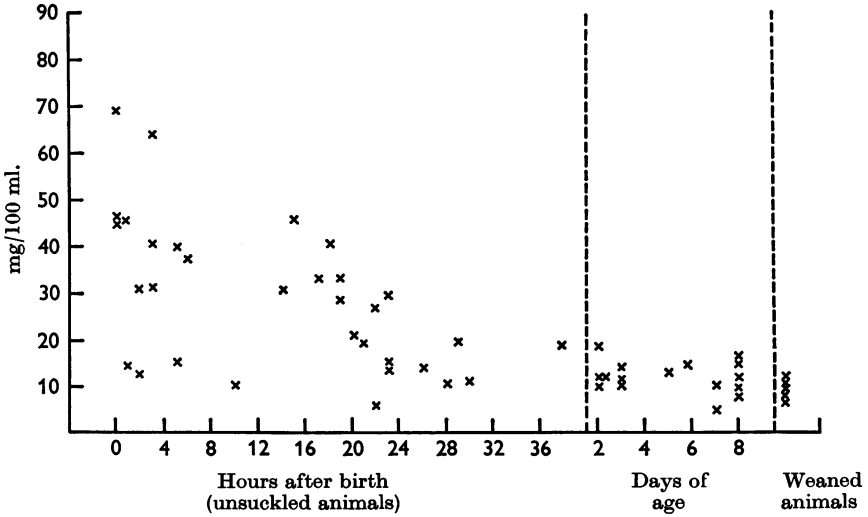

Fig. 2. Concentration of lactic acid in the venous blood of calves over the first 36 hr after birth, from 2 to 8 days of age and in weaned animals. Values obtained over the first 36 hr after birth were all taken from animals which had not been fed.

The effect of insulin on the calf

The effects of intravenous injections of insulin in doses of 1–8 u./kg have been assessed in calves of different ages by recording both the incidence of hypoglycaemic convulsions and the changes in the concentrations of metabolites in the blood.

TABLE 1. Incidence of convulsions in calves given 1 u. of insulin/kg intravenously

Age	No. of animals	No. of animals convulsing		No. of animals not convulsing		% of animals convulsing
		Observed	Expected	Observed	Expected	
0–24 hours	12	0	5·20	12	6·80	0·00
2–3 days	4	0	1·73	4	2·27	0·00
4–5 days	10	5	4·33	5	5·67	50·00
6–7 days	11	7	4·77	4	6·23	63·64
1–7 weeks	18	14	7·80	4	10·20	77·78
4–6 months	5	0	2·17	5	2·83	0·00
Total	60	26	26·00	34	34·00	43·33

χ^2 with 5 D.F. = 26·78: $P < 0{\cdot}01$.

Incidence of convulsions. The incidence of convulsions in the young calf during hypoglycaemia varied with age. This was shown most clearly when the animals were tested with a single injection of insulin (1 u./kg body wt., Table 1). The administration of this dose was not followed by convulsions in calves under 4 days of age, whereas older animals usually convulsed.

Signs of hypoglycaemia were difficult to detect in calves less than 4 days

of age. During the first hour after the injection the animals were somnolent but could readily be roused; thereafter their behaviour was indistinguishable from that of normal animals. By 6 days of age convulsions generally occurred within 50–100 min. The animals became recumbent within 40–50 min of the injection of insulin, the ears were retracted, the head started to droop, muscular tremors occurred and there was an increase in the respiratory rate. Some calves fell asleep, others became restless and a few succeeded in standing before convulsions occurred. Violent convulsions

TABLE 2. The effect of increasing the dose of insulin on the incidence of convulsions in new-born calves

Age (hr)	Dose of insulin (u./kg)	No. of calves tested	No. of calves convulsing	Result of test for 2×2 contingency table at each age
2	1	4	0	$P < 0{\cdot}05$
	8	9	2	
24	1	7	0	$P < 0{\cdot}05$
	4	8	2	
48	1	5	0	$P < 0{\cdot}01$
	2	4	4	

generally lasted from 5 to 10 min and were followed by periods of relative quiescence, characterized by opisthotonus, nystagmus and weak paddling movements of the limbs. Spontaneous recovery was slow but the overt signs disappeared rapidly after the intravenous injection of 5 g glucose in 100 ml. NaCl (solution 0·9 g/100 ml.).

The resistance to hypoglycaemia in the new-born calf is not absolute. Convulsions sometimes followed the injection of much larger amounts of insulin but even then they only occurred after a delay of several hours. Thus, when the dose was increased to 8 u./kg only two out of the nine calves which were tested at 2 hr of age convulsed and then only after a delay of 6–8 hr (Table 2).

Changes in the respiratory rate, rectal temperature and heart rate were observed in all the calves which were tested with 8 u./kg at 2 hr of age (Fig. 3). A rise in the rectal temperature from a mean resting value of 101·3 °F (38·5 °C) to a mean value of about 103 °F (39·4 °C) started 60 min after the administration of insulin. It was associated with a marked increase in the respiratory rate, from about 35/min to approximately 100/min, after the same latent period. Both effects persisted for many hours. During this time the pH of the blood did not exceed the limits of 7·25–7·45 (Fig. 3). Comparable changes in the respiratory rate, heart rate and rectal temperature were not observed in calves between 4 and 50 days of age which failed to convulse after receiving 1 u. insulin/kg.

Convulsions did not occur in calves over 4 months of age which had been

completely weaned, even after doses of up to 8 u. insulin/kg, but the animals became lethargic and muscular tremors were also observed.

Changes in the blood during hypoglycaemia

Glucose. The degree of hypoglycaemia varied with the dose of insulin and the duration with the age of the calf. Figure 4 compares the effects of single injections of insulin (1 u./kg) on the blood glucose concentration in calves of different ages.

At 2 hr of age hypoglycaemia was prolonged; the blood glucose concentration fell to between 10 and 15 mg/100 ml. within 1–3 hr, remained

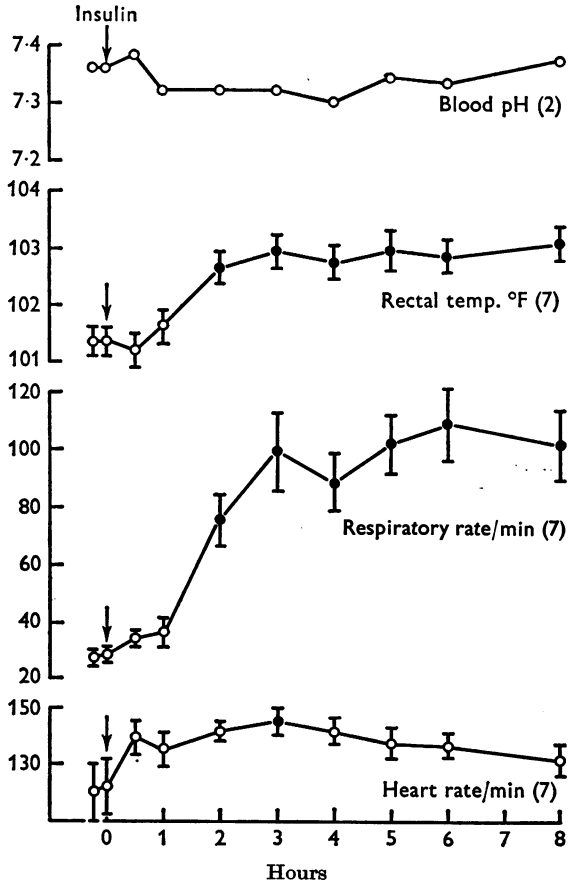

Fig. 3. 2–5-hr-old calves. Changes in the blood pH, heart rate, respiratory rate and rectal temperature in calves which were tested with insulin (8 u./kg). Figures in parentheses refer to the number of experiments represented by the mean values given. ● Represent significant variation from the mean value at time 0 ($P < 0{\cdot}01$). Vertical lines represent the standard error of each mean.

below 10 mg/100 ml. for a further 3 hr and even after 8 hr was still below 20 mg/100 ml. in every case. A similar though less prolonged hypoglycaemia occurred in calves which were tested at 24 hr of age which had not

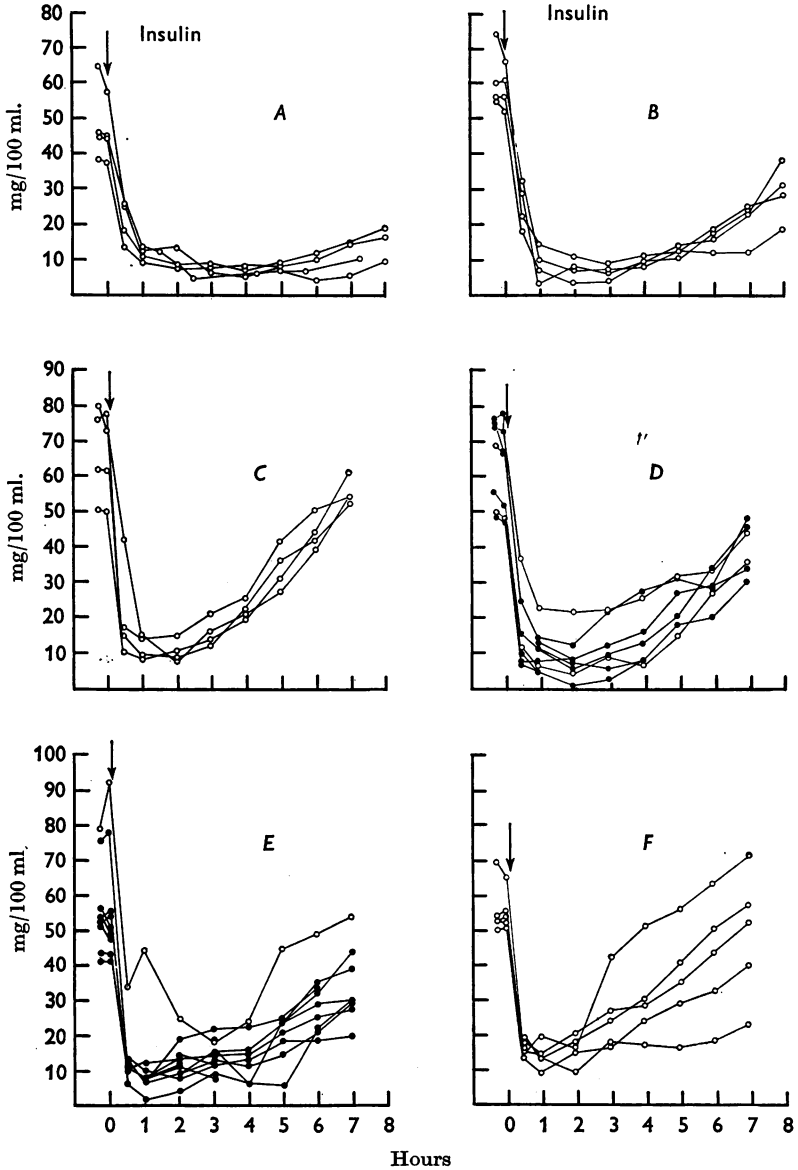

Fig. 4. The effect of intravenous injections of insulin (1 u./kg) on the blood glucose concentration of calves at different ages. *A*: 2 hr. *B*: 24 hr unsuckled. *C*: 2–4 days. *D*: 7–9 days. *E*: 3–4 weeks. *F*: 4–7 months. ●—●: calves which convulsed. ○—○: calves which did not convulse.

been fed since birth. With increasing age the blood glucose regained its resting values more rapidly and by 2–4 days the concentration of glucose in the blood started to rise within 3 hr of the injection. The majority of calves tested at 7 days and 3–4 weeks of age convulsed during the course of the experiment (Fig. 4). A significantly higher blood glucose concentration was maintained in two out of three animals tested at these ages which failed to convulse. However, a similar explanation cannot account for the absence of convulsions in younger calves. Figure 4 shows that the blood glucose concentration in 2- and 24-hr-old calves fell to the same or to lower levels than those at which convulsions occurred in 7-day and 3- to 4-week-old animals.

The absence of any clear relationship between the occurrence of convulsions in calves up to 24 hr of age and the concentration of glucose in the blood during hypoglycaemia was confirmed by increasing the dose of insulin. Intravenous injections of 8 u. insulin/kg caused an intense and prolonged hypoglycaemia in which blood glucose concentrations of below 5 mg/100 ml. persisted for up to 12 hr and did not exceed 10 mg/100 ml. for 24 hr (Fig. 5). The majority of these animals did not convulse (Table 2) and, although they were somnolent for a period, later recovered and appeared to be alert in spite of the virtual absence of glucose from the venous blood. Very similar results were obtained when 24-hr-old calves were tested with insulin at a dose of 4 u./kg body wt. (Fig. 7).

These results show that the response of the calf to insulin varies with age. Hypoglycaemia is more prolonged when the animals are tested immediately after birth although convulsions rarely occur at this age. By 5–7 days, calves frequently convulse, although recovery from hypoglycaemia is more rapid.

Lactic acid. The effect of insulin on the blood lactate concentration also varied with age. The resting concentration in the blood of 2-hr-old calves was normally high (30–50 mg/100 ml. blood), and rose abruptly about 60 min after the injection of 8 u. insulin/kg to exceed 100 mg/100 ml. after 4–5 hr (Fig. 6). This abrupt rise in the lactate concentration coincided with the increase in rectal temperature and respiratory rate (Fig. 3).

Changes in the blood lactate concentration also occurred in 24-hr-calves which had not been fed since birth, after they were given 4 u. insulin/kg body wt. A transitory fall from the relatively high resting levels (15–40 mg/100 ml. blood) followed the injection of insulin and the blood lactate concentration then rose to between 35 and 50 mg/100 ml. during hypoglycaemia (Fig. 7*a*). Two 24-hr-old calves in which the blood glucose concentration fell relatively slowly failed to show this response (Fig. 7*b*) and also failed to convulse. At 6–7 days of age the blood lactate concentration did not alter during hypoglycaemia unless the animals con-

vulsed (Fig. 8). The blood lactate concentration then rose abruptly and the extent appeared to be related to the duration of the convulsions.

Weaned animals, over 100 days of age, were also tested with 8 u. insulin/kg. Convulsions did not occur although the blood glucose concentration was lower than 10 mg/100 ml. blood for about 9 hr. There was a slight but maintained rise in the concentration of lactate in the venous blood which exceeded 25 mg/100 in only one animal (Fig. 9).

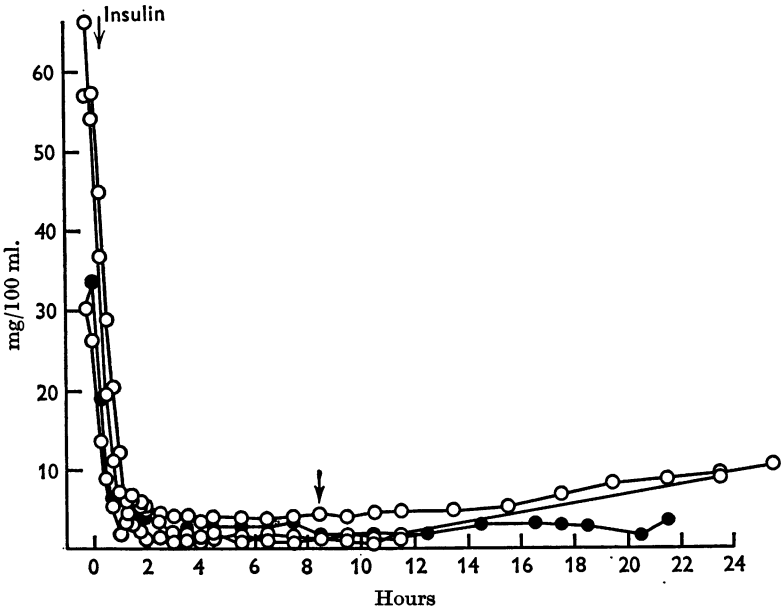

Fig. 5. 2–5-hr-old calves. The effect of large doses of insulin (8 u./kg) on the blood glucose concentration of four calves, one of which convulsed 8 hr after the administration of insulin. ●—●: animal which convulsed 8 hr after insulin injection (↓). ○—○: animals which did not convulse.

The absence of convulsions in the new-born calf appears to be associated with the occurrence of high concentrations of lactic acid in the blood during hypoglycaemia. This response is not present in older calves, in which convulsions frequently follow the administration of small doses of insulin, nor in weaned animals which do not normally convulse during insulin hypoglycaemia.

Fructose. The rate at which fructose disappeared from the circulating blood in the new-born calf did not appear to be altered by the administration of insulin in doses of 8 u./kg. The resistance to the convulsing effect of insulin hypoglycaemia was still present in 24-hr-old calves although the blood concentration had fallen to low values by 14 hr of age (Fig. 1).

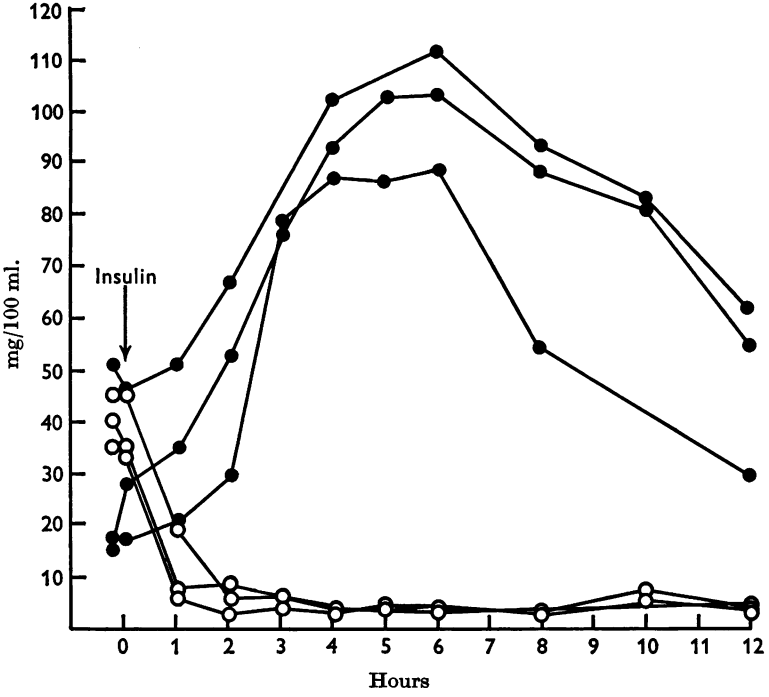

Fig. 6. 2–5-hr-old calves. Effect of 8 u. insulin/kg on blood glucose (○—○) and blood lactate (●—●) concentrations. None of these animals convulsed.

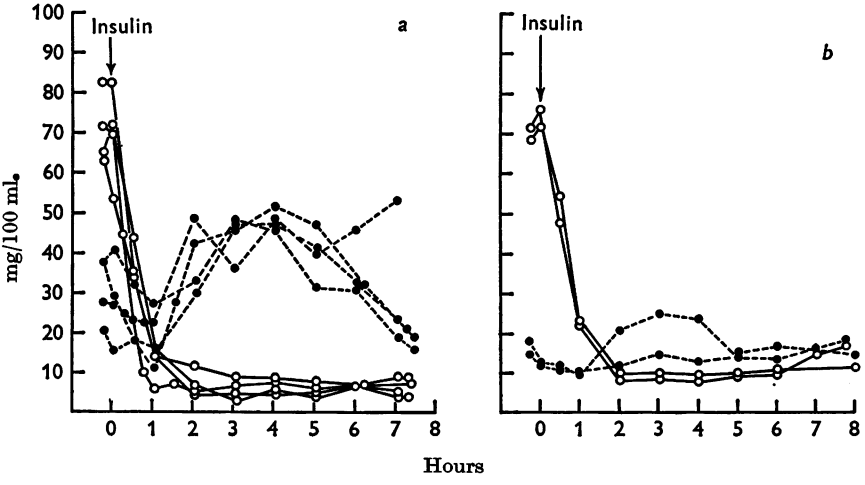

Fig. 7*a* and *b*. 24-hr-old calves. Effect of 4 u. insulin/kg on the blood glucose (○—○) and blood lactate (●---●) concentration (see text).

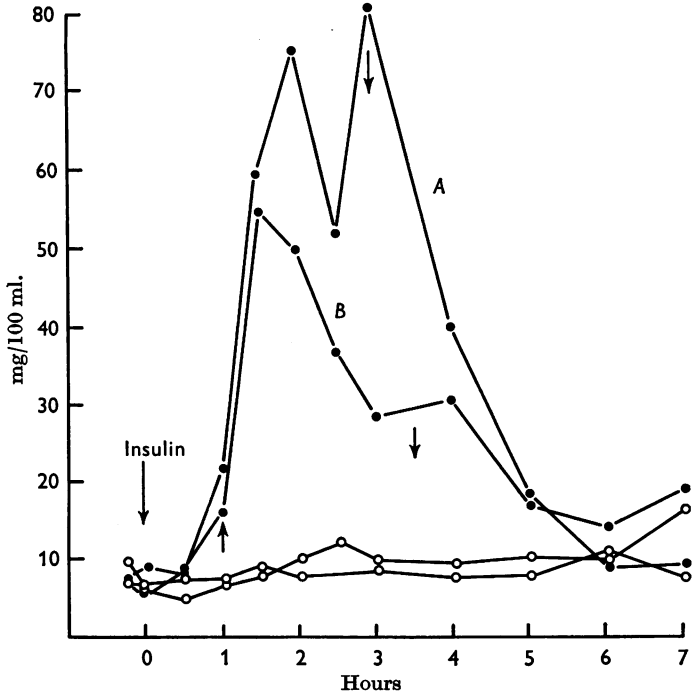

Fig. 8. 7-day-old calves. Blood lactate concentrations of four calves given 1 u. insulin/kg at time 0, two of which convulsed (●—●) and two of which did not (○—○). Onset of convulsions in both animals (*A* and *B*) started 60 min after insulin (↑) and recovery is marked (↓). Convulsions were more vigorous in *A* than in *B*.

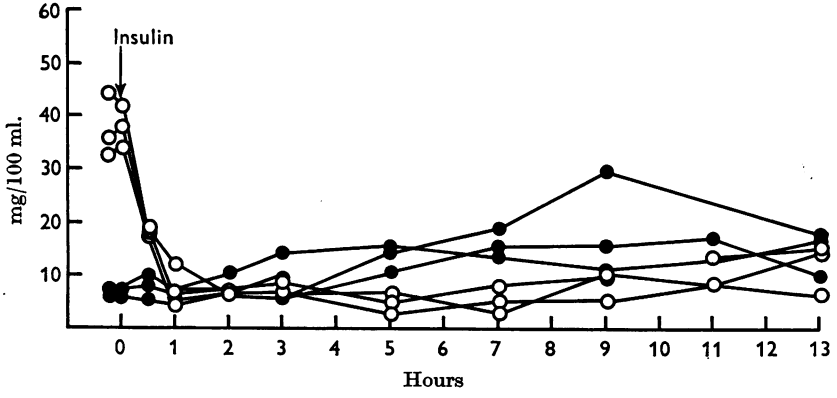

Fig. 9. Calves over 3½ months of age. Effect of insulin (8 u./kg) on the blood glucose (○—○) and the blood lactate concentrations (●—●), of weaned calves.

The effect of section of the splanchnic nerves on the sensitivity to insulin

Preliminary experiments showed that single injections of adrenaline (25 μg/kg) terminated hypoglycaemic convulsions within 3–5 min in calves both at 24 hr and at 7 days of age, whereas the same dose of noradrenaline was quite ineffective. The recovery which followed the intravenous injection of a comparatively large dose of adrenaline was associated with a transient rise in the blood glucose concentration at both these ages but in the 24-hr-old calf the blood lactate concentration was raised to a high value for a much longer period than in the 7-day-old calf. This response was examined further in calves in which the splanchnic nerves had previously been cut. The experiments were confined to animals at either 24 hr or 7 days after birth, since these are convenient ages at which to compare the sensitivity to insulin hypoglycaemia.

24-hr-old calves. The splanchnic nerves were cut within 3 hr of birth and the experiments carried out 24 hr after birth.

Cutting the splanchnic nerves completely altered the effects of insulin in these calves. All convulsed within 3 hr and during this period the normal rise in the blood lactate concentration did not occur (Fig. 10); there was no increase in rectal temperature or respiratory rate.

7-day-old calves. Since all these animals convulsed in response to small doses of insulin, it was difficult to demonstrate any differences in their sensitivity to hypoglycaemia by cutting the splanchnic nerves.

Infusions of adrenaline and noradrenaline after cutting the splanchnic nerves

The possibility that infusions of either adrenaline or noradrenaline would have a comparable effect on the metabolites in the blood to that of the secretion of the adrenal medulla, and eliminate convulsions during hypoglycaemia, was therefore examined.

The experiments were again confined to animals at 24 hr or 7 days of age in which the splanchnic nerves had been cut and which were given 4 u./kg of insulin. The effects of adrenaline were dependent upon the rate at which it was infused as well as on the age of the calves.

Infusions of adrenaline

24-hr-old calves. Infusions were started 60 min after insulin was injected (Fig. 11) at a time when, in the intact animal, other signs such as the increase in blood lactate concentration and the rise in rectal temperature and respiratory rate indicated the possible onset of a sympathetic discharge (Fig. 3). A relatively small dose of adrenaline (0·165 μg/kg/min)

prevented convulsions for the duration of the infusion and although there was little effect on the blood glucose concentration which always remained below 10 mg/100 ml., the concentration of lactate in the blood rose to levels comparable with those found in the normal animal at this age

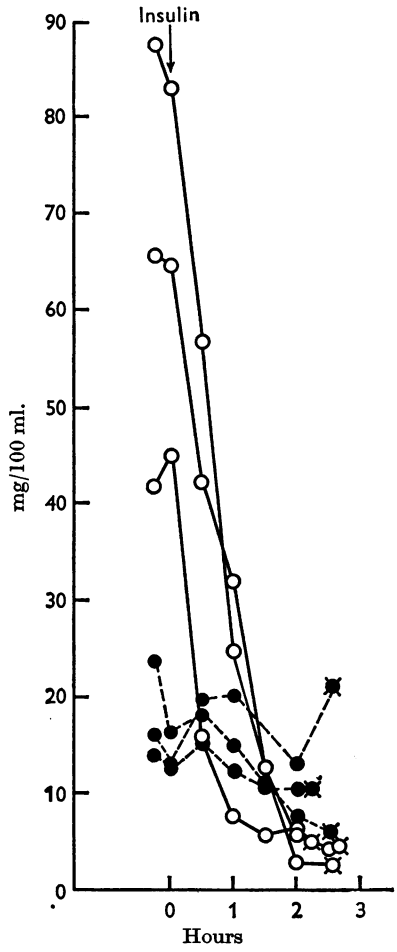

Fig. 10. 24-hr-old calves. The effect of insulin (4 u./kg) on the blood glucose (○—○) and the blood lactate concentration (●—●) of three calves in which both splanchnic nerves had been cut. All these animals convulsed at the points marked ⊗.

during insulin hypoglycaemia (Fig. 11*a*). When the infusion was discontinued, the blood lactate concentration fell abruptly, the blood glucose concentration showed a slight decrease and most of the animals convulsed within 30–60 min.

Larger amounts of adrenaline (0·5 μg/kg/min) produced a more obvious

although transient rise in the blood glucose concentration, especially at the start of the infusion (Fig. 11*b*). The rise in the blood lactate concentration was more abrupt and higher levels (80–90 mg/100 ml.) were reached than in the preceding experiments (Fig. 11*a*). Convulsions did not occur until 120–200 min after the infusion by which time the blood lactate concentration had fallen to between 40 and 60 mg/100 ml.

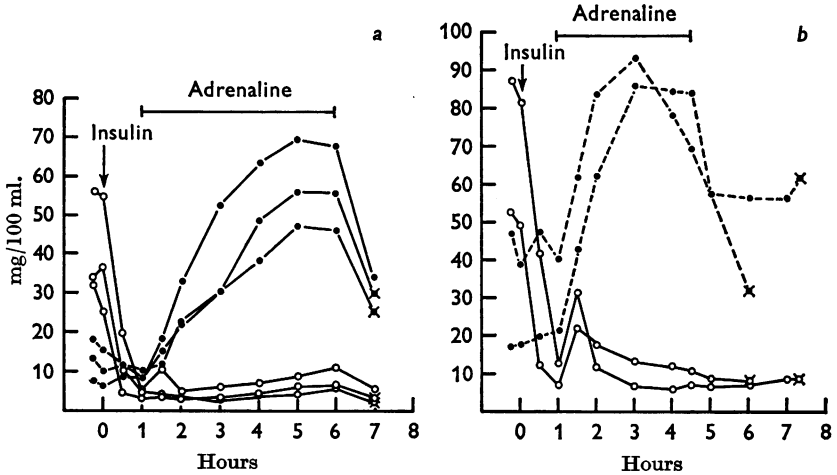

Fig. 11. 24-hr-old calves. Effect of intravenous infusions of adrenaline in calves given 4 u. insulin/kg after both splanchnic nerves had been cut. O—O: Blood glucose concentration. ●—●: Blood lactate concentration. ⨯: Convulsions. (*a*) 0·165 μg adrenaline/kg/min infused from 1 to 6 hr (⊢⊣). (*b*) 0·5 μg ad renaline/kg/min infused from 1 to 4½ hr (⊢⊣).

7-*day-old calves*. Infusions of adrenaline had to be started at the same time as insulin was injected since even normal animals of this age convulsed within 50–100 min (Fig. 4). Small amounts of adrenaline (0·165 μg/kg/min) did not alter the time at which convulsions occurred. A slight increase to 0·25 μg/kg/min produced a rise in the blood lactate concentration and slowed the rate at which the blood glucose concentration fell but the animals still convulsed during the infusion (Fig. 12).

Convulsions were prevented during infusions of larger amounts of adrenaline (0·5 μg/kg/min) but they occurred in two out of three calves within 15–60 min when the infusions were discontinued (Fig. 13*a*). This dose of adrenaline always raised the blood glucose concentration to higher values than those found in control experiments and the blood lactate concentration rose to 30–50 mg/100 ml. In the normal 7-day-old calf, the blood lactate concentration does not rise during hypoglycaemia until the animal convulses (Fig. 8). A more direct comparison of the effect of this dose of adrenaline in calves at these two ages was obtained by starting the infusion in 24-hr-old calves immediately after the injection of insulin

(Fig. 13*b*). The blood lactate concentration rose rapidly to considerably higher values (100–140 mg/100 ml. blood) than in 7-day-old calves. This dose of adrenaline also had a marked effect on the blood glucose concentration and delayed the onset of hypoglycaemia.

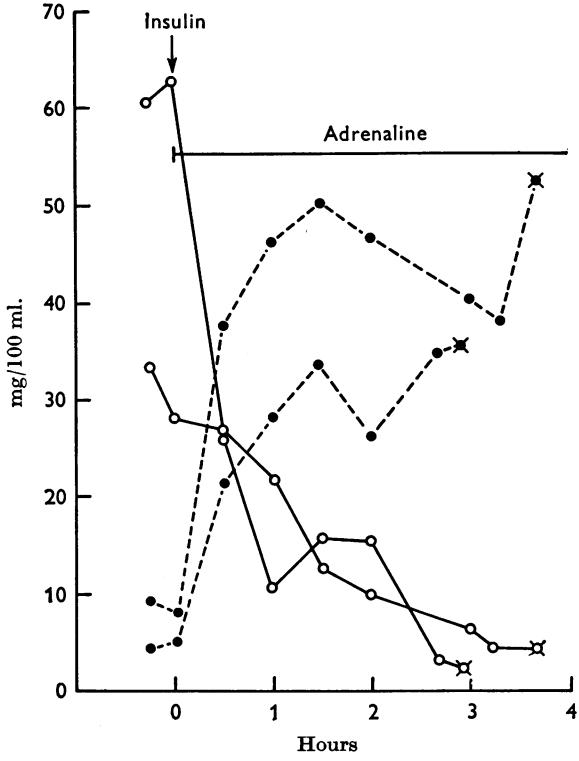

Fig. 12. 7-day-old calves. Effect of intravenous infusions of adrenaline (0·25 μg/kg/min (⊢—) in two calves given 4 u. insulin/kg after both splanchnic nerves had been cut. ○—○: Blood glucose concentration. ●---●: Blood lactate concentration. ⋈: Convulsions.

Infusion of noradrenaline

Infusions of relatively large doses of noradrenaline (0·5 μg/kg/min) had little or no effect on the onset of convulsions in 24-hr-old calves. The blood lactate concentration was not increased and there appeared to be no effect on the blood glucose concentration (Fig. 14). Noradrenaline was also quite ineffective in preventing or postponing convulsions in calves at 7 days of age.

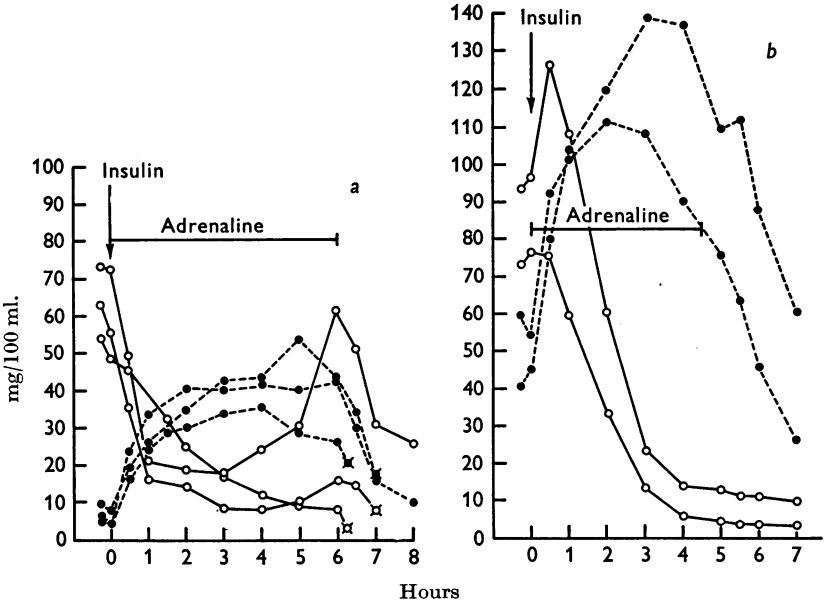

Fig. 13. Effect of infusions of 0·5 μg adrenaline/kg/min (⊢⊣) in calves given 4 u. insulin/kg after both splanchnic nerves had been cut. ○—○: Blood glucose concentration. ●—●: Blood lactate concentration. (*a*) 7-day-old calves. ⋇ Convulsions. (*b*) 24-hr-old calves. Neither of these animals convulsed.

DISCUSSION

The remarkable ability of the central nervous system of the new-born calf to withstand the virtual absence of glucose from the blood for long periods probably represents an extension of foetal metabolism into the period immediately after birth, for it disappears within 3–4 days and is succeeded by a period of increased sensitivity to hypoglycaemia. The glucose concentration of foetal blood is lower than that of the mother (Shelley & Neligan, 1966), especially in ruminants in which the foetal blood glucose concentration is extremely low until the end of gestation. However, a direct comparison of the responses of the foetal and the new-born central nervous systems to hypoglycaemia is complicated both by the transfer of glucose across the placenta and by the relative quiescence of the foetus during gestation (Barcroft & Barron, 1937). Nevertheless, the absence of convulsions in both foetal sheep and puppies after large doses of insulin reported in earlier work (Passmore & Schlossman, 1938; Schlossman, 1938) has recently been confirmed during hypoglycaemia in perfused foetal lambs between 89 and 127 days of gestation (Alexander, Britton & Nixon, 1966). A comparable resistance to hypoglycaemia to that of the calf probably also occurs in the human since low blood glucose con-

centrations rarely produce signs of hypoglycaemia during the first 24–48 hr after birth (Brown & Wallis, 1963; Neligan, 1964; Shelley & Neligan, 1966). There is also evidence that the absence of clinical signs during hypoglycaemia in new-born babies may be associated with an increase in the urinary excretion of adrenaline (Greenberg, Lind & Euler, 1960).

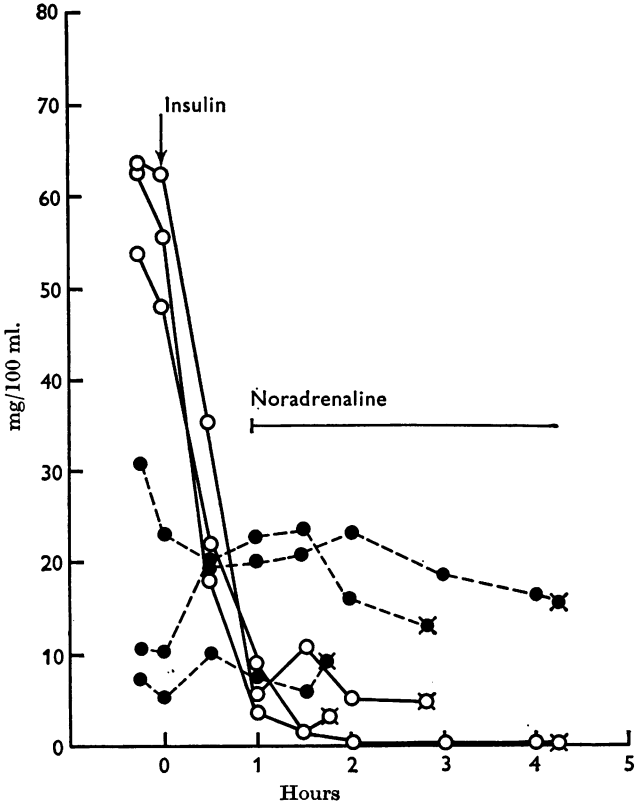

Fig. 14. 24-hr-old calves. Effect of intravenous infusions of 0·5 μg noradrenaline/kg/min (⊢—) in three 24-hr-old calves given 4 u. insulin/kg in which both splanchnic nerves had been cut. ○—○: Blood glucose concentration. ●—●: Blood lactate concentration. ⋈: Convulsions.

The changes in the concentration of glucose, fructose and lactic acid in the blood of the new-born calf during the first 12–24 hr after birth are similar to those reported in the lamb (Shelley, 1960, 1961). The steady rise in the blood glucose concentration from the low values found in the foetus probably reflects the redistribution of glycogen, for it is accentuated by, but is not dependent upon, feeding. During the same period fructose disappears from the blood at a similar rate and from about the same initial values at birth as in the lamb (Cole & Hitchcock, 1946; Huggett,

Warren & Warren, 1951; Shelley, 1960). There is, however, this difference, that in the calf the concentration of fructose in the foetal blood immediately before term was almost twice that found at birth. A discrepancy of this order has not been reported in the lamb, in which the placenta is known to be the sole source of fructose during gestation (Huggett, Warren & Winterton, 1949; Huggett *et al.* 1951). The present results indicate that there may be an abrupt reduction in the placental synthesis of fructose several hours before the calf is delivered and it is possible that other functions of the placenta are affected in a similar way.

In general, the blood lactate concentration falls gradually from the high foetal values within 24 hr of birth (Barker & Britton, 1958; Dawes, Jacobson, Mott & Shelley, 1963; Comline & Silver, 1966). The wide individual variations shortly after birth may, however, be associated with either excessive hypoxia or struggling at parturition. Adrenaline appears to be especially effective in raising the lactate concentration of the blood in the calf during the first 24 hr of life and a similar response may also be present in the foetus.

All the evidence indicates that the absence of convulsions in the new-born calf during hypoglycaemia depends on the secretion of adrenaline from the adrenal medulla, for the convulsions which occurred, if the splanchnic nerves were cut before the injection of insulin, could be prevented by infusions of adrenaline in amounts which, although small, were nevertheless comparable to those released during anoxia or in response to direct stimulation of the splanchnic nerves (Comline & Silver, 1966). In contrast, noradrenaline, which forms by far the largest proportion of the catecholamines released by such stimuli from the adrenal medulla immediately after birth, did not prevent convulsions. These relatively small amounts of adrenaline were only effective during the first 24 hr and much larger doses were required to prevent convulsions at 7 days of age. This may be one factor responsible for the increased incidence of convulsions in the older animals, for all the direct evidence indicates that the output of adrenaline from the adrenal medulla does not alter appreciably during the first week of life.

Other changes in the effects of adrenaline within the first week after birth are, however, apparent and the elimination of convulsions during infusions of adrenaline in new-born calves is associated with insignificant hyperglycaemic effects. Thus in new-born calves there were no obvious changes in the blood glucose concentration during infusions of small doses of adrenaline, although the concentration of lactate was raised to levels comparable with those found in normal calves during hypoglycaemia. Conversely, the absence of convulsions in the older calves could be related more specifically to the existing blood glucose concentration which was

invariably raised by the larger doses of adrenaline required to prevent convulsions in these animals during hypoglycaemia after previous section of the splanchnic nerves.

One of the more attractive explanations for the action of adrenaline in the new-born calf would be the ability of the central nervous system to utilize other substances derived from the blood as an alternative to glucose during hypoglycaemia. If this were so, lactate would appear to be an obvious possibility. It is known from the results of numerous *in vitro* studies that the enzyme systems necessary for lactate metabolism are present in the brain (McIlwain, 1953, 1955) and that brain suspensions from animals treated with insulin metabolize lactate more efficiently (Elliot, McNair Scott & Libet, 1942). Other possible sources of energy such as free fatty acids and glycerol do not appear to act as substitutes for glucose, at least in the absence of lactate, since noradrenaline which raised their concentration but not that of lactate, did not prevent convulsions.

The contribution of fructose can probably be ignored; the resistance to hypoglycaemia was still present at 24 hr of age although the fructose concentration had fallen to very low values. Preliminary experiments have also shown that infusions of fructose will not prevent hypoglycaemic convulsions in 24 hr old calves after section of the splanchnic nerves (Edwards, 1964). The ability of the liver to remove fructose appears to develop only gradually after birth in both the lamb and the new-born pig (Andrews, Britton, Huggett & Nixon, 1960; Kidder, Manners, McCrea & Weaver, 1963). There is no evidence that the new-born calf can utilize fructose, indeed infusions of it cause hypoglycaemia in these animals at this age (Edwards & Powers, 1967).

While the evidence suggests that lactate is used by the central nervous system during hypoglycaemia, certain discrepancies indicate that other factors may be involved. Thus, although the amount of lactate in the blood normally increases during hypoglycaemia, in two animals at 24 hr of age it did not do so and yet convulsions did not occur. Furthermore, although the concentration of lactate invariably fell when infusions of adrenaline were discontinued, the levels at which animals convulsed varied widely. The values obtained in these experiments may not, however, give a true indication of the concentration which precipitates convulsions, since rapid fluctuations in the concentration of lactate in the blood occur during periods of excessive muscular activity. The results therefore do not provide conclusive evidence that lactate is the sole means by which convulsions are prevented in the calf during hypoglycaemia; other actions of adrenaline may be involved in protecting the central nervous system at this age.

Comparable amounts of adrenaline produce much higher blood lactate

concentrations at 24 hr than at 7 days of age. This difference could be ascribed to an increase in the rate of production but the uptake of lactate by the liver is probably less efficient immediately after birth. Marked changes in hepatic function have been described in new-born animals of many species and, more specifically, a deficiency in the capacity of the liver of foetal sheep to metabolize lactate has been postulated to account for the high concentrations found in the blood during perfusion of the organ (Andrews *et al.* 1960).

The resistance to hypoglycaemia in the new-born calf resembles that in the adult ruminant in that it is abolished by cutting the splanchnic nerves (Strand, Anderson & Allcroft, 1934; Hitchcock & Phillipson, 1946; Reid, 1951*a*, *b*; Jarrett & Potter, 1953; Jasper, 1953). However, the rise in the blood lactate concentration during hypoglycaemia in the adult is slight and cannot account for the resistance to hypoglycaemia.

The duration of insulin hypoglycaemia is most prolonged immediately after birth, which may indicate that the factors which stabilize the blood glucose concentration are least efficient at this time. During this transitional period the central nervous system is, however, protected from hypoglycaemia by reactions which are more pronounced than in older animals. At this time the high concentration of lactic acid in the blood which is dependent upon the release of adrenalin from the adrenal medulla and possibly also on hepatic insufficiency, may go far to meet the metabolic requirements of the brain.

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