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THE DEVELOPMENT OF THE ADRENAL MEDULLA OF THE FOETAL AND NEW-BORN CALF

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SUMMARY

1. The output of adrenaline and noradrenaline from the adrenal medulla during asphyxia, stimulation of the splanchnic nerves or the intra-arterial injection of acetylcholine, has been investigated in foetal and new-born calves up to 3 weeks of age.

2. Between 180 days' gestation and term (~ 281 days) the response of the foetal adrenal gland of the calf to asphyxia appeared to be independent of its nerve supply and the discharge consisted largely of noradrenaline. A similar type of discharge was obtained after the intra-arterial injection of acetylcholine, but stimulation of the splanchnic nerves resulted in only a small discharge of both adrenaline and noradrenaline.

3. Rapid changes occurred in the response of the adrenal medulla to all forms of stimulation during the first 24 hr after birth. For the first 4–6 hr the adrenal medulla was hypersensitive; thereafter the response rapidly declined and a variable period of depressed excitability followed. The changes affected the output of noradrenaline rather than that of adrenaline and were more pronounced during asphyxia or after the intra-arterial injection of acetylcholine. Within 24 hr of birth the amount of noradrenaline released in response to either form of stimulation was less than 25% of that found immediately after birth.

4. During the hypersensitive phase immediately after birth splanchnic nerve activity appeared to potentiate the direct effect of asphyxia on the noradrenaline cells since the maximum output of noradrenaline was attained more rapidly and at a higher P_{O_2} if the splanchnic nerves were intact.

5. The non-nervous direct response of the adrenal medulla to asphyxia decreased rapidly after birth and disappeared within 24 hr. It did not reappear at any age and was a feature of foetal life.

6. The recovery of the response to acetylcholine occurred between 3 and 8 days after birth with a return of the high level of noradrenaline secretion; no similar increase in the output of adrenaline occurred at this stage.

7. The response to asphyxia was not restored to the level found in the new-born calf until 2–3 weeks after birth. At this time the effect on the adrenal medulla appeared to be mediated almost entirely by the splanchnic nerves.

8. The effects of chloralose and pentobarbitone anaesthesia on the changes in the nervous response to asphyxia after birth were compared. Essentially the same pattern of changes was found with both anaesthetics although the absolute level of discharge under chloralose was greater and a considerably larger amount of adrenaline was secreted at 3 weeks of age.

9. At certain ages stimulation of the splanchnic nerves enhanced the response of the adrenal medulla to subsequent injections of acetylcholine. The noradrenaline output was only significantly increased by this procedure during the period of depressed excitability whereas the adrenaline discharge was always increased throughout the first 3 weeks of life.

10. The changes in adrenaline and noradrenaline content of the adrenal glands during the 3-week period after birth were investigated. The noradrenaline concentration was low immediately after birth during the hypersensitive phase and increased during the period of reduced sensitivity. The output of this amine was thus inversely related to its content in the adrenal gland. A similar relation did not occur with adrenaline, the concentration of which remained relatively constant during the first 3 weeks of life.

INTRODUCTION

The relative amounts of noradrenaline and adrenaline in the adrenal medulla at birth vary considerably from species to species (Shepherd & West, 1951), but in nearly all there is a gradual increase in the proportion of adrenaline with age until the adult concentrations are reached. Apart from the lamb, little is known about the release of these amines before or after birth, although it is generally assumed that a high percentage of noradrenaline in the adrenal medulla is indicative of immaturity. Measurements of content alone may, however, be misleading and, especially in the foetus and new-born animal, may not reflect the composition of the secretion. Both adrenaline and noradrenaline are present in the adrenal medulla of the calf and lamb at birth, but whereas the new-born lamb secretes these amines in the same way and in almost the same proportions as the adult (Comline & Silver, 1961), the response to direct stimulation of the splanchnic nerves of the calf does not resemble that of the adult until several months after birth (Silver, 1960).

The full development of the innervation of the adrenal medulla of the foetal lamb occurs *in utero* during the last few days of gestation. In the immature sheep foetus, however, another mechanism for the release of

amines from the adrenal medulla is present long before the development of the splanchnic innervation. A discharge, consisting largely of noradrenaline, occurs during asphyxia in foetuses as young as 80–90 days and is present after cutting the splanchnic nerves or pithing the spinal cord (Comline & Silver, 1961). This response is due to the direct effect of hypoxia on the noradrenaline cells (Comline, Silver & Silver, 1965). It is eventually almost completely replaced by the nervous mechanism so that, at term, little or no anoxic discharge is obtained after denervation.

Since the presence of a similar direct response of the adrenal medulla to a low P_{O_2} in the mature foetal or new-born calf might compensate for the slow development of the nerve supply after birth, the discharge from the adrenal medulla of the calf during different types of stimulation has now been examined during the latter part of gestation and immediately after birth. Preliminary reports of some of the present investigations have already been published (Comline & Silver, 1962, 1963).

METHODS

Animals

All experiments were carried out on adult cattle or calves of the Jersey breed. Foetuses were used from 180 days gestation until term; care was taken to ensure that pregnancy had resulted from a single service, the date of which was accurately known. The duration of pregnancy in the cow is normally accepted as 280 days, although considerable variations can occur (Brakel, Rife & Salisbury, 1952). Since some of the critical experiments in the present investigation were performed either immediately before or after the time of parturition, it was essential to know whether the gestational ages of such calves were within the normal range. Information on the duration of pregnancy in Jersey cows was therefore collected from farms in which reliable and accurate records were kept and which had supplied the majority of animals for the present experiments. The frequency distribution of parturition in 730 Jersey cows and heifers from these farms has been plotted in Fig. 1, and shows the wide range of the duration of pregnancy. Calvings were distributed normally, about a mean of 280·5, with 95% of all calvings occurring between 267 and 294 days' gestation. The apparent decrease in the frequency of parturition at 290 days was not statistically significant; the distribution was symmetrical but there was highly significant kurtosis in the upward direction ($t = 34{\cdot}319$).

Calves for experiments were used from 1 hr after birth. The exact time of birth was known as well as the duration of pregnancy. No colostrum or milk was fed to animals used within the first 24–30 hr of birth; all other calves received colostrum and a normal milk diet, and food was withheld from the animals on the morning of the experiment. When weaned animals were used, solid food but not water was withheld for 24–36 hr before the start of the experiment.

These experiments on foetal and new-born calves were carried out before, and simultaneously with, those on the adrenal medulla of the foetal lamb (Comline & Silver, 1961; Comline *et al.* 1965) but in contrast with the sheep the number of animals used was limited not only by their cost but also by their availability from reliable sources.

Preparation

Anaesthesia. In the majority of experiments on both calves and pregnant cows anaesthesia was induced and maintained by the intravenous injection of sodium pentobarbitone (in

earlier experiments Nembutal, Abbott; in the majority of experiments and in all pregnant animals, sodium pentobarbitone (May & Baker) 6 g/100 ml. of 0·9 % (w/v) NaCl). Chloralose anaesthesia was used in a number of calves between 4 hr and 3 weeks of age; in these animals chloralose (60 mg/kg) was injected intravenously in a concentration of 7·5 mg/ml. of 0·9 % (w/v) NaCl.

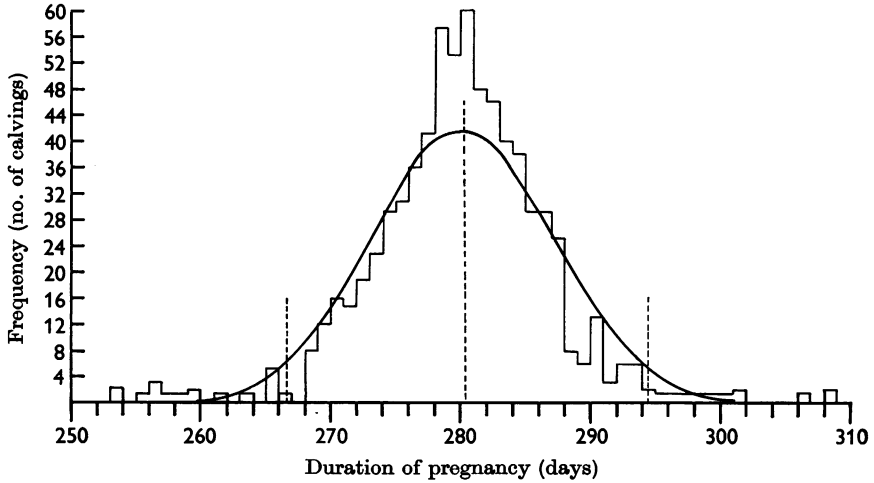

Fig. 1. The duration of pregnancy in 730 Jersey cows and heifers. The data fit a normal frequency–distribution curve (heavy line) with parturition occurring between 266·7 and 294·3 days of pregnancy in 95 % of all calvings.

Operatives procedures. The foetal calves were removed from the uterus and prepared for the collection of adrenal effluent blood in a manner similar to that used in the foetal sheep (Comline & Silver, 1961). Care was taken to keep the placental circulation intact. In the present experiments, owing to the greater weight of the foetus and uterus, it was found most convenient to place the cow on her side and to expose the uterus through an incision on the right side. Foetal adrenal effluent blood was collected immediately before and during the period of stimulation, and the forceps on the junction between the renal vein and vena cava were removed during the recovery periods. When required, a cannula for intra-arterial injections was inserted into the anterior mesenteric artery after ligature of the coeliac artery and the insertion of a strong loose ligature round the aorta, caudal to the left adrenal. In experiments in which denervation of the adrenal was tested the splanchnic nerves were ligated and cut 10–20 min before the first adrenal effluent sample was taken: the peripheral ends were used for stimulation. The splanchnic nerves were also cut in all experiments in which the effect of intra-arterial acetylcholine was tested. The preparation of the new-born calf for the collection of effluent blood from the left adrenal gland, the stimulation of the splanchnic nerves and intra-arterial injection of acetylcholine has already been described (Silver, 1960).

When continuous arterial P_{O_2} measurements were required, the method used was essentially similar to that employed previously for the foetal lamb (Comline *et al.* 1965). Polythene arterio-venous loops were inserted into the carotid artery and jugular vein of the foetus and the brachial or facial artery and vein of the mother; in the new-born calf the femoral artery and vein were used.

Asphyxia and hypoxia. In all experiments with foetal calves a slow stream of O_2 (2–6 l./min) was delivered through the slide arm of the tracheal cannula of the cow. This was

generally sufficient to ensure good foetal oxygenation and little change in foetal blood pH throughout the experiment. When continuous measurements of foetal and maternal arterial P_{O_2} were made in cows near term, more precise control was possible and the foetal carotid P_{O_2} was maintained above 24 mm Hg by adjusting the level of supplementary O_2 given to the cow. Data obtained previously on the foetal lamb showed that the carotid P_{O_2} under normal resting conditions was usually at or above this level. Foetal and maternal arterial blood pH and P_{CO_2} were checked at intervals in these experiments. Full details of these procedures have been given previously (Comline *et al.* 1965). Asphyxia was produced by clamping the umbilical cord. Only one such experiment was possible in any given foetus, although in some of the younger foetuses with cut splanchnic nerves the effect of nerve stimulation or acetylcholine was also tested; in these animals a rest period of at least 20 min was allowed between stimulation and clamping the cord.

In the new-born and older calves asphyxia was induced either by rebreathiug N_2 from a balloon (volume about 1100 ml.) or, in certain experiments, by positive pressure ventilation from an 'Ideal' Respiratory Pump with gas mixtures which contained 10% CO_2 in N_2 to simulate conditions in the foetus after occlusion of the umbilical cord as nearly as possible. Not more than two such periods of asphyxia were tested in any one calf and a 30 min recovery period was allowed between them. Preliminary experiments had shown that the response of the adrenal medulla to two identical periods of asphyxia was similar, provided that the original resting conditions were restored during the recovery period. Arterial P_{O_2} was measured continuously in a number of experiments and the pH and P_{CO_2} were measured at intervals throughout the experiment. The resting P_{O_2} levels remained relatively stable before a test period and were usually rapidly restored after asphyxia without the use of continuous supplementary O_2. The only exceptions to this were in 2–4 hr calves in which more rigorous control of resting P_{O_2} levels was necessary; further details are given in the text.

Administration of drugs. Heparin was given intravenously to the mother at 1000 i.u./kg and to the foetus at 2000 i.u./kg after all operative procedures were completed. Coagulation of the blood was similarly prevented in new-born and other calves by the administration of 1000 i.u./kg heparin intravenously. Hexamethonium bromide was given intravenously at a dose of 10 mg/kg. In all experiments in which acetylcholine was given intra-arterially, atropine sulphate was administered intravenously at a dose of 0·2 mg/kg, either at the beginning of the experiment or, in some animals, after the first test with acetylcholine.

Close arterial injections of acetylcholine (2–4 mg) were given through the anterior mesenteric artery in a volume of either 2 or 4 ml. of 0·9% (w/v) NaCl containing 1·0 mg NaH_2PO_4/ml. The aorta was occluded caudal to the adrenal, during and after the acetylcholine injection, for a maximum period of 15 sec in the new-born animals and 10 sec in the foetuses. This procedure in the foetus caused a fall in arterial P_{O_2} due to cessation of flow in the umbilical arteries, but this was usually only about 5 mm Hg and was never sufficient to bring about a discharge due to the direct effect of hypoxia on the adrenal gland. The injection in a similar manner of an equivalent volume of saline, or solute for the acetylcholine, did not cause a detectable increase in the resting rate of secretion from the adrenal medulla.

Stimulation of splanchnic nerves. The peripheral ends of the cut splanchnic nerves were stimulated with platinum electrodes which delivered stimuli at 30/sec (40 V exponential wave form). The duration of stimulation varied between 1½ and 5 min depending on the rate of blood flow from the adrenal gland.

Adrenal glands. In the foetus these were removed after death from asphyxia. The expense of pregnant animals did not justify the use of foetuses solely for the assay of the content of adrenaline and noradrenaline in the glands. In the new-born calves a series of animals was used specifically for the removal of their adrenal glands, since anaesthesia and other operative interference had been shown previously to have a marked effect on the content of

amines in the adrenal medulla (Comline & Silver, 1961). The glands were removed with minimum operative procedures as soon as possible after the induction of anaesthesia with sodium pentobarbitone.

Samples

Arterial blood samples

Samples of 2–4 ml. for the measurement of pH, P_{CO_2}, glucose and lactic acid were taken from the renal or femoral artery at rest and at 1, 3 and 5 min after the onset of asphyxia. If the asphyxia was continued for a longer period further samples were taken at 2 min intervals.

The volume of blood removed as samples in the foetal calves did not exceed 5% of the total estimated blood volume. In the new-born animals with their very high rate of adrenal blood flow and lower total blood volume, larger amounts of blood were of necessity removed; excess blood was returned to the animal and the total volume withdrawn did not exceed 10% of the estimated blood volume.

Adrenal venous blood samples

The duration of the collecting periods and the intervals between them varied with the type of stimulation and the blood flow through the gland. The procedures used can be summarized as follows.

Asphyxia. In foetal calves up to 240 days adrenal effluent blood was normally collected over 5 min periods. In the foetuses nearer term shorter collection times were possible, and in a large number of foetuses consecutive 1 min samples were taken from the onset and throughout the period of asphyxia. In new-born calves in which asphyxia was induced by re-breathing N_2, attainment of a P_{O_2} comparable with that in the foetus after cord occlusion was slower. In the majority of animals consecutive 1 min samples were collected throughout the whole 5 min period of asphyxia, but since little or no output occurred in the first 2 min of asphyxia, in some animals sampling was delayed for this period, and three 1 min samples were then collected.

Nerve stimulation. In foetuses up to 240 days a 3–5 min collecting period was again employed. After this age, a 1½–2 min period was used, both for foetuses and new-born animals, with a 30 sec time lag before the onset of collection.

Intra-arterial acetylcholine. In foetuses less than 230 days a collecting period of 1½–2 min was used, with a time lag of 15 sec before collection was started. In foetuses near term, in which the rate of blood flow from the gland was very much greater, the collection of each sample was begun at the same time as the injection of acetylcholine and continued for 1½ min. In the new-born calves the volume of the tubing collecting the adrenal venous blood was considerably larger than that used in the foetuses, and therefore collection of the sample was not begun until 10–15 sec after the injection of acetylcholine to allow for this dead space. Preliminary experiments had shown that the discharge due to the injected acetylcholine was detectable in the effluent blood for only about 1 min after this lag period and that samples taken during the following minute contained only small amounts of adrenaline and noradrenaline.

The secretion of both adrenaline and noradrenaline during the 15 sec lag period was also tested in a number of calves. The output of both amines was very variable and appeared to depend partly on the rate of blood flow through the gland. In some calves a significantly higher proportion of adrenaline was secreted during this period, but the amounts were insufficient to make any substantial difference to the results obtained in the whole series.

A period of 10 min was allowed between tests with either acetylcholine injections or stimulation of the splanchnic nerves.

Measurements

pH, P_{CO_2} and P_{O_2}. pH and P_{CO_2} were measured in individual arterial blood samples according to the procedures described previously (Comline *et al.* 1965). P_{O_2} was measured continuously in flowing blood throughout the experiments. Full details of the O_2 electrodes

used and the method of calibration and measurement have been given in previous papers (Silver, 1963; Comline *et al.* 1965).

Chemical determinations. The method of extraction, chromatography and measurement of adrenaline and noradrenaline in blood and adrenal glands was the same as that described previously (Silver, 1960; Comline & Silver, 1961). Blood glucose was determined by glucose oxidase method of Huggett & Nixon (1957) and lactic acid by the lactic dehydrogenase method of Barker & Britton (1957).

The levels of catecholamines in the adrenal effluent blood are given throughout as μg adrenaline or noradrenaline base secreted by the left adrenal gland per minute, and are uncorrected for losses during extraction and chromatography. The recovery of standard amounts of both amines was tested in most experiments; the mean percentage recovered was $84{\cdot}0 \pm 1{\cdot}06\,\%$ for 1·0 μg noradrenaline and $81{\cdot}5 \pm 1{\cdot}09\,\%$ for 1·0 μg adrenaline (20 estimations taken at random between 1962 and 1964). The data have not been expressed as output/kg body wt./min in these experiments since accurate measurements of the foetal weight could not be made at the end of the experiment and changes in body weight during the first 3 weeks after birth were negligible (see Table 8).

RESULTS

Composition of the blood and resting output from the adrenal medulla in foetal and new-born calves under anaesthesia

Since asphyxia was to be tested for its effect on the adrenal medulla, it was important to ensure that the animals, particularly the foetuses, were well oxygenated before any form of stimulation was given, and that other changes in blood composition which might give rise to stimulation of the adrenal medulla did not occur.

Table 1 shows the mean arterial blood gas tensions, pH, glucose and lactic acid levels found in foetal and new-born calves under anaesthesia at the beginning of experiments, after all operative procedures had been completed. The values obtained for the foetuses were comparable in all respects with those reported previously for the foetal lamb under similar conditions (Comline *et al.* 1965) and, as in the lamb, the resting level of secretion from the adrenal medulla was very low (Table 2).

Large changes in many of the blood constituents normally occur at birth and although a full investigation of these was beyond the scope of this paper, a comparison of the levels of blood gases and other metabolites before and after anaesthesia was carried out in certain new-born calves. In those over 24 hr of age no large changes in blood composition occurred although in some animals anaesthesia resulted in a slight elevation of P_{CO_2}, lactic acid and glucose levels, and a corresponding fall in P_{O_2} and pH. The P_{O_2} was normally maintained at 80–90 mm Hg with supplementary O_2 when necessary, and the pH had generally returned to between 7·36 and 7·40 before any samples were taken (Table 1).

The conditions in calves within 6 hr of birth were much more variable, both before and after anaesthesia. This is shown in Table 3, which gives

TABLE 1. Composition of arterial blood in anaesthetized foetal and new-born calves (after completion of operative procedures)

Age (days)	No. of animals	P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)	pH	Glucose (mg/100 ml.)	Lactic acid (mg/100 ml.)
Foetus*						
265–280 (pentobarbitone)	6	33·8 ± 4·6 (n = 5)	52·0 ± 2·9	7·29 ± 0·02	31·3 ± 2·3	47·5 ± 3·0
Calves†						
2–5 hr (chloralose or pentobarbitone)	11	80·9 ± 6·8	63·0 ± 5·1 (n = 7)	7·24 ± 0·02	67·8 ± 13·9 (n = 6)	23·0 ± 2·09 (n = 6)
24–48 hr (chloralose or pentobarbitone)	5	83·4 ± 7·4	48 (n = 3)	7·36 ± 0·01	—	—
14–21 days (chloralose)	4	86·0	43	7·40	—	—

* Mothers receiving supplementary O_2 (2–6 l./min).

† Receiving supplementary O_2 when required ($\frac{1}{2}$ l./min via side arm of tracheal cannula).

No. of observations (n) = no. of animals unless stated otherwise.

TABLE 2. Levels of pressor amine output from the adrenal medulla under resting conditions in foetal and new-born calves

Age (days)	No. animals	Mean rate of secretion from adrenal gland (μg/min)	
		Noradrenaline	Adrenaline
Foetus			
180–220	4	0·06	0·025
230–250	4	0·33	0·14
260-term			
Nerve intact	2	0·40	0·10
Nerve cut	5	0·34	0·20
New-born (1–5 hr)			
Nerve intact	4	0·42	0·23
Nerve cut	4	0·53	0·12

the changes in the composition of the blood in six animals before and after anaesthesia and during the course of experiments involving asphyxia. The poor oxygenation often observed in such animals, even before anaesthesia, could usually be countered by the administration of supplementary oxygen, but this had little or no effect on the pH, which remained low throughout the experiments. Despite these variations resting secretion of the adrenal medulla in these calves, as well as the older animals, was invariably low.

Output from the adrenal medulla during asphyxia in foetal and new-born calves

Foetal calves

Asphyxia was induced by ligature of the umbilical cord; this resulted in an immediate drop in foetal blood P_{O_2} and more gradual changes in pH and P_{CO_2}. These changes, which were essentially similar to those observed during asphyxia in the foetal lamb (Comline *et al.* 1965) are included among the data shown in Fig. 5.

The cardiovascular changes associated with asphyxia in the foetal sheep were also observed in both foetal and new-born calves during asphyxia. The initial increase in blood pressure and heart rate were followed 2–3 min later by the abrupt cardiac slowing and increased pulse pressure which appears to be characteristic of the foetus.

The effect of asphyxia on the response of the adrenal medulla was investigated with the splanchnic nerves intact and after they had been cut in a series of foetuses between 180 days and term. The results, given in Fig. 2, showed, first, that the output of noradrenaline at all stages during the latter part of pregnancy was considerably higher than that of adrenaline and, secondly, that the splanchnic nerves appeared to play no obvious part in this response to asphyxia. Two exceptions were found which are also shown in Fig. 2; in these foetuses, aged 270 and 276 days, noradrenaline secretion was about 2·5 μg/min which was below the level expected during asphyxia at this age. In both animals parturition had already started and this may have contributed to the exceptionally low discharge.

As a check on the efficacy of denervation the effect of hexamethonium (10 mg/kg) was tested since this abolished the nervous response to asphyxia in the near-term foetal lamb. These results are also included in Fig. 2, and give further evidence for the absence of participation of the nerve supply to the adrenal medulla in the response of the foetal calf to asphyxia.

The time course and pattern of the adrenal response to asphyxia is shown in Fig. 3 for two groups of foetuses (mean age 230 and 270 days). As in the case of immature foetal lambs the adrenal gland continued to secrete for as long as the arterial blood pressure was maintained and the

TABLE 3. The composition of the blood of 6 new-born calves before and during course of experiments involving positive pressure ventilation with 10 % CO_2 in N_2 (pentobarbitone)

Sample details	No. obs.	pH	P_{CO_2} (mm Hg)	P_{O_2} (mm Hg)	Glucose (mg/100 ml.)	Lactic acid (mg/100 ml.)
Before anaesthesia (jugular blood)	6	7·33 ± 0·01	51·4 ± 2·2	—	28·8 ± 3·2	22·9 ± 1·6
After anaesthesia (arterial blood)						
After operative procedures completed (1–1½ hr)						
(*a*) Calves not requiring supplementary O_2	2	7·28	56·1	75	51 (n = 1)	—
(*b*) Calves requiring supplementary O_2 (½–1 l.)	4	7·24	68·8	Pre O_2 35–52 Post O_2 76–130	79·4	24·1
Before onset of 1st period of asphyxia	6	7·25 ± 0·02	63·7 ± 6·0	86 ± 12·4	67·8 ± 13·9	23·0 ± 2·1
After 30 min rest period and before onset of 2nd period of asphyxia	6	7·23 ± 0·02	58·5 ± 2·4	87·5 ± 13·8	99·1 ± 11·3 (n = 5)	32·8 ± 4·9 (n = 5)

circulation to the gland continued. The output fell slowly after the first 5 min of asphyxia. There was no obvious difference between the pattern of discharge from the innervated gland and that after denervation or the administration of hexamethonium, in either group.

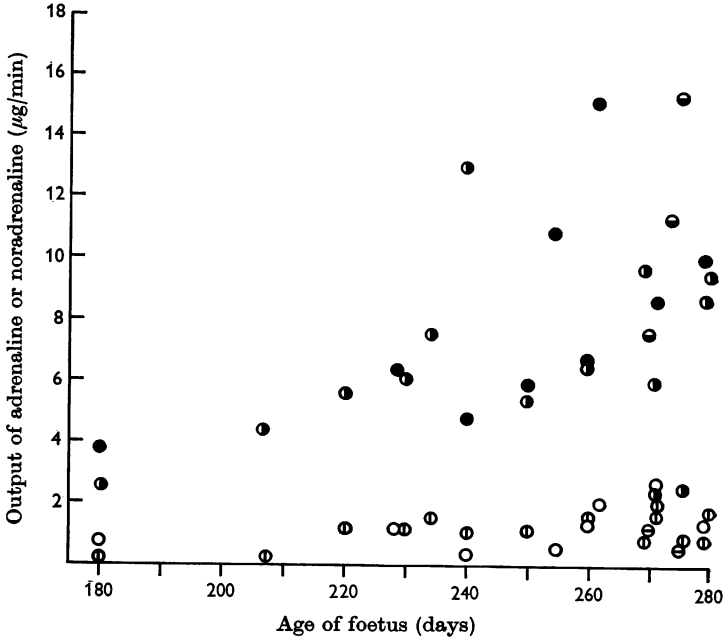

Fig. 2. The output of adrenaline and noradrenaline from the adrenal medulla during asphyxia in foetal calves of different ages. Each point represents the mean rate of discharge over the 5 min period immediately after ligation of the umbilical cord. *Noradrenaline:* ●, splanchnic nerves intact; ◑, splanchnic nerves cut; ◒, after hexamethonium. *Adrenaline:* ○, splanchnic nerves intact; ⓘ, splanchnic nerves cut; ⊖, after hexamethonium.

A detailed analysis of the factors responsible for the adrenal discharge during asphyxia in the foetal calf could not be carried out but, in those experiments in which the P_{O_2} was recorded throughout the experiment, it was clear that the rate of secretion from the gland did not increase to any great extent until the P_{O_2} had fallen to below 4–5 mm Hg. It seems probable therefore that in the calf as well as the lamb the rate of adrenal discharge might be related to the level of hypoxia. Evidence for this conclusion is given in Fig. 4. In this foetus, aged 270 days, the left splanchnic nerve had been cut and the umbilical cord clamped in the usual way, when a leakage occurred in the tracheal tube of the foetus before the P_{O_2} had fallen to zero. The onset of respiration in the foetus led to a rise in P_{O_2} of the foetal arterial blood and a decline in the discharge from the adrenal medulla which had not at that point reached its maximum output.

The rate of secretion from the gland fell to near resting levels and did not show any further increase, although the arterial P_{CO_2} and pH levels continued to change. The animal was then resuscitated by positive-pressure ventilation and, after a 20 min recovery period, was asphyxiated with N_2 by positive-pressure ventilation; the P_{O_2} fell rapidly to less than 1 mm Hg and a high output of noradrenaline was obtained. About 4 min after

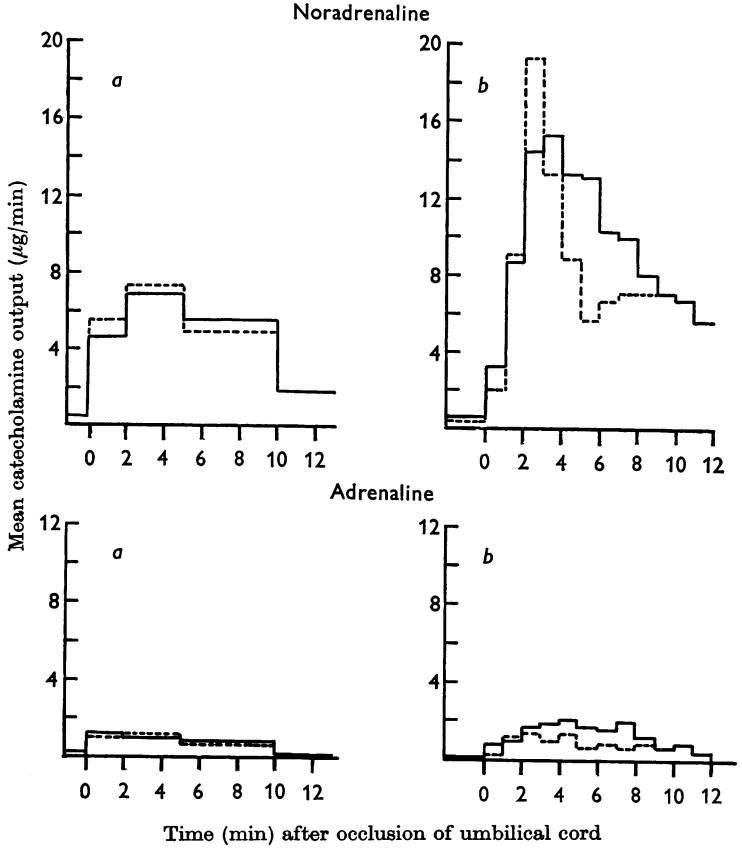

Fig. 3. Time course of the asphyxial discharge of adrenaline (lower frames) and noradrenaline (upper frames) from the intact (——) or acutely denervated (- - - -) adrenal medulla in two groups of foetuses. (*a*) 220–240 days gestation: nerve intact, two animals; nerve cut, four animals; (*b*) 260–280 days gestation: nerve intact, four animals; nerve cut (or 10 mg hexamethonium/kg), eight animals.

the onset of asphyxia the animal swallowed several times. This led to a slight rise in the blood P_{O_2} level and was promptly accompanied by a temporary fall in the output of noradrenaline. The pH and P_{CO_2} levels changed between the two periods of hypoxia, due primarily to a large increase in blood lactate from 30 to 70 mg/100 ml., but these changes

were small in comparison with those which occurred during the periods of asphyxia. In any case the new pH and P_{CO_2} levels did not cause any increase of the resting output from the gland between the two tests and the changes in noradrenaline output during both hypoxic periods were closely related to the concomitant P_{O_2} changes.

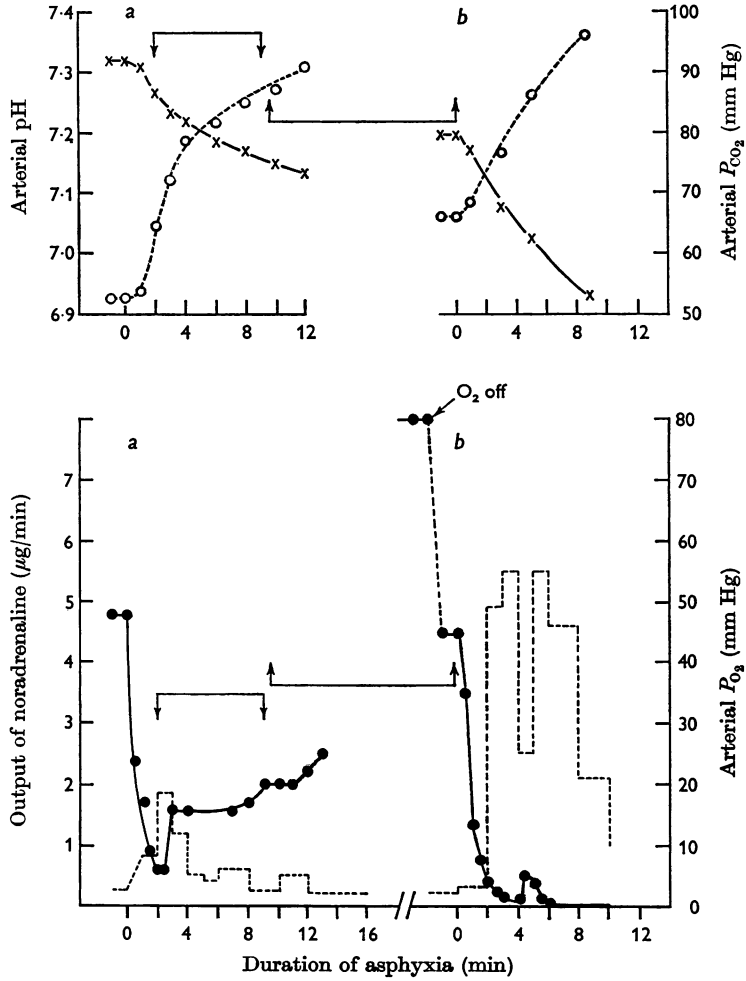

Fig. 4. The relation between noradrenaline output (-----) and foetal arterial P_{O_2} (●——●) after (*a*) umbilical cord occlusion, and (*b*) during positive-pressure ventilation with N_2 in a foetal calf aged 270 days (splanchnic nerve cut). ↓——↓ leak in tracheal tube and attempted respiration; ↑——↑ period of artificial ventilation with supplementary O_2. Corresponding changes in foetal arterial pH (×——×) and P_{CO_2} (○-----○) are shown in the upper graphs (*a*) and (*b*).

New-born calves: pentobarbitone anaesthesia

In the majority of experiments the new-born and older calves were allowed to re-breathe from an N_2-filled balloon. The initial resting blood gas levels in these calves were very different from the foetuses and the course and severity of asphyxia was to some extent dependent upon these levels. Thus, although there was an immediate fall in P_{O_2} after the onset of asphyxia, the final level attained after 5 min asphyxia was between 2 and 4 mm Hg (Fig. 5), whereas in the foetuses a P_{O_2} of zero was reached within 3 min.

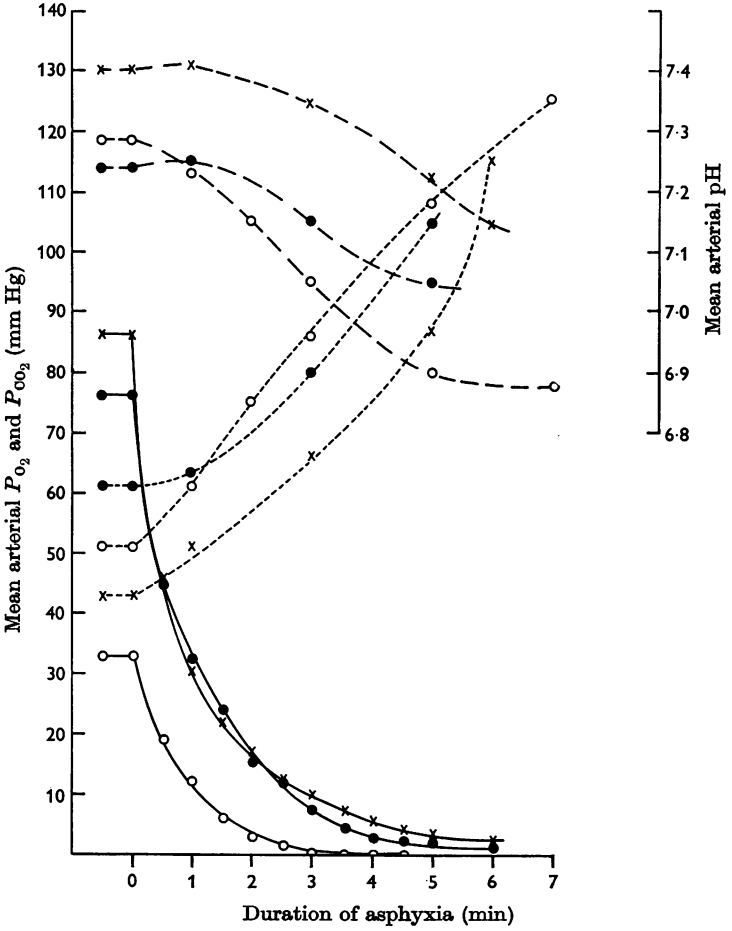

Fig. 5. Mean changes in arterial blood P_{O_2} ——; P_{CO_2} ----; and pH, ———, during asphyxia in foetal, new-born and older calves. Asphyxia was induced by umbilical cord occlusion in the foetuses and by N_2 rebreathing after birth. ○, foetuses (265–278 days, five animals); ●, new-born calves (1–5 hr, five animals); ×, older calves (7–21 days, four animals).

The pH and P_{CO_2} changes during asphyxia in calves after birth differed slightly from those in the foetus (Fig. 5). pH changes were negligible in the first minute, probably due to the initial hyperventilation; a drop in pH occurred between 1 and 5 min and the rate of rise in P_{CO_2} was greatest towards the end of this period. In the majority of calves neither the absolute nor the relative pH fall or the rise in P_{CO_2} was as great as that which occurred in the foetus over a 5 min period of asphyxia. The rise in the concentration of lactic acid during asphyxia was also smaller in the new-born animals than in the foetus. An increase in lactic acid of 40 mg per 100 ml. occurred during 5 min after occlusion of the umbilical cord; in the calves, after a similar period of re-breathing N_2, an increase of 25–35 mg per 100 ml. was the highest observed and frequently an increase of only 10–15 mg per 100 ml. was found.

Splanchnic nerves intact. The mean outputs of noradrenaline and adrenaline per min during asphyxia in calves aged between 1 hr and 3 weeks are shown in Fig. 6, together with comparable data for the late-term foetus. The mean level of secretion in both foetuses and new-born calves has been calculated from values obtained over a 2–3 min period after the response to asphyxia had become maximal.

At 1–5 hr of age the response of the adrenal medulla to asphyxia was high, and, as in the foetus, noradrenaline was the predominant amine released. This ability to secrete large amounts of noradrenaline was not maintained after parturition; within 15 hr of birth the response to asphyxia had diminished by nearly 50 % (Fig. 6) and at 24 hr it was only 25 % of the high output at birth. The response of the gland to this form of stimulation remained low for a week or more and it was not until 2–3 weeks after birth that the output of noradrenaline during asphyxia rose to, or exceeded, that found immediately after birth (Fig. 6).

The proportion of adrenaline released during asphyxia immediately after birth was somewhat higher than in the foetus at term, but the output of this amine was variable. It appeared to diminish slightly at 7 days but even at 3 weeks the level of adrenaline released was only 20 % of the total output of catecholamines.

Analysis of the duration of the response to asphyxia in calves of different ages (Fig. 7) showed that the high level of noradrenaline output immediately after birth was maintained for several minutes, whereas the response at 24 hr or 7 days remained low. Prolongation of the stimulus during this period did not increase the response in any way.

Splanchnic nerves cut. In order to determine whether the splanchnic nerves were involved in any of the different stages of adrenal secretion after birth, the direct response of the gland to asphyxia was investigated after cutting the splanchnic nerves or, in a few animals, after the admini-

stration of hexamethonium. The results from these experiments can be compared in Fig. 6 with those in which the splanchnic nerves were intact. Again, the most striking features of the direct response of the adrenal medulla to asphyxia in the new-born calf were the high proportion of noradrenaline released and the very rapid decline in output after birth,

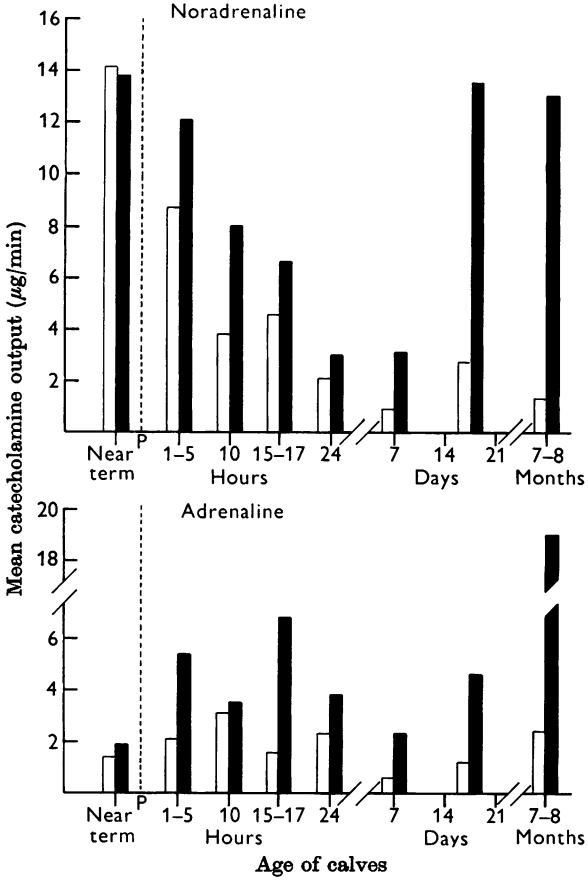

Fig. 6. The change in the response of the innervated ■, and acutely denervated, □, adrenal medulla to asphyxia in the calf with age. Each histogram represents the mean rate of discharge/min in three to six animals; the level of secretion in each animal (except at 7–8 months, see text) was calculated from values obtained over a 2–3 min period after the response to asphyxia had become maximal. P, parturition. Asphyxia was induced by N_2-rebreathing (calves) or by umbilical cord occlusion (foetuses).

but whereas the ability of the gland with an intact nerve supply to secrete noradrenaline was regained by 2–3 weeks, the direct response was permanently lost. The property of the adrenal medullary cells to react directly to asphyxia therefore appears to be confined to the foetus.

The differences between the mean output from intact and denervated glands (Fig. 6) implied that at birth the splanchnic nerves were in part responsible for the asphyxial discharge and that the direct response of the adrenal medulla, even at 2 hr after birth, had already regressed from the foetal condition. On the other hand, in view of the extremely low P_{O_2} which appeared to be required to elicit a direct response in the foetus (see Fig. 4), it was possible that the smaller response from the denervated

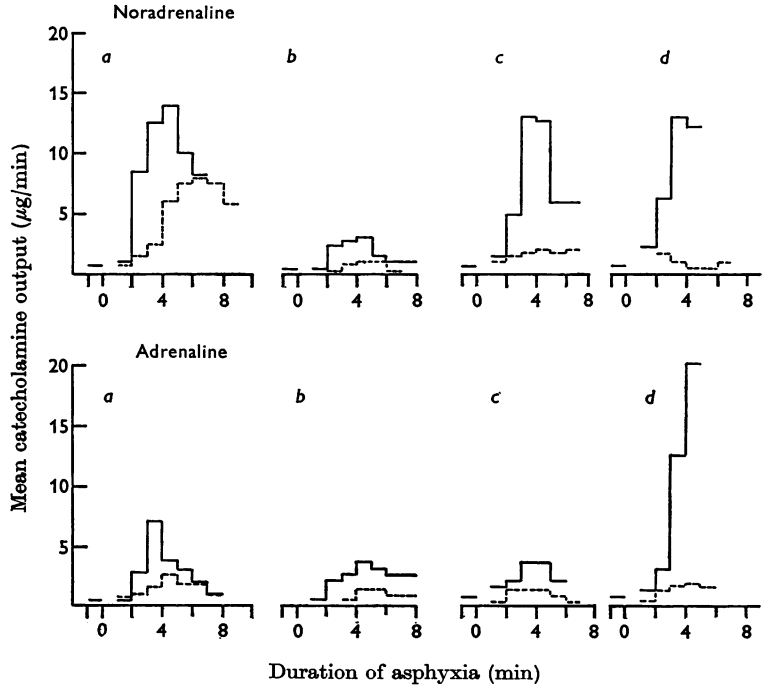

Fig. 7. Time course of the asphyxial discharge of adrenaline and noradrenaline from the intact (——) or acutely denervated (---) adrenal medulla in the calf at different ages after birth (three to six animals in each group). (*a*) 1–5 hr; (*b*) 24 hr; (*c*) 2–3 weeks; (*d*) 7–8 months. Asphyxia was induced by N_2-rebreathing. The response at 7 days was similar to (*b*).

gland of the new-born animal was due to an insufficient stimulus. This was tested in a series of experiments on new-born calves in which the P_{O_2} was lowered much more rapidly to the critical level by means of positive-pressure ventilation with N_2 containing 10% CO_2. The changes in P_{O_2} during asphyxia were then very similar to those of the foetus. A comparison between the mean levels attained in five foetuses and in five new-born calves during positive-pressure ventilation showed that after 5 min the P_{O_2} levels in both groups were zero; at $2\frac{1}{2}$ min foetal levels were $1{\cdot}7 \pm 0{\cdot}29$ mm Hg, while in the new-born animals the mean P_{O_2} was

3·1 ± 0·95 mm Hg. By contrast, the mean P_{O_2} level attained at 2½ min in six calves asphyxiated by rebreathing N_2 was significantly higher (12·0 ± 0·86 mm Hg).

In Fig. 8 the mean changes in arterial blood P_{O_2}, P_{CO_2} and pH produced by the two methods of inducing asphyxia in the new-born calves (1–5 hr) are compared. While the changes in P_{CO_2} and pH were comparable in the two groups the rate of fall in P_{O_2} was considerably faster in the artificially

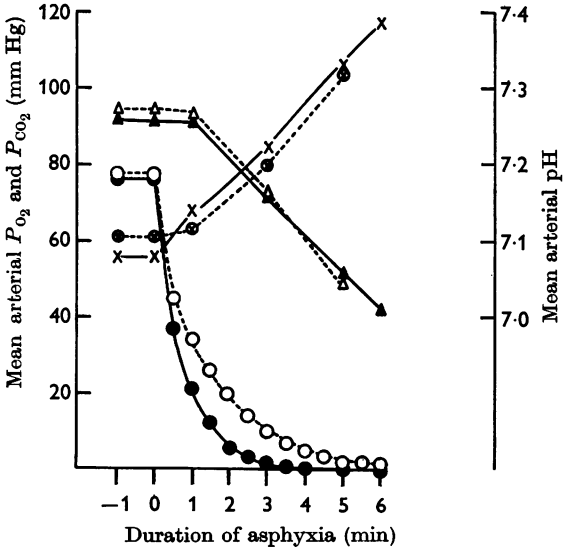

Fig. 8. A comparison of the changes in arterial blood P_{O_2}, P_{CO_2} and pH in two groups of new-born calves asphyxiated either by rebreathing N_2, ----- (five animals), or by positive-pressure ventilation with 10 % CO_2 in N_2, —— (five animals). Mean levels of P_{O_2}, ○ and ●; P_{CO_2} ⊗ and ×; pH, △ and ▲, for each group, respectively.

ventilated animals. The mean discharge of adrenaline and noradrenaline from the acutely denervated adrenal gland obtained in these new-born animals during positive-pressure ventilation with 10 % CO_2 in N_2 is shown in Fig. 9*a* as solid lines: the corresponding response obtained previously, during asphyxia induced by N_2-rebreathing, is shown in the same figure as broken lines. The most striking difference between the two types of response was the more rapid onset and high level of noradrenaline output obtained when the arterial P_{O_2} was rapidly reduced to zero. The maximum level of secretion in this group was, in fact, as great or greater than the maximum output attained previously with splanchnic nerves intact (Fig. 7*a*). The response of the intact adrenal medulla to this much more effective stimulus was therefore tested. The mean results for three calves, given as solid lines in Fig. 9*b*, showed that although the response of the innervated gland to a more abrupt reduction in P_{O_2} was slightly more

rapid than the discharge obtained during N_2-rebreathing (interrupted lines, Fig. 9*b*), the maximum discharge obtained was no greater than the maximum output of the denervated gland (Fig. 9*a*).

Some stimulation of the splanchnic nerves thus appeared to occur in these calves during asphyxia at a P_{O_2} which appeared to be higher than that which stimulated the gland directly. A similar situation has been

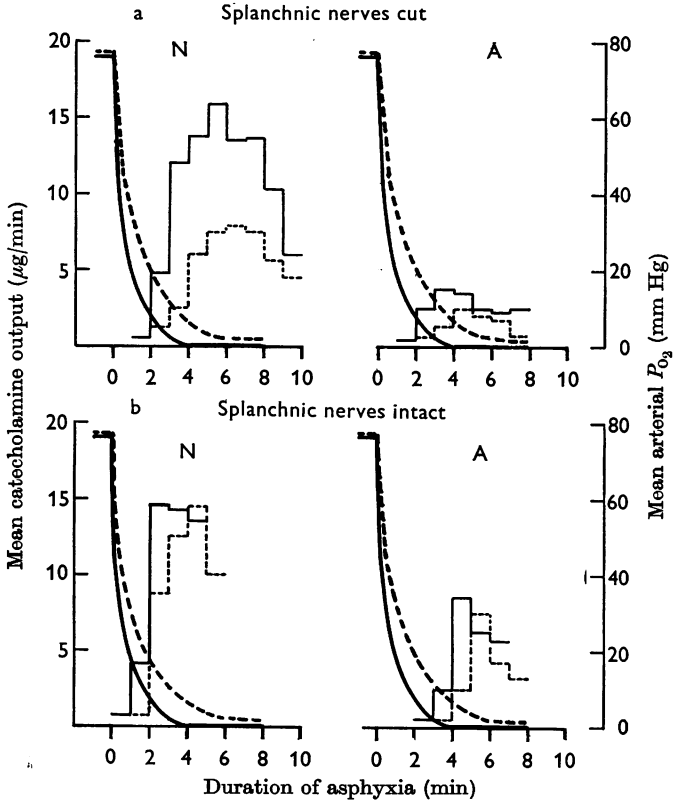

Fig. 9. A comparison of the mean rates of noradrenaline (N) and adrenaline (A) output during the induction of a rapid (——) or a slow (----) fall in arterial P_{O_2}. The curves superimposed on each graph, taken from Fig. 8, show the mean rate of fall in P_{O_2} during asphyxia induced by positive-pressure ventilation with 10 % C_{O_2} in N_2 (——) or by N_2-rebreathing (– – – –). (*a*) *Upper graphs:* splanchnic nerves cut, five animals in each group. (*b*) *Lower graphs:* splanchnic nerves intact, three animals (positive-pressure ventilation) and five animals (N_2-rebreathing).

observed in the mature foetal lamb, in which the threshold for the nervous response to asphyxia was about 16 mm Hg, while that for the direct response was 4–6 mm Hg (Comline *et al.* 1965). In the new-born calf, however, the present results suggest that the nervous mechanism is probably not complete at this stage, since direct stimulation of the nerves to

the gland resulted in a discharge which was only 20–30 % of the response to asphyxia (see Table 4). It seems more likely that any splanchnic nerve impulses caused by asphyxia probably potentiated the direct response of the gland to this stimulus.

A rapid reduction to a low P_{O_2} was essential for a high rate of discharge from the denervated adrenal medulla in the new-born calf as well as the foetus. It was important, therefore, to determine whether this high noradrenaline output could be maintained during the period of adrenal insensitivity after birth by a rapid reduction to a low P_{O_2}. This was tested in two calves aged 15 and 24 hr by positive-pressure ventilation with 10 % CO_2 in N_2; in each case the rate of secretion, especially of noradrenaline, was as low as that found previously during N_2 rebreathing. The sensitivity of the noradrenaline cells to low P_{O_2} levels thus appeared to be lost within a few hours of birth.

The fall in noradrenaline output in response to asphyxia in calves between birth and 24 hr of life was assumed to be a consequence of the change from foetal to extra-uterine life, but the wide variation in the duration of pregnancy, which has already been mentioned (Fig. 1) suggested an alternative explanation; that changes in output might be related to gestational age. The relation between mean output of noradrenaline during asphyxia, and during other forms of stimulation, and the gestational age of calves between 0 and 24 hr of birth, was therefore investigated further. There was no increase or decrease in the secretion of noradrenaline with increased gestational age, and the abrupt changes after birth could not be explained on this basis.

New-born calves: chloralose anaesthesia

In all the above experiments sodium pentobarbitone was used for anaesthesia, and the nervous response to asphyxia, although greatest immediately after parturition, was clearly diminished during the first 24 hr of life and remained low for 1–2 weeks after birth. Although this might be ascribed to some deficiency in the adrenal cells or in the mechanism whereby they are excited by their preganglionic nerves the possibility remained that it might be due to some depression of pathways in the central nervous system at this time. Barbiturates have a particularly long-lasting action on young calves and it was important to ascertain whether the decreased secretion was determined by the anaesthetic rather than the development of the adrenal medulla. The same series of experiments were therefore repeated with chloralose anaesthesia. Asphyxia was induced by rebreathing N_2 from a balloon and continuous samples of adrenal effluent blood were taken, for periods of 1 min, during asphyxia. The pooled results are given in Fig. 10 as histograms together with the

corresponding results with pentobarbitone anaesthesia. A similar fall in the response to asphyxia was observed 24 hr after birth. A slight increase in the response to asphyxia appeared to occur at 40 hr after birth, although the discharge was very variable at this stage; a complete restoration of the very high levels of secretion of noradrenaline equivalent to those found immediately after birth was again not evident until 3 weeks of age.

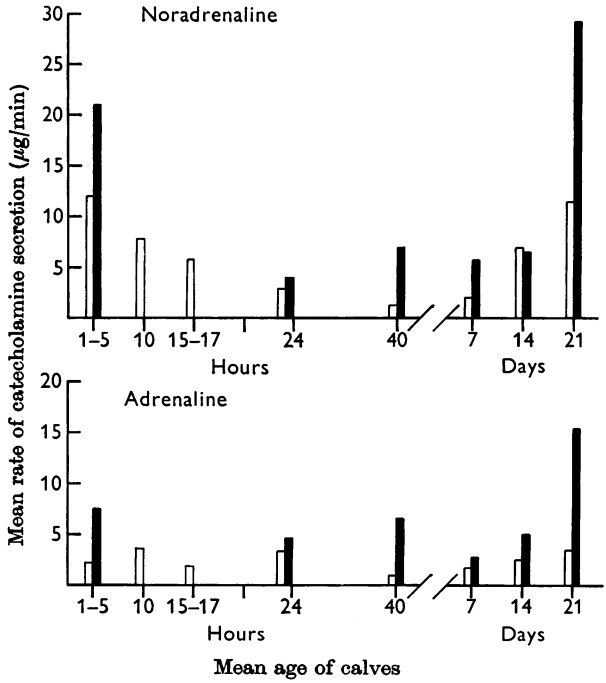

Fig. 10. A comparison between the mean outputs of adrenaline and noradrenaline from the adrenal medulla during asphyxia induced by N_2 rebreathing in calves under sodium pentobarbitone (□) or chloralose (■) anaesthesia; three to six animals in each group.

The values obtained under chloralose anaesthesia at all ages were higher than those with pentobarbitone, although the same over-all pattern emerged irrespective of the type of anaesthetic used. With chloralose anaesthesia there was, however, a considerable increase in adrenaline output in response to asphyxia 2–3 weeks after birth, which was absent with pentobarbitone anaesthesia.

Older calves

It seemed probable that the high output of noradrenaline which occurred during asphyxia at 3 weeks of age would later be replaced by the adult

type of secretion. More direct evidence on this point was obtained in three animals at 7–8 months. Previous experiments had shown that at this age adrenaline was secreted in slightly larger amounts than noradrenaline during direct stimulation of the splanchnic nerves (Silver, 1960).

Pentobarbitone anaesthesia was used and asphyxia was induced by the rebreathing technique, using a Douglas bag filled with N_2; the mean rates of adrenaline and noradrenaline secretion, once maximum outputs had been attained in this group, are included in Fig. 6. The output of adrenaline in these animals was considerably higher than at earlier stages of development although the rate of noradrenaline secretion had not changed. However, the mean values for rates of secretion during asphyxia in these calves may be misleading since considerable changes in the relative proportion of the two amines occurred during the course of 5 min asphyxia. Figure 7*d* shows that noradrenaline was released earlier and reached its maximum rate of secretion before adrenaline, but that during the last 2 min of asphyxia the discharge of adrenaline increased rapidly and eventually exceeded that of noradrenaline. Section of the splanchnic nerves in these animals confirmed that the very small residual discharge during asphyxia amounted to about 10 % or less of the normal output.

Response of the adrenal medulla to stimulation of the splanchnic nerves

In view of the apparent lack of activity of the nerve supply to the adrenal medulla during foetal asphyxia it was important to establish whether in fact the gland would respond to direct splanchnic nerve stimulation and what changes, if any, occurred during the first 24 hr after birth.

Foetal calves

The mean rates of secretion during splanchnic nerve stimulation are given in Table 4. The output of both amines was greater than the resting levels encountered throughout the series (Table 2), but, compared with asphyxia, the rate of noradrenaline secretion during splanchnic nerve stimulation was extremely low. Adrenaline output was similar to that found during asphyxia, i.e. with either form of stimulation only small amounts of this amine were released.

New-born calves

In previous experiments on the young calf, no detailed analysis of the response to direct stimulation of the splanchnic nerves was made during the first 24 hr of life (Silver, 1960). The results obtained in the present series are summarized in Table 4. At 2 hr after birth there was a slightly larger secretion in response to splanchnic nerve stimulation. This was not maintained, and little or no change in the low level of secretion could be

found during the first week of life. By 3 weeks, i.e. the time at which the nervous response to asphyxia was present, the response to nerve stimulation also increased, but noradrenaline remained the predominant amine released. The amounts of both amines released in the present series of experiments with sodium pentobarbitone anaesthesia were lower than those obtained previously when chloralose anaesthesia was used. In view of the different levels of output obtained with these two anaesthetics during asphyxia the discrepancy between the results with nerve stimulation can probably be ascribed to the type of anaesthesia.

TABLE 4. Mean output from the adrenal medulla of foetal and new-born calves during 1½–2 min stimulation of the splanchnic nerves to the gland

Age	No.	Mean rate of secretion μg/min		Adrenaline (%)
		Noradrenaline	Adrenaline	
Foetuses				
180–207 days	2	0·68	0·09	11·7
221–240 days	4	1·49	0·83	35·8
250–280 days	5	2·46	1·71	41·0
Calves				
2 hr	3	5·1	5·5	52·0
6–12 hr	2	2·5	2·6	51·0
24–30 hr	5	1·8	2·6	59·0
2 days	2	1·9	2·4	55·8
3 days	3	2·1	3·6	63·2
7 days	8	3·1	2·8	47·5
3 weeks	3	8·0	6·0	43·0
7–8 mths	3	20·6	23·0	52·8

Output from the adrenal medulla after intra-arterial acetylcholine

The effect of the intra-arterial injection of acetylcholine was tested in foetal, new-born and older calves to ascertain whether the small response to splanchnic nerve stimulation could be related to deficiency of the transmitter or to any changes in the cells of the adrenal medulla.

Foetal calves

It was not possible, in the limited number of foetuses available, to carry out the extensive series of tests which were subsequently made on new-born calves. The results for two foetal calves at 210 and 230 days, respectively, and two calves near term have been added to the series for the new-born animals (Fig. 11). The discharge obtained was as great as that during asphyxia in the younger foetuses, but at term the response to acetylcholine was smaller. In both groups a high proportion (80 %) of noradrenaline was secreted and contrasted sharply with the response to nerve stimulation (Fig. 11, lower graph). The absolute amounts of the amines secreted under these conditions are probably of less significance

than the proportions, since the response after a single injection is almost bound to be exponential and any variation in blood flow may affect the total amount collected.

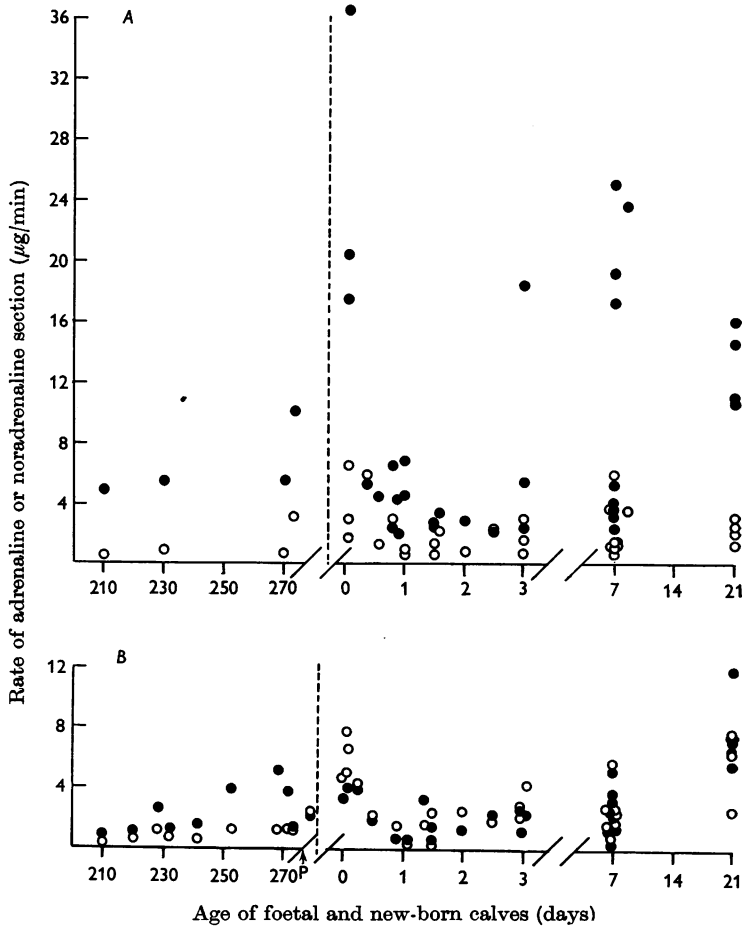

Fig. 11. The output of adrenaline (○) and noradrenaline (●) after the intra-arterial injection of acetylcholine (*A*) or during splanchnic nerve stimulation (*B*). *A*: each point represents the mean rate of secretion in a given animal in response to 2 mg acetylcholine (two or more tests). In the 210–230 day foetuses 1 mg acetylcholine was used. *B*: each point is the rate of secretion in a given animal during 1½–2 min stimulation of the peripheral ends of the cut splanchnic nerves. P, parturition.

New-born calves

The response to acetylcholine (2 mg) immediately after birth was extremely high and, as with asphyxia, the discharge consisted primarily of noradrenaline (Fig. 11). Similarly the response fell rapidly within the first 24 hr of life as the adrenal medulla became less and less sensitive

to the same dose of acetylcholine; the output then remained low for about a week. The sensitivity to acetylcholine appeared to return suddenly, although the exact age at which this change occurred varied with individual animals. This is shown in Fig. 11; in one of the three 3-day-old calves, a maximum discharge was obtained with 2 mg acetylcholine, while in nine animals examined at 7–8 days, five were still refractory to this stimulus. In fact, there seemed to be no intermediate level of secretion during this period after birth; the gland released either above 10 μg/min or about 4 μg/min noradrenaline in response to the same dose of acetylcholine.

Adrenaline output was low in response to 2 mg acetylcholine throughout the first 3 weeks after birth although a slight increase in secretion occurred in the hypersensitive phase immediately after birth and the output varied between 1 and 6 μg/min at 7 days of age.

During the course of the experiments with acetylcholine, tests on the efficacy of splanchnic nerve stimulation were always made and it was noticed that any subsequent injections of acetylcholine had a much greater effect on the pressor amine discharge. This phenomenon was therefore examined in more detail. The effect of doubling the dose of acetylcholine was also investigated, in view of the apparent loss of sensitivity of the noradrenaline cells and the almost complete insensitivity of the adrenaline-secreting cells to this stimulus after birth.

A comparison of the effect of 2 *and* 4 *mg acetylcholine.* Preliminary experiments had shown that two successive tests with the same dose of acetylcholine did not result in any significant change of output of either noradrenaline or adrenaline. An investigation into the effect of doubling the dose of acetylcholine on the secretion of the adrenal medulla was therefore carried out and it became apparent that the extent to which noradrenaline secretion was increased by the intra-arterial injection of 4 mg acetylcholine was dependent upon the initial rate of secretion of this amine after the injection of 2 mg. The whole series could be arranged in two arbitrary groups (Table 5); in those animals in which the initial noradrenaline output was over 10 μg per minute (Group I, mean: 18·8 $\pm$ 2·4 μg/min, $n = 11$) an increase in the dose of acetylcholine had no significant effect on the rate of noradrenaline secretion. On the other hand, when the initial response to 2 mg acetylcholine was low (Group II, mean 3·8 $\pm$ 0·39 μg/min, $n = 17$), then 4 mg acetylcholine elicited a 100% increase in noradrenaline output. This increase was statistically significant ($P < 0{\cdot}01$).

Group I included all animals aged 1–4 hr and 2–3 weeks and some of the week-old calves; all responded to 2 mg acetylcholine with a maximum response. Group II consisted of calves between 6 hr and 7 days; all were

comparatively insensitive to 2 mg acetylcholine and although 4 mg increased the output of noradrenaline it did not restore the response to the level found in Group I. Adrenaline secretion was not raised significantly in either group of calves by doubling the dose of acetylcholine.

TABLE 5. Mean output of noradrenaline and adrenaline in response to 2 mg and 4 mg acetylcholine given (*a*) before and (*b*) after splanchnic nerve stimulation in two groups of calves

Group	Catechol	Mean catecholamine output (μg/min) ± S.E. 2 mg ACh	4 mg ACh	*n*	Mean difference
		(*a*) Before stimulation			
I*	Noradrenaline	19·10 ± 3·60	21·20 ± 2·68	7	N.S.
	Adrenaline	2·93 ± 0·51	3·20 ± 0·51	8	N.S.
II†	Noradrenaline	3·64 ± 0·50	7·14 ± 1·13	12	3·5 ± 0·80, $P < 0·01$
	Adrenaline	1·18 ± 0·19	1·84 ± 0·45	12	N.S.
		(*b*) After stimulation			
I*	Noradrenaline	17·50 ± 3·01	18·60 ± 3·30	9	N.S.
	Adrenaline	6·30 ± 0·71	7·40 ± 1·22	9	N.S.
II†	Noradrenaline	6·74 ± 0·71	11·34 ± 1·46	14	4·61 ± 1·52, $P < 0·01$
	Adrenaline	3·58 ± 0·65	4·48 ± 0·83	14	N.S.

* Group I—high initial noradrenaline response to 2 mg ACh (mean for eleven animals, 18·77 ± 2·44).

† Group II—low initial noradrenaline response to 2 mg ACh (mean for seventeen animals, 3·79 ± 0·39).

TABLE 6. The effect of splanchnic nerve stimulation on the output of adrenaline and noradrenaline in response to 2 or 4 mg acetylcholine in two groups of calves

Group	Catechol	Mean catecholamine output (μg/min) ± S.E. Nerve stimulation: Before	After	*n*	Mean difference
		Response to 2 mg ACh			
I*	Noradrenaline	18·77 ± 2·44	21·47 ± 2·45	11	N.S.
	Adrenaline	3·00 ± 0·44	6·74 ± 1·17	11	3·72 ± 0·90, $P < 0·01$
II*	Noradrenaline	3·79 ± 0·39	7·18 ± 0·68	17	3·41 ± 0·65, $P < 0·001$
	Adrenaline	1·33 ± 0·14	3·56 ± 0·62	15	2·24 ± 0·54, $P < 0·01$
		Response to 4 mg ACh			
I*	Noradrenaline	20·70 ± 2·34	20·40 ± 3·20	8	N.S.
	Adrenaline	3·20 ± 0·60	7·00 ± 1·51	7	3·80 ± 1·30, $P < 0·05 > 0·02$
II*	Noradrenaline	7·60 ± 1·04	10·40 ± 1·30	11	2·60 ± 1·16, $P = 0·05$
	Adrenaline	2·01 ± 0·45	4·20 ± 0·68	11	1·92 ± 0·72, $P < 0·05 > 0·02$

* Groups I and II as in Table 5.

The effect of splanchnic nerve stimulation on the response to acetylcholine. Previous stimulation of the splanchnic nerves increased the discharge of noradrenaline to the same dose of acetylcholine only in calves in which there was a small initial output (Group II). In others, in which the secre-

tion was already high (Group I), this effect was not found with either 2 or 4 mg of acetylcholine (Table 6).

The secretion of adrenaline which followed the injection of acetylcholine was significantly increased after stimulation of the splanchnic nerves (Table 6). These results have to be compared with the failure of doubling the dose of acetylcholine to increase the discharge of adrenaline in the absence of splanchnic nerve stimulation.

Blood flow changes through the adrenal gland. It was possible that changes in the rate of blood flow through the adrenal gland might, in part, account for some of the different effects of the same dose of acetylcholine when given at different stages after birth, or before and after nerve stimulation in any given experiment. However, the values for mean rates of blood flow through the gland, at rest, and during different forms of stimulation,

TABLE 7. Mean rates of blood flow (ml./min ± S.E.) from the adrenal gland of the calf during different forms of stimulation

	Age of calf				
Stimulus	1–5 hr	24–30 hr	2–3 days	7 days	3 weeks
Resting flow	16·2 ($n = 3$)	11·6 ± 1·27 ($n = 6$)	12·4 ± 0·94 ($n = 5$)	11·7 ± 1·58 ($n = 5$)	9·8 ($n = 4$)
2 mg ACh (before atropine)	29·6 ($n = 3$)	21·0 ± 2·52 ($n = 6$)	29·9 ± 4·09 ($n = 5$)	25·3 ± 2·38 ($n = 5$)	22·0 ($n = 1$)
Animals atropinized in all tests listed below					
2 mg ACh	18·5 ($n = 3$)	12·1 ± 1·19 ($n = 8$)	15·4 ± 1·38 ($n = 5$)	15·4 ± 1·20 ($n = 9$)	17·3 ($n = 4$)
4 mg ACh	17·5 ($n = 3$)	13·9 ± 1·53 ($n = 5$)	16·5 ($n = 2$)	15·5 ± 1·88 ($n = 6$)	20·7 ($n = 4$)
Splanchnic nerve stimulation	9·1 ($n = 3$)	6·4 ± 1·05 ($n = 7$)	7·7 ± 0·61 ($n = 5$)	8·5 ± 0·77 ($n = 9$)	10·8 ($n = 4$)
2 mg ACh (after stimulation)	21·8 ($n = 3$)	14·3 ± 1·73 ($n = 7$)	17·8 ± 1·40 ($n = 5$)	14·6 ± 1·08 ($n = 9$)	19·9 ($n = 4$)
4 mg ACh (after stimulation)	17·0 ($n = 3$)	14·6 ± 2·12 ($n = 7$)	19·3 ± 1·06 ($n = 5$)	15·0 ± 0·64 ($n = 8$)	19·9 ($n = 4$)

n calves of different ages do not offer any convincing evidence for this view (Table 7). After atropine, the rates of blood flow during acetylcholine stimulation were remarkably similar in any given group of calves and there was no significant change in flow in the tests following splanchnic nerve stimulation in any group although during nerve stimulation adrenal blood flow invariably fell. In each group there was a very marked difference in blood flow during acetylcholine stimulation before and after atropine. This apparent vaso-dilatation caused by acetylcholine before atropine did not appear to affect the discharge from the gland, since tests with 2 mg acetylcholine given before and after atropine showed no significant change in the output from the gland in twelve calves.

When grouped according to age there appeared to be a somewhat higher

rate of adrenal blood flow at 1–5 hr and at 21 days compared with the intervening age groups. In view of the sharp distinction between the two levels of noradrenaline output in the whole series which led to the arbitrary arrangement into two groups, it seemed possible that significant differences in adrenal blood flow between these two groups might also be detectable. Although the mean blood flow in the calves showing a high sensitivity to acetylcholine (Group I) was slightly greater (16–18 ml./min) than in Group II (12–15 ml./min) the variation was considerable and the differences were not statistically significant.

Adrenaline and noradrenaline in the adrenal glands

Foetal calves

The foetal adrenal glands were removed for extraction and estimation of amine content after death of the foetus by asphyxia. This procedure has been shown in the lamb to cause losses and changes in the relative proportions of amines (Comline & Silver, 1961) but since no other material was available, the values have been included in Table 8, to show that, despite the asphyxial discharge and the stimulation before removal, the amine content was generally over 1·0 mg/g tissue.

TABLE 8. Adrenaline and noradrenaline in the adrenal glands of foetal and new-born calves

		Mean catecholamine concentration (mg/g adrenal tissue, ± S.E.)		Mean weight ± S.E.	
Age	No.	Noradrenaline	Adrenaline	L. adrenal (g)	Body wt.(kg)
Foetuses					
180 days	1	0·65	0·29	0·35	4·1
220–240 days	3	2·04	1·25	0·62	12·1
260–280 days	5	1·26 ± 0·12	1·23 ± 0·07	1·11 ± 0·09	18·9 ± 0·7
Calves					
1–5 hr	7	1·25 ± 0·15	1·11 ± 0·13	1·48 ± 0·10	24·4 ± 0·9
5½–10 hr	5	1·2 ± 0·22	1·00 ± 0·27	1·42 ± 0·18	23·9 ± 1·2
11–20 hr	4	1·51 ± 0·30	1·27 ± 0·12	1·34 ± 0·06	23·6 ± 0·6
21–28 hr	4	1·81 ± 0·35	1·16 ± 0·23	1·47 ± 0·05	25·1 ± 0·4
30–72 hr	7	2·27 ± 0·19	1·52 ± 0·12	1·37 ± 0·12	23·8 ± 1·2
5–8 days	6	2·97 ± 0·36	1·50 ± 0·20	1·43 ± 0·13	24·4 ± 1·2
14 days	5	2·37 ± 0·31	1·61 ± 0·24	1·30 ± 0·10	22·5 ± 1·1
21 days	5	1·58 ± 0·15	1·88 ± 0·18	1·44 ± 0·08	26·1 ± 1·0
60 days	1	1·01	2·42	2·1	34·0

New-born calves

A series of calves from 1 hr after birth to 3 weeks was used specifically for the estimation of the amine content of the adrenal glands. The results are given in Table 8. The noradrenaline concentration increased within 15 hr of birth at the time when the sensitivity of the noradrenaline cells had decreased. After the first day the amount of noradrenaline per gram

of adrenal tissue was significantly greater than at 5 hr after birth; it remained high for 2 weeks and then appeared to fall. The adrenaline concentration, on the other hand, showed no significant changes over the period examined, although the slight rise at 21 days appeared to continue with age, and was similar to the earlier findings on material obtained with chloralose anaesthesia (Silver, 1960).

The weight of the adrenal glands remained remarkably constant throughout this period after birth (Table 8) and the abrupt rise in adrenal weight, due to growth of the cortex, which was found in the lamb at or immediately after birth (Comline & Silver, 1961), was absent in the calf.

DISCUSSION

The properties of the adrenal medulla of the foetal and new-born calf are quite different from those of the adult. Throughout the period of gestation investigated in the present experiments the gland reacted directly to asphyxia, independently of its nerve supply, and the discharge consisted largely of noradrenaline. The greater secretion towards the end of pregnancy could not be ascribed to the development of the innervation of the adrenal medulla, since section of the splanchnic nerves had practically no effect upon the discharge from the gland, and, conversely, stimulation of the peripheral ends of the cut splanchnic nerves released less than 10% of the noradrenaline discharged during asphyxia. The evidence indicates that the direct response of the foetal adrenal gland to asphyxia, previously observed in the immature sheep foetus (Comline & Silver, 1961) predominates in the foetal calf throughout gestation. In the lamb, splanchnic nerve stimulation suddenly becomes effective during the last 3 weeks of gestation and at the same time the direct response to asphyxia wanes, so that at term ($\sim$ 147 days) it is almost completely replaced by a nervous mechanism. The development of the adrenal medulla of the calf is much slower and the properties of the gland at term thus resemble those of the foetal lamb at 110–120 days gestation before the development of an effective adrenal innervation.

As yet the reasons for this ability of the foetal adrenal medulla to respond directly to asphyxia at certain stages in its development are not known, but the results in the calf confirm and extend the previous observations on the sheep foetus. In both species the response is almost entirely confined to the noradrenaline cells and, although it is usually associated with the absence of an effective innervation, the present results show that cells with this property can respond to acetylcholine and, at least shortly after birth in the calf, may even be sensitized to asphyxia by splanchnic nerve impulses. The preliminary experiments on the foetal calf at term

showed that the arterial P_{O_2} at which a substantial increase in noradrenaline output occurred, was very low, about 2–4 mm Hg, and was similar to that found in the younger sheep foetus (Comline *et al.* 1965). It is probable that in the calf as well as the lamb, hypoxia is the stimulus primarily responsible for the direct effect of asphyxia on the adrenal medulla, and that rapid depression of the arterial P_{O_2} to below the critical level is essential in order to secure a maximal discharge. In the new-born calf, within the first 4–6 hr of birth, the direct response of the acutely denervated adrenal gland was as great or greater than that of the foetus provided that the rate at which the arterial P_{O_2} reached the critical level was comparable with that found after ligation of the umbilical cord; this could only be ensured by the use of positive-pressure ventilation.

The degree of development of the adrenal medulla at birth may well be related to the severity and duration of hypoxia during normal parturition which probably varies in different species. Certainly in the lamb a discharge would be evoked at much higher levels than in the calf, in which almost complete anoxia is required to stimulate the adrenal medulla of the mature foetus. A situation similar to that in the calf may exist in primates in which early placental separation is often encountered and very low P_{O_2} values have been reported for human umbilical cord blood at delivery (Bartels, 1959). The extent of the development of the innervation of the human foetal adrenal medulla at term is, however, still uncertain. The gland itself appears to be structurally immature at birth and the organs of Zuckerkandl, which contain large amounts of noradrenaline (West, Shepherd, Hunter & MacGregor, 1953), may reinforce the discharge from the adrenal medulla in the foetus and the new-born infant. Since these chromaffin cell bodies are probably not innervated (Muscholl & Vogt, 1964) they may have properties similar to the adrenal medulla of the foetal calf and the immature sheep foetus. If so, they could be expected to release noradrenaline as a direct response to asphyxia, at least in the foetus and immediately after placental separation, but whether this property would be as evanescent as that of the calf adrenal medulla and decline as rapidly after birth is still a matter for speculation.

The abrupt and marked changes which occurred in the response of the adrenal medulla to all forms of stimulation within 24 hr of birth of the calf always followed the same pattern and primarily affected the noradrenaline output. A short period of increased sensitivity during the first 4–5 hr after birth was followed by a rapid decrease in the discharge so that by 24 hr the adrenal medulla was extremely insensitive to all forms of stimulation and the output of noradrenaline was only 10–20 % of its previous high level. This decrease in noradrenaline output, which occurred irrespective of either the duration of pregnancy or the type of stimulus used,

might be attributed to many factors, but two which appear to be immediately relevant are, first, the probable excitation of the adrenal medulla during parturition and, secondly, the effect of changes associated with the transition from foetal to extra-uterine life upon the undeveloped adrenal medulla.

Stimulation of the adrenal medulla no doubt occurs during parturition but the extent of the discharge and the effects on the adrenal medulla itself are by no means certain. The after-effects of such prolonged stimulation might explain the hypersensitive phase, but it is difficult to relate the period of depressed excitability which follows to the discharge during parturition. The changes in the noradrenaline and adrenaline content of the adrenal glands after birth are reminiscent of the process of resynthesis in the adrenal medulla of the adult cat after drastic depletion by acetylcholine (Butterworth & Mann, 1957) or by reserpine (Callingham & Mann, 1958). It is most unlikely, however, that stimulation during parturition and even some increased secretion which might occur during the hypersensitive phase, could result in such excessive losses, except under very adverse conditions. At this time the adrenal medulla of the calf still contained large amounts of noradrenaline and, in any case, the highest rate of secretion could be elicited during the first 4 hr after birth, the time at which the gland contained the lowest concentration of this amine. In fact, during the first 72 hr after birth the output of noradrenaline was inversely related to the content so that depletion of the cells during parturition does not appear to be a tenable explanation for the rapid decrease in output during this time.

A more probable reason for the changes in output would appear to be the effect of the change in environment at birth on the immature adrenal medulla of the new-born calf; similar changes are not found in the lamb, in which the transition from the foetal to the adult type of response appears to be completed within the last few days of foetal life (Comline & Silver, 1961). In the calf it is significant that it is the noradrenaline cells which lose their ability to respond to asphyxia and to acetylcholine after birth. This suggests that the mechanism for release of noradrenaline from the foetal cell is different from that in the adult and that it is not immediately replaced by the adult form after its abrupt decline in the new-born animal. During this transitional period the cells are unresponsive, even to acetylcholine which normally elicits a discharge in both the foetus and the adult. This apparent inability to release noradrenaline would explain its accumulation within the gland, provided synthesis continued during this time. An analogous increase in the noradrenaline concentration of the adrenal medulla has been reported in other species, such as the rabbit, cat and guinea-pig after birth (Shepherd & West, 1951), but it remains to be shown

whether the same inverse relation between the output and content is present in these animals as well.

The adrenal medulla is not alone in the retention of foetal characteristics for only a short period after birth. Other examples, such as the rapid change in the form of haemoglobin and the absorptive capacity of the small intestine for macromolecules are well known. The high pulmonary arterial pressure reported by Reeves & Leathers (1964) during the first 2–3 hr of extra-uterine life in the calf, and its rapid decrease in the next 9–10 hr, is probably another example. Their report that severe hypoxia could increase the pulmonary arterial pressure to systemic levels only on the day of birth and not thereafter might well be explained in part by the discharge of large amounts of noradrenaline from the adrenal medulla at this time.

The duration of the period of depressed sensitivity of the adrenal medulla in the young calf is variable and difficult to define. The ability to respond to acetylcholine reappeared abruptly between 3 and 8 days of age, but the development of the response to asphyxia through the splanchnic nerves was much slower and the output of noradrenaline did not equal or exceed that found immediately after birth until about 21 days of age. Since this delay in the return of the asphyxial response corresponded with the relatively slow increase in the rate of secretion on direct stimulation of the cut splanchnic nerves, it can probably be attributed to deficiencies in the effect of splanchnic nerve impulses on the cells of the adrenal medulla.

Further examination showed, however, that in the young calf intra-arterial injections of acetylcholine never completely mimicked the effects of splanchnic nerve impulses, whether these were produced by direct stimulation of the cut nerve or through the central nervous system. Two discrepancies were most evident. First, injections of acetylcholine released disproportionately large amounts of noradrenaline, not only in the hypersensitive phase after birth but also in the 2–3 week old calf and even in the more mature animal; in contrast splanchnic nerve stimulation, however brought about, was relatively more effective in stimulating the secretion of adrenaline.

A second discrepancy between nerve stimulation and the intra-arterial injection of acetylcholine was found when the after-effects of these two forms of stimulation were investigated. A short period of splanchnic nerve stimulation, although it produced only a small response, had a prolonged sensitizing effect on subsequent doses of acetylcholine. Acetylcholine itself had no such action and successive tests with the same dose gave identical responses. The sensitizing effect of nerve stimulation could be shown most readily at times when the discharge from the cells was low: with noradrenaline the critical time corresponded with the period of

depressed sensitivity, but with adrenaline a significant increase in output was found, after a period of nerve stimulation, during the whole 3-week period after birth.

Changes in the blood flow from the adrenal gland did not appear to account for the change in sensitivity brought about by nerve stimulation for, although the venous outflow was reduced during stimulation, the volume of blood obtained during acetylcholine tests was similar before and after stimulation of the splanchnic nerves.

These discrepancies between the effects of nerve stimulation and acetylcholine injections may be accentuated by the organization of the different types of cell within the adrenal medulla of the calf. Palkama (1962), by differential staining of the cells, showed that the ox was one of the species in which a peripheral zone of adrenaline-containing cells surrounds a mass of noradrenaline cells and later (Palkama, 1964) found that this species is exceptional in that cholinergic nerves can only be identified in the peripheral adrenaline cell zone. This distribution of cells and nerve fibres supports the explanation, given previously, that the apparent hypersensitivity of the noradrenaline cells might be attributed to the absence of innervation (Silver, 1960) and suggests that even in adult cattle the secretion from the central mass of noradrenaline cells may be stimulated by diffusion of a transmitter rather than by a direct nervous mechanism. It may also confer upon the adrenal medulla of the young calf properties which are not found in species such as the cat and dog, which have a different pattern of cells in the adrenal medulla with islands of innervated noradrenaline cells scattered within the mass of adrenaline cells.

While the arrangement of cells within the adrenal medulla may explain some of the responses, at least of the noradrenaline cells in the young calf, it does not account for the prolonged enhancement of the response to acetylcholine after splanchnic nerve stimulation. This enhancement was long lasting and could easily be demonstrated 30 min after the end of the sensitizing period of stimulation. The failure of acetylcholine to produce an analogous response, and the apparent absence of cholinergic nerves to the noradrenaline cells, suggests that the effect can only be mediated by diffusion into this region. Many of the discrepancies between nerve stimulation and acetylcholine injections would be resolved if substances were known to be released by nerve impulses which sensitized both types of cell to the secretory effect of acetylcholine.

The study of the return of sensitivity of the noradrenaline components in the adrenal medulla was complicated by the apparently different results obtained under pentobarbitone or chloralose anaesthesia. On the present evidence there is no means of differentiating between the possibility that pentobarbitone depressed the cells of the adrenal medulla and of the

C.N.S., or whether, under chloralose anaesthesia, a background of excitation of the adrenal medulla, which occurred during the preparation of the adrenal gland, increased its sensitivity. The latter possibility seems more likely in view of the apparent potentiation of the adrenaline component in the nervous response to asphyxia at 3 weeks of age in animals under chloralose anaesthesia, and also the higher rate of secretion of both amines during nerve stimulation obtained in the previous series of experiments on calves under chloralose anaesthesia.

The inability of the adrenal medulla to release adrenaline in amounts comparable to those of noradrenaline was evident with all forms of stimulation. Only previous stimulation of the nerve supply to the gland appeared to potentiate the output, and even then the amounts released were very small when compared with the concentration in the gland, which remained remarkably constant until about 3 weeks of age. Thereafter an increased secretion was associated with a raised concentration in the gland and the gradual development of an effective nerve supply (Silver, 1960). The relation between synthesis, storage and release of adrenaline from the adrenal medulla during the first 3 weeks of life of the calf would therefore appear to be quite different from that of noradrenaline with which changes in output showed an inverse relation to the amount stored in the gland.

The conditions which normally stimulate a discharge from the adrenal medulla of the new-born animal, after recovery from the anoxia of parturition, have not been studied here, but it should be noted that the secretion of adrenaline during the first 24 hr after birth, although small, appears to be sufficient to prevent convulsions during prolonged and severe periods of hypoglycaemia for up to 24 hr or more. If this secretion is eliminated by cutting the splanchnic nerves convulsions readily occur but can be prevented by the infusion of adrenaline but not of noradrenaline (Edwards, 1964). The rate of infusion required (0·16–0·25 μg/kg/min) is similar to the total rate of secretion induced by nerve stimulation. These results emphasize the dependence of the adrenal medulla upon its nerve supply for the secretion of adrenaline even at this stage of incomplete development.

There is little information on the stimuli responsible for noradrenaline secretion in the young calf. Its calorigenic action, which is probably associated with fat metabolism (Dawkins & Hull, 1964) appears to be important in the new-born lamb (Van Duyne, Havel, Parker & Holm, 1960) especially in adverse environmental conditions and the absence of food (Alexander, 1962). Whether noradrenaline released at parturition will contribute to heat production in the immediate post-parturient stage is not known, although Scopes & Tizard (1963) have shown that this amine

will bring about a rise in O_2 consumption and rectal temperature in new-born kittens and rabbits. Significant effects were obtained with infusions of 0·25–1·0 μg noradrenaline/kg/min, levels which are well within the maximum rate of secretion in the calf at birth, but in view of the rapid onset of the period of insensitivity of the noradrenaline cells it seems unlikely that much noradrenaline can be discharged at later stages after birth in this species. The combination of inexcitability of the noradrenaline cells and comparative inactivity of the adrenaline cells may well account in part for the vulnerability of the young calf to adverse conditions during the first week or so of life.

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