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FACTORS RESPONSIBLE FOR THE STIMULATION OF THE ADRENAL MEDULLA DURING ASPHYXIA IN THE FOETAL LAMB

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Previous investigations on the release of catecholamines from the adrenal medulla of the foetal lamb during asphyxia showed that certain characteristic changes occurred during the development of the foetus (Comline & Silver, 1961). From relatively early in pregnancy, 80–90 days (full term $\sim$ 147 days), the adrenal medulla appeared to react directly to asphyxia with a secretion which contained a high proportion of noradrenaline. This response remained unaffected by section of the splanchnic nerves, destruction of the spinal cord, or by hexamethonium and it appeared to be due to the direct effect of asphyxia on the medullary cells. About 15–20 days before the end of gestation a nervous component was demonstrable, which was dependent upon the integrity of the splanchnic nerves, and the secretion then contained large amounts of both adrenaline and noradrenaline. The mechanism for this response, which at the end of pregnancy superseded the direct effect, appeared to lie in the spinal cord, since section at C1 made little difference to the secretion.

In all these experiments (Comline & Silver, 1961) asphyxia was induced by ligation of the umbilical cord, and at that time it was not practicable either to identify the exact nature of the stimuli responsible for the two types of discharge from the adrenal gland, or to determine whether a relation existed between the level of secretion and the severity of asphyxia in these foetuses. The present investigations have been concerned largely with these problems. The changes of P_{O_2}, P_{CO_2}, pH, lactic acid and glucose in the foetal blood during asphyxia which accompany the discharge from the gland have been examined in lambs at different stages of gestation, and the possible factors involved in the response studied under more carefully controlled conditions. It was soon apparent that the output of catecholamines from the adrenal medulla was primarily affected by a fall in foetal arterial P_{O_2}; changes in arterial P_{CO_2}, pH and lactic acid had little effect. Accurate and rapid measurement of both foetal and maternal

blood P_{O_2} levels therefore became essential, and an O_2 electrode for direct measurement of P_{O_2} in flowing blood was developed for this purpose (Silver, 1963). The sensitivity and rapid response time of this electrode not only enabled continuous records of blood P_{O_2} to be made during the collection of adrenal effluent samples under different conditions, but also offered a unique opportunity for the examination of the relation between foetal and maternal blood P_{O_2} levels.

A preliminary account of some of these findings has already been published (Comline, Silver & Silver, 1963).

METHODS

Animals

Welsh mountain ewes (25–40 kg) were used in the majority of the experiments during breeding seasons between 1960 and 1963. A small number of Border Leicester ewes (60 kg) were used in experiments which did not require positive pressure ventilation. The date of service was known and measurements of foetal body weight afforded additional checks on foetal age.

Preparation

Anaesthesia was induced and maintained by the intravenous injection of sodium pentobarbitone (May & Baker, 6 g/100 ml. of 0·9 % (wt./vol.) NaCl). Depression and disappearance of the pedal reflex was used as a guide to the level of anaesthesia. After cannulation of the trachea, polythene catheters were placed in the femoral artery and vein of each side. In the majority of experiments the uterus was exposed by a mid-line abdominal incision with the ewe in a supine position.

Foetal lambs. The method of preparation of the foetus and collection of adrenal effluent blood from the left foetal adrenal gland has already been described (Comline & Silver, 1961). In the present experiments the oesophagus as well as the trachea was ligated immediately after removal of the foetus from the uterus since swallowing, after umbilical cord occlusion, was found to result in a slight rise in foetal arterial P_{O_2}. As a routine, polythene cannulae were inserted into the carotid and femoral arteries and the jugular vein of the foetus for blood samples and P_{O_2} measurements. In certain experiments cannulation of the anterior mesenteric artery for close arterial injections or of the umbilical vessels for P_{O_2} measurements was carried out.

Position of P_{O_2} electrodes. The maternal P_{O_2} electrode was placed in a femoral arteriovenous loop. In the foetus the P_{O_2} electrode for routine measurements was placed in a carotid–jugular loop. A second foetal P_{O_2} electrode in a femoral arteriovenous loop was used when comparisons between carotid and abdominal aortic blood were required. For comparison of P_{O_2} in the umbilical vessels polythene loops placed in one umbilical vein and one umbilical artery allowed blood from each to pass over a P_{O_2} electrode before returning to the same vessel.

Adrenal effluent blood samples. Adrenal venous blood was collected immediately before and during an experimental period by placing curved forceps across the junction of the renal vein with the vena cava (Comline & Silver, 1961). The timing of samples varied with the conditions of the experiment: after clamping the umbilical cord it was dependent upon the rate of the blood flow from the adrenal gland, which changed with the age of the foetus. In foetuses between 80 and 105 days a 30 sec time lag was allowed between tying the cord and taking the first sample,which was of 3 min duration; subsequent samples were all of 5 or even 10 min duration. In foetuses from 115 to 135 days the initial time lag was only

10–15 sec and the sampling periods were 2, 3 and 5 min; at 140 days sampling was begun immediately after tying the cord, and the majority of samples were of 2 min duration.

In short periods of partial asphyxia, hypoxia or hypercapnia the samples were generally taken before and at 0–1, 1–3, 3–5 and 5–6 or 7 min after the introduction of the gas mixture to the ewe. Further details of these experiments are given in the relevant part of the text. After close arterial injections of lactic acid or sodium cyanide blood was collected during 1–2 min periods for up to 6 min after the injection had been given.

The total amount of blood removed from any one foetus varied with its age; up to 125-days gestation less than 4 % of the estimated total blood volume (Barcroft, 1946) was removed for sampling while in foetuses near term only about 2 % of the estimated blood volume was taken.

Asphyxia, hypoxia and hypercapnia. Total asphyxia of the foetus was produced by clamping the umbilical cord; under these conditions only one experiment was possible in any given animal.

Partial asphyxia or hypoxia of the foetus was induced by positive pressure ventilation of the ewes at about 6·5 l./min from an Ideal Respiratory Pump with gas mixtures which contained a low percentage of O_2 in N_2 with or without additional CO_2. This permitted predictable alterations of the foetal arterial P_{O_2} and P_{CO_2} to be made without irreversible interference with the placental or general circulation. Foetal carotid P_{O_2} levels of between 6 and 16 mm Hg required the use of 6, 10 or occasionally 8 % O_2 in N_2; ventilation of the ewe with 3 % O_2 in N_2 was required to obtain foetal P_{O_2} levels of below 4 mm Hg. Foetal hypercapnia was induced by administration of 10–15 % CO_2 in O_2 or in air to the ewe. All gas mixtures were made up in Douglas bags by means of a gas meter.

After a period of about 3 min the maternal blood came into equilibrium with the O_2 in the administered gas mixture and the positive pressure ventilation was continued for a further 3–4 min (see Fig. 1). The maternal P_{CO_2} and pH levels did not reach equilibrium during this period, although the major changes in both occurred within the first min of the period of positive pressure ventilation.

Since careful control of the resting arterial P_{O_2} levels was essential in these experiments, the arterial P_{O_2} of both the ewe and the foetus was continuously measured and checks on the pH and P_{CO_2} made during the course of the experiment. Any fall in P_{O_2} was immediately countered by the administration through the side arm of the tracheal cannula of either 5 % CO_2 in O_2, or 100 % O_2 if the maternal blood pH was below 7·4. The rate of administration was adjusted so that the original resting level of P_{O_2} in the foetal arterial blood was restored. Such precautions were rarely necessary at the beginning of an experiment but often became essential after a period of hypoxia.

Not more than two tests with hypoxic gas mixtures were imposed on any one foetus and an interval of 25 min was allowed between them. During the recovery phase the ewe was first given O_2 or 5 % CO_2 in O_2 and then allowed to breathe air with or without additional O_2. Under these conditions, provided the P_{O_2} was carefully controlled during the recovery phase, the foetal blood gas levels and pH returned to the original resting values measured before the onset of the first period of hypoxia. This is shown in Table 1. However, a significant rise in the foetal blood glucose and lactic-acid concentrations occurred after the first period of hypoxia. The former appeared to be directly related to the changes in the maternal glucose levels which doubled during the first period of hypoxia; maternal lactic-acid concentrations did not change significantly. These changes in foetal blood glucose and lactic acid did not appear to affect the problem under investigation since the level of secretion from the foetal adrenal medulla was unchanged when the same hypoxic gas mixture was administered in two successive tests to any given animal.

Failure to raise a low foetal P_{O_2} within a few minutes of the administration of O_2 to the ewe was taken to be indicative of poor placental exchange. This was usually reflected also in a high foetal P_{CO_2} and low pH and all preparations in which this occurred were discarded.

Administration of drugs. Heparin was given intravenously to the ewe at 1000 i.u./kg and to the foetus at 2000 i.u./kg after all operative procedures were completed. The dose of hexamethonium bromide given intravenously to certain foetuses was 10 mg/kg; its effects have been described previously (Comline & Silver, 1961). The method of Dawes, Jacobson, Mott & Shelley (1963) was used to maintain constant the pH of the foetal blood during asphyxia and the infusions of Na_2CO_3 (2–4 g/100 ml.) were given intravenously at rates which varied with the size of the foetus; quantitative details are given in the text. Close arterial injections of lactic acid (4–10 mg) and sodium cyanide (2–5 mg) in 0·5–1·0 ml. were given to the foetus through the anterior mesenteric artery with the coeliac artery tied and the aorta occluded posterior to the adrenal during and up to 10–15 sec after the injection. The injection, in a similar manner, of an equivalent volume of saline or 0·1 M neutral sodium phosphate buffer (which was used as a vehicle for the NaCN) caused no detectable increase in the resting rate of secretion from the adrenal medulla in six lambs.

TABLE 1. Changes in the composition of the foetal blood during the course of experiments which involved two periods of hypoxia

Mean difference ± S.E. between initial (R_1) and final (R_2) resting levels

No. of observations	P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)	pH	Lactic acid (mg/100 ml. blood)	Glucose (mg/100 ml. blood)
11	−0·87 (±0·56)	+0·3 (±1·12)	−0·022 (±0·009)	+18 (±3·5)	+18 (±3·1)

R_1 = values obtained immediately before first period of hypoxia.
R_2 = values obtained after 25 min recovery from first period of hypoxia, immediately before second period of hypoxia.

Measurements

Arterial blood pH and P_{CO_2}. Blood samples of 0·5–1 ml., kept under liquid paraffin, were taken simultaneously from the femoral or renal artery in the foetus and from the femoral artery in the ewe. They were immediately stored on ice, and measurements were made within 1–3 hr. During the tests with gas mixtures, samples were taken just before and at 1, 2½, 4 and 5–6 min after the onset of artificial ventilation.

pH was measured with an E.I.L. capillary electrode and P_{CO_2} with an electrode modified from the design of Hertz & Siesjö (1959). Both were kept in a water-bath at 39° C, and readings were made on an E.I.L. blood pH meter. The response time of the P_{CO_2} electrode was too long for it to be used in series with the P_{O_2} electrodes for continuous readings in flowing blood. Tests with different concentrations of CO_2 (10, 7, 5, 3 and 2 % CO_2 in O_2, British Oxygen Co.) showed that a 99 % response was achieved within 2 min when changing from 2 to 10 % CO_2; the electrode responded more slowly when the concentrations were reversed but the response to a change from 10 to 3 % CO_2 was complete within 3 min. The electrode gave the same response whether the CO_2 was tested as a gas or in solution. All readings were made 3 min after the introduction of a sample or gas mixture for calibration into the cuvette.

Arterial blood P_{O_2}. As a routine the P_{O_2} in the foetal carotid and maternal femoral arteries was measured simultaneously and continuously throughout the experiments. The P_{O_2} in the foetal abdominal aorta and the umbilical vessels was also measured continuously in certain experiments.

P_{O_2} was measured with a Teflon-covered 1, 5 or 10 μ Pt cathode (Silver, 1963). This electrode showed a linear relation between current and P_{O_2} from 0 to 713 mm Hg, and was insensitive to changes in hydrostatic pressure and blood flow. It was housed in a stainless-steel cuvette through which blood was passed in the course of an arteriovenous loop. The whole assembly was kept in a water-bath at 39° C and the output from the electrode was

measured with a high impedance DC amplifier. The size of the cuvette used depended upon the blood flow; in the foetus its capacity was of the order of 0·1 ml., whereas that used for the ewe had a capacity of about 0·75 ml. These electrodes could also be used for measuring P_{O_2} in single samples of blood or in gas and their general characteristics have been described previously (Silver, 1963). A combination of 12μ Teflon membrane with 5μ Pt cathode was used as a routine. This gave 1 % difference in current output between unstirred blood and gas with the same P_{O_2} and no difference between blood and saline. The time for 100 % response to a change from N_2 to O_2 or vice versa was 4·5 sec but the time for 97 % response to small changes of P_{O_2}, e.g. 0–50 mm Hg, was 0·75 sec.

The electrodes were calibrated with saline equilibrated with specially prepared gas mixtures containing 0·5, 2·5, 5, 10, 20 and 50 % O_2 in N_2 with 5 % CO_2. The oxygen content of the gas mixtures was checked paramagnetically by the Cambridge Instrument Co. The zero current was found by flushing the electrode with O_2-free N_2 or cyclopropane or with N_2-saturated saline. At the end of each experiment the electrode calibration was checked immediately with N_2 and with saline equilibrated with air. All calibrations were carried out at the same temperature as the experimental readings.

Chemical determinations. Samples of 0·5 ml. arterial blood placed in tubes containing NaF were taken simultaneously from the ewe and foetus before and after an experimental period. Glucose was estimated by the glucose oxidase method of Huggett & Nixon (1957). Lactic acid was determined according to the method of Barker & Britton (1957). All figures are expressed as mg/100 ml. whole blood.

Details of the treatment of the plasma, the subsequent extraction procedures, chromatography and the fluorimetric method for the estimation of adrenaline and noradrenaline have already been described (Comline & Silver, 1961).

RESULTS

Composition of foetal and maternal blood

Initial resting levels

The first measurements of foetal and maternal arterial blood P_{O_2} were made within 1–2 hr of induction of anaesthesia in the ewe immediately after removal of the foetus from the uterus and cannulation of the necessary vessels. At the same time samples were withdrawn for the estimation of P_{CO_2}, pH, lactic acid and glucose.

Oxygen tension. Although Barron (1953) and Kaiser & Cummings (1957) have reported adverse effects of barbiturate anaesthesia on pregnant ewes, particularly with animals in a supine position, in the present experiments the majority of ewes and foetuses appeared to be well oxygenated when the initial measurements were made; this was confirmed by the values obtained for foetal and maternal arterial P_{O_2} given in Table 2. There was a significant fall in foetal carotid P_{O_2} towards the end of pregnancy ($P < 0{\cdot}01$) but this was not accompanied by any corresponding change in maternal arterial P_{O_2} (Table 2).

CO_2 tension and pH. The values for maternal blood, given in Table 2, did not change between 115 days and term. In the foetal blood there was a significant rise in P_{CO_2} ($P = 0{\cdot}01$) and a slight fall in pH throughout the period of pregnancy examined in these experiments. Similar though more

pronounced changes in pH have been reported by Dawes, Mott & Shelley (1959).

Glucose and lactic acid. The initial values for maternal blood glucose were variable, and depended upon the condition of the ewes and the number of foetuses. Foetal levels were generally about half those found in the maternal blood; a similar relation between foetal and maternal blood glucose was reported by Shelley (1960). Maternal values below 20 mg/100 ml. were confined to ewes with twins, but a wide variation was found in animals with single lambs. There was no apparent change in foetal blood glucose with increased foetal age (Table 2).

The increase in foetal-blood lactic-acid concentration between 115 days and term was statistically significant ($P < 0{\cdot}01$), while the levels in the maternal blood remained between 10 and 25 mg/100 ml. throughout pregnancy.

TABLE 2. Initial composition of foetal and maternal arterial blood

Age range (days)	No. of animals	P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)	pH	Glucose (mg/100 ml.)	Lactic acid (mg/100 ml.)
		Mean foetal levels ± S.E.				
100	2	40·0	37·2	7·40	—	—
113–120	6	36·0 ± 2·96	41·2 ± 1·41	7·34 ± 0·003	9·1 ± 4·3 (n = 5)	24·2 ± 4·2 (n = 5)
126–132	3	32·3	45·3	7·33	—	—
140–144	8	24·6 ± 1·15	48·4 ± 1·01	7·32 ± 0·011	13·0 ± 2·5	46·0 ± 5·1
		Mean maternal levels ± S.E.				
100	—	—	—	—	—	—
113–120	6	94·3 ± 5·88	35·0 ± 1·34 (n = 5)	7·45 ± 0·018	23·4 ± 4·50 (n = 5)	18·8 ± 2·33 (n = 5)
126–132	1	90	38	7·42	—	—
140–144	8	90·6 ± 2·32	35·0 ± 0·45 (n = 6)	7·48 ± 0·014	36·4 ± 4·54 (n = 7)	16·9 ± 1·89

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

The relation between foetal and maternal arterial P_{O_2}

Efficient placental exchange was essential for the success of these experiments, particularly in the series in which specific P_{O_2} and P_{CO_2} levels in the foetus were induced by ventilation of the ewe with suitable gas mixtures. The response to a rise or fall in the level of inspired O_2 was normally very rapid in both ewe and foetus. A change in maternal arterial P_{O_2} was detectable within a few sec of the administration of the gas mixture, and this was normally followed ½–1 min later by a change in foetal carotid P_{O_2}. The rapidity of response and the attainment of equilibrium for P_{O_2} in maternal and foetal blood during the administration of 6 % O_2 in N_2 is illustrated in Fig. 1.

The relation between maternal and foetal P_{O_2} after equilibrium had been attained is shown for a wide range of maternal P_{O_2} levels in Fig. 2. It appeared to be fairly constant between 30 and 150 mm Hg maternal P_{O_2}, irrespective of the gas mixtures used to ventilate the ewes. A level of 2 mm Hg P_{O_2} was reached in the foetal carotid blood at a maternal level of 10–25 mm Hg P_{O_2}.

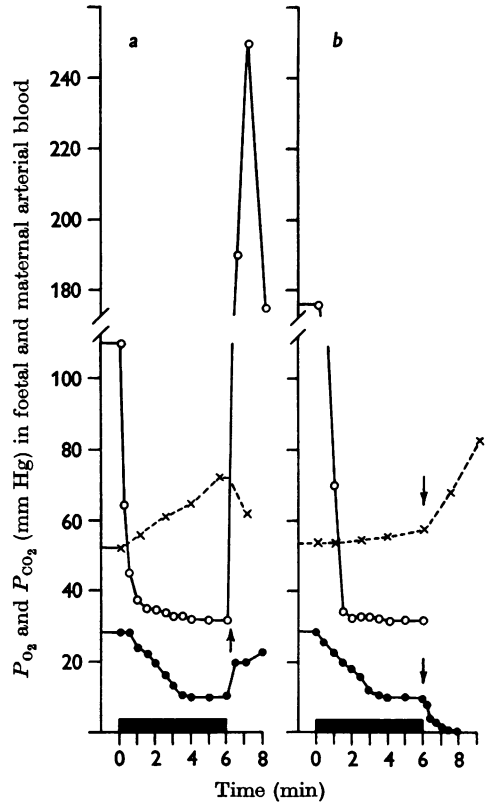

Fig. 1. The change in foetal and maternal blood gas tensions during and after two successive periods of maternal ventilation with (*a*) 6 % O_2, 15 % CO_2 and 79 % N_2, and (*b*) 6 % O_2 and 94 % N_2. Maternal arterial P_{O_2}, O—O; foetal arterial (carotid) P_{O_2}, ●—●; foetal arterial (abdominal aorta) P_{CO_2}, ×– – –×. Interval between *a* and *b* 25 min; O_2 given to ewe at ↑; umbilical cord clamped at ↓. Age of foetus 140 days.

The foetal arterial P_{O_2} did not, however, follow the maternal level if the latter rose to or was maintained at 250 mm Hg or more. A sudden rise in the maternal P_{O_2} to such levels often occurred after a period of hypoxia, when the ewe was ventilated with O_2 for a few minutes; no comparable rise in foetal P_{O_2} was observed at this time and the foetal levels were merely restored slowly to their original resting values (Fig. 1). However,

during this period maternal blood pressure also rose abruptly and it is possible that uterine vasoconstriction contributed to the increased O_2 gradient at this time. The usual relation was normally restored during the 20–25 min rest period. On the other hand, when ewes were maintained on a high level of O_2 intake for long periods and a new equilibrium appeared to be established between ewe and foetus, the foetal P_{O_2} did not rise above 60 mm Hg (Fig. 2) and was sometimes considerably below this, although maternal levels ranged from 150 to 350 mm Hg.

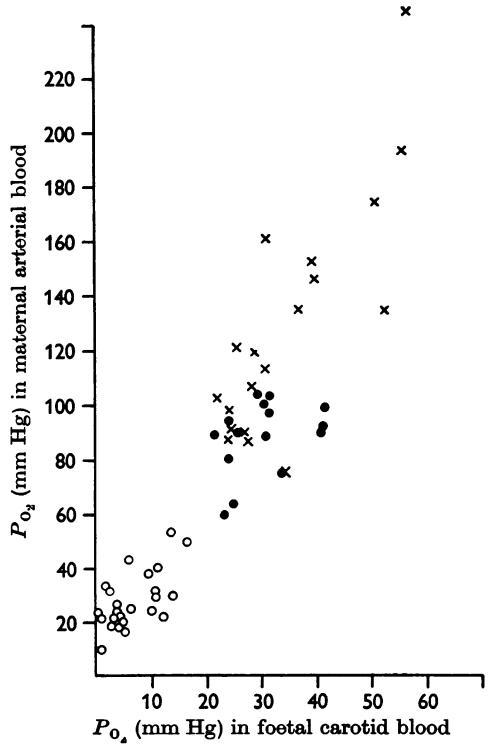

Fig. 2. The relation between foetal and maternal arterial blood P_{O_2}. Simultaneous measurements of P_{O_2} in ewe and foetus were made under the following conditions: ewes breathing air, ●; ewes receiving supplementary O_2, ×; ewes ventilated with 3, 6 or 10% O_2, ○.

Comparative measurements of P_{O_2}, P_{CO_2} and pH at different points of the foetal circulation

Continuous routine measurements of foetal P_{O_2} were made from the carotid artery, while single samples for analysis of pH and P_{CO_2} were usually taken from a branch of the abdominal aorta. It was therefore essential to determine what differences might be encountered in P_{O_2} and

P_{CO_2} levels between foetal carotid and aortic blood over a wide range of gas tensions. Because of the scarcity of data on the subject a few measurements were also made on gas tensions in umbilical vein blood in some of these experiments.

Measurements of pH and P_{CO_2} made over a wide range of conditions showed that differences between aortic and carotid blood were very small. The pH differed by 0–0·02 units and P_{CO_2} by 0–3 mm Hg and since, in the experiments designed to test the effect of increased P_{CO_2}, the tension was altered by 20–50 mm Hg and the pH by 0·2–0·4 units, these small differences between carotid and aortic blood were considered unimportant.

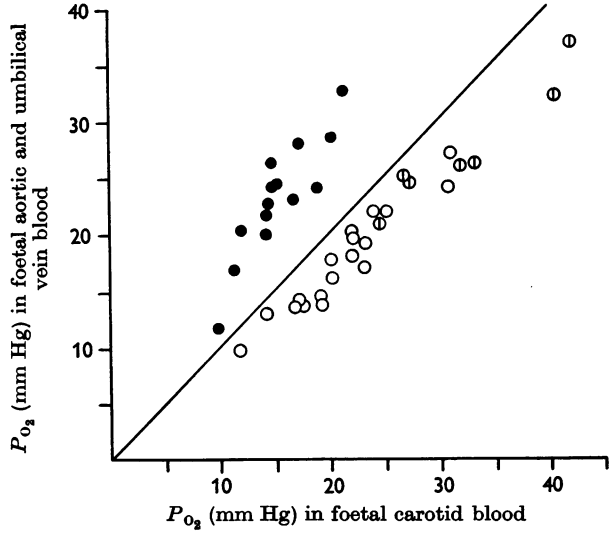

Fig. 3. The relation between umbilical vein and carotid blood P_{O_2} (●) and between abdominal aortic and carotid blood P_{O_2} (○, 140 days; ⓘ, 115–120 days) in the foetal lamb. The solid line indicates equality.

The P_{O_2} in the abdominal aorta was lower than that in the carotid which was expected on the basis of the O_2 saturation measurements of Dawes, Mott & Widdicombe (1954) and recently extended by Dawes & Mott (1964). The relation between P_{O_2} in the carotid artery and abdominal aorta, shown in Fig. 3, was constant over a side range of P_{O_2} levels, and a 10–15 % difference in tension between the blood in the two vessels was found at both 115 and 140 days of pregnancy.

Samples of umbilical vein blood taken at the same time as aortic blood enabled estimates of arteriovenous differences in foetal P_{CO_2} to be made. A mean value of 8·5 mm Hg was obtained in this series, which is in good agreement with previously published figures obtained under different

experimental conditions (Barron & Meschia, 1957; Kaiser & Cummings, 1957). A limited number of continuous measurements of P_{O_2} levels in the umbilical vein and the femoral or umbilical artery were also made, and the arteriovenous P_{O_2} differences in these foetuses ranged from 7 to 12 mm Hg. In the same experiments, a series of simultaneous P_{O_2} recordings from the umbilical vein and carotid artery were taken; the relation between the oxygen tension in these two vessels, shown in Fig. 3 (●), was fairly constant. The P_{O_2} of the carotid blood was 30–40 % lower than that returning from the placenta. These results were, however, obtained over a relatively restricted range of foetal oxygen tensions, since cannulation of the umbilical vein led to a drop in foetal P_{O_2}, which was not followed by a return to pre-existing levels. The umbilical blood gas differences recorded under these conditions, in which carotid P_{O_2} remained at 12–20 mm Hg, are thus not necessarily applicable to higher levels of foetal oxygen tension.

Variations in resting output from the foetal adrenal medulla

The presence or absence of an initial resting discharge from the adrenal medulla could not be related to the P_{O_2}, P_{CO_2} or lactic-acid levels in the foetal blood if these were within the normal range. At 115 days the resting levels of noradrenaline output (mean: 0·04 μg/min) were similar to those reported previously (Comline & Silver, 1961); at 140 days the initial levels for both amines ranged from 0 to 0·05 μg/min. There was sometimes a slight rise in the basal discharge after the first period of hypoxia, bringing the mean resting levels to 0·07 μg/min, but this did not appear to be related to the rise in lactic acid concentration which was a constant finding. In any event, the increased resting discharge at this time was considerably below the high rates of secretion encountered during stimulation of the adrenal medulla.

Effects of ligature of the umbilical cord

In previous experiments (Comline & Silver, 1961) on the release of adrenaline and noradrenaline after ligation of the umbilical cord, the results were expressed as mean output over a 5 min period of asphyxia. In the present series, more information was required on the pattern of secretion throughout the duration of asphyxia so that any changes could be related to the condition of the foetus. The experiments were therefore repeated at the critical times during gestation, before and after the development of the innervation of the adrenal medulla; at the same time concomitant changes in the composition of the foetal arterial blood which occurred during asphyxia were also measured. The mean rates of secretion of noradrenaline and adrenaline which preceded and followed ligature of

the umbilical cord are shown in Fig. 4 for six groups of foetal lambs between 80 and 145 days.

Secretion after acute denervation or hexamethonium

In these preparations, in which nerve stimuli were absent or had been eliminated, the cells of the adrenal medulla appeared to react directly to a stimulus which became most effective 2–5 min after the onset of asphyxia, and which continued to excite until the circulation failed. A high proportion of noradrenaline continued to be secreted throughout the entire period of asphyxia, whether it lasted 10–15 min in the foetus near term, or more

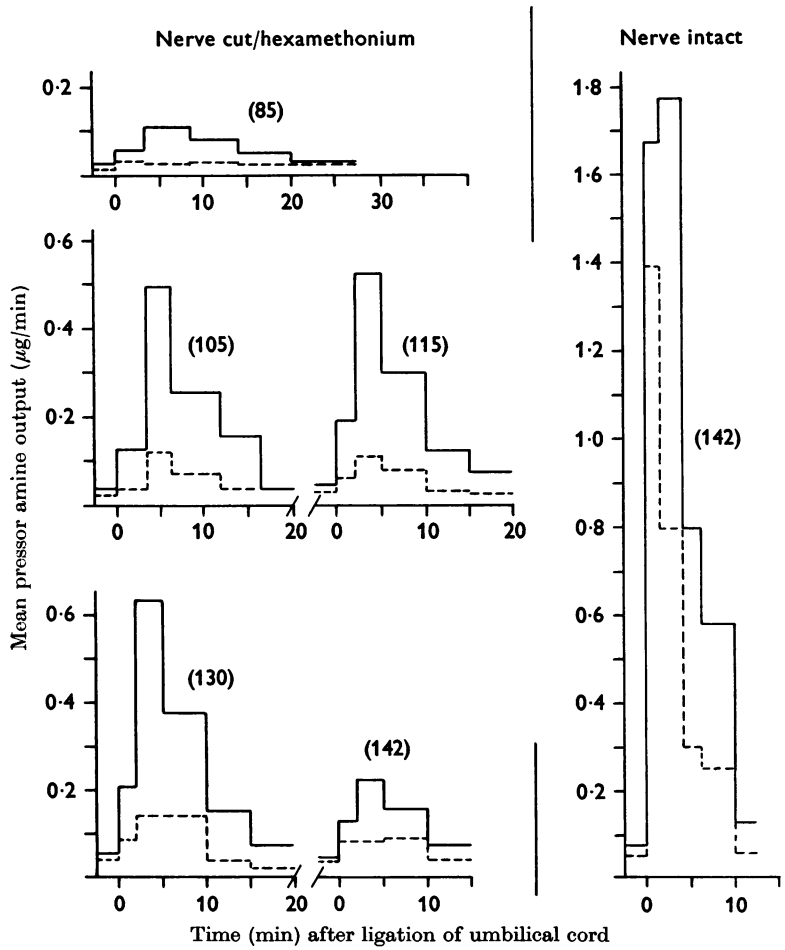

Fig. 4. Secretion from the intact and denervated adrenal medulla of the foetal lamb during asphyxia. Mean rates of adrenaline (– – –) and noradrenaline (—) discharge before and after ligation of the umbilical cord. Mean age of each group of foetuses (4–10 animals) is given in parentheses.

than 30 min in some of those younger than 115 days. In all groups the output increased significantly immediately after clamping the umbilical cord, but the maximum rate of discharge was not attained until 2–5 min later; thereafter the output fell. The pattern of secretion in foetuses between 105 and 130 days was very similar, but by 140–145 days the adrenal medulla was much less sensitive to the direct effect of asphyxia and the rate of secretion had waned considerably.

Secretion from adrenal glands with intact innervation

Previous experiments have shown that the response of the intact adrenal gland to asphyxia was indistinguishable from that of the denervated gland until about the last 3 weeks of gestation (Comline & Silver, 1961). By 140–145 days, however, the divergence between the two types of response was very great; this is shown in Fig. 4 in which the two rates of discharge throughout 15 min asphyxia may be compared. The output from the intact adrenal medulla at 140 days was 10 times greater than the maximum rate of secretion of the denervated gland at this age, and nearly 4 times the output at any other age after cutting the splanchnic nerves.

The most striking feature of the response of the intact gland of the 140 day foetus to asphyxia was the high proportion of adrenaline released initially; this was completely different from the discharge of the denervated gland at this age. Large amounts of both amines were secreted from the innervated gland shortly after tying the cord, whereas the maximum output during the direct response to asphyxia was invariably delayed.

Changes in blood gases and other metabolites

The changes in the composition of the foetal blood which followed ligature of the umbilical cord in a foetus at 125 days of age are shown in Fig. 5. These changes were typical of foetuses from 100 days until 145 days.

The outstanding feature was the extremely rapid fall in P_{O_2} of the foetal arterial blood which occurred irrespective of the initial resting level. During the first 60–90 sec after umbilical cord occlusion the P_{O_2} fell at a rate of 15–30 mm Hg/min, and thus in the majority of experiments a foetal carotid P_{O_2} of 2–5 mm Hg was reached within 2 min. The subsequent fall to 0–1 mm was slower, but was complete within 4–5 min of cord occlusion.

By comparison with the fall in P_{O_2} all the other changes after ligation of the cord were slower and more prolonged. The large decrease in foetal blood pH, which continued for 10–15 min, has been reported previously by Dawes *et al.* (1959) and was ascribed to the concomitant rise in the lactic acid concentration of the foetal blood. However, a considerable rise in

foetal arterial blood P_{CO_2} from about 45 mm Hg to over 100 mm Hg also occurs at the same time (Fig. 5) and will contribute to the fall in pH. The small changes in blood glucose under these conditions were similar to those reported by Dawes *et al.* (1959).

The changes in P_{O_2}, P_{CO_2}, pH and lactic acid in the foetal arterial blood were sufficiently large to suggest that any one, or a combination of these factors, could excite the foetal adrenal medulla either directly and, in the older foetuses, indirectly through the splanchnic-nerve supply from the central nervous system.

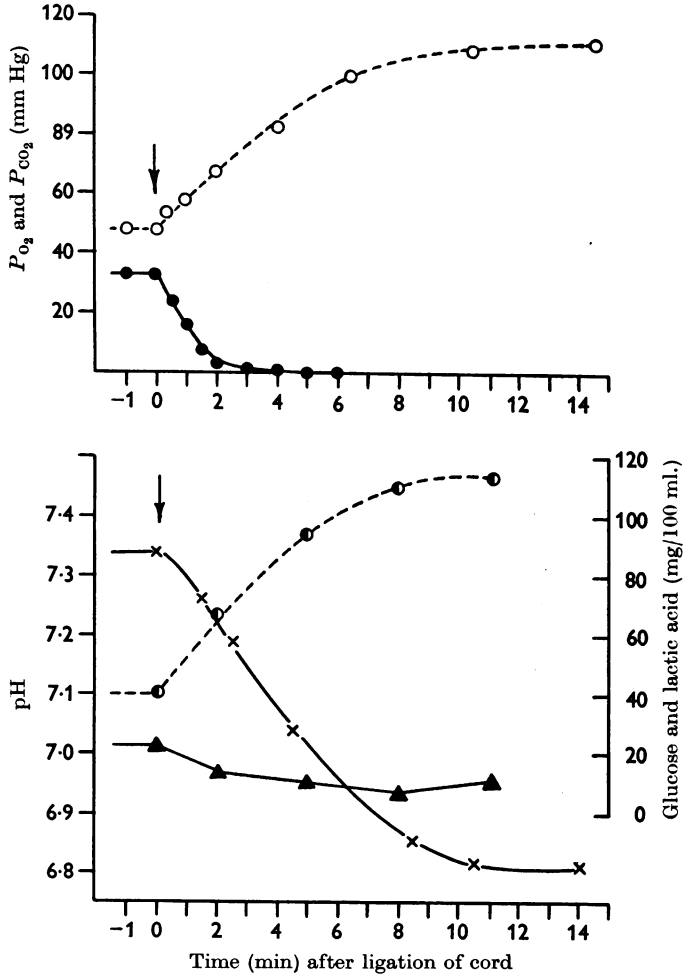

Fig. 5. The effect of ligation of the umbilical cord (↓) in a foetal lamb aged 125 days on the composition of its arterial blood: ●—●, P_{O_2} in carotid blood. Other data from abdominal aortic blood: ○- - -○, P_{CO_2}; ×—×, pH; ◐- - -◐, lactic acid; ▲—▲, glucose.

Factors involved in the direct response of the foetal adrenal medulla to asphyxia

The direct effect of asphyxia on the foetal adrenal medulla is most pronounced at 130 days (Fig. 4) but the nervous mechanism is also detectable at this stage (Comline & Silver, 1961); therefore, investigations were largely confined to foetuses at 115 days, an age at which the splanchnic nerves have no effect on secretion. A few animals between 120 and 130 days were used in the controlled pH experiments and in these foetuses possible interference by any nervous discharge was eliminated either by cutting the splanchnic nerves or by administration of hexamethonium.

The previous experiments had shown that the secretion ascribed to the direct action of asphyxia was characteristically slow in onset, with a maximum output between 2 and 5 min, but was prolonged often for as long as 20–30 min. This pattern of discharge appeared to parallel the relatively slow changes in the acidity of the foetal blood after occlusion of the cord, and therefore the importance of these changes on adrenal activity during asphyxia was assessed in the following experiments.

Maintenance of a constant blood pH after ligature of the umbilical cord

In these experiments the pH of the foetal blood was kept constant for 10 min after tying the cord by the intravenous infusion of Na_2CO_3 (2–4 g/100 ml.) at a rate which delivered about 15 mg Na_2CO_3/min/kg of foetus. The response in each experiment was compared with that of a foetus of the same age and size in which the pH was allowed to fall in the normal manner after cord occlusion. In all the experiments the pH and the noradrenaline concentrations were measured in samples taken before and every 2 min after the onset of asphyxia. At this age the concentration of adrenaline was so low that slight changes in output were of dubious significance and the observations were therefore confined to noradrenaline output.

The pooled results from seven pairs of lambs between 115 and 130 days of age are compared in Fig. 6. It is clear that although the pH of the foetal arterial blood remained almost constant in the foetuses which received an infusion of Na_2CO_3, there was no diminution in the output of noradrenaline. Furthermore, there was no trace of a rise in the rate of noradrenaline secretion when the pH fell at the end of the Na_2CO_3 infusion.

The maintenance of a constant pH in the foetal arterial blood did not always result in a constant blood P_{CO_2} which in some preparations rose slightly. The amount of noradrenaline released could not, however, be related to any changes in P_{CO_2}; all values for noradrenaline output were

within the normal range of variation found in the control experiments. The direct action of CO_2 on the adrenal medulla was tested more thoroughly in the following experiments.

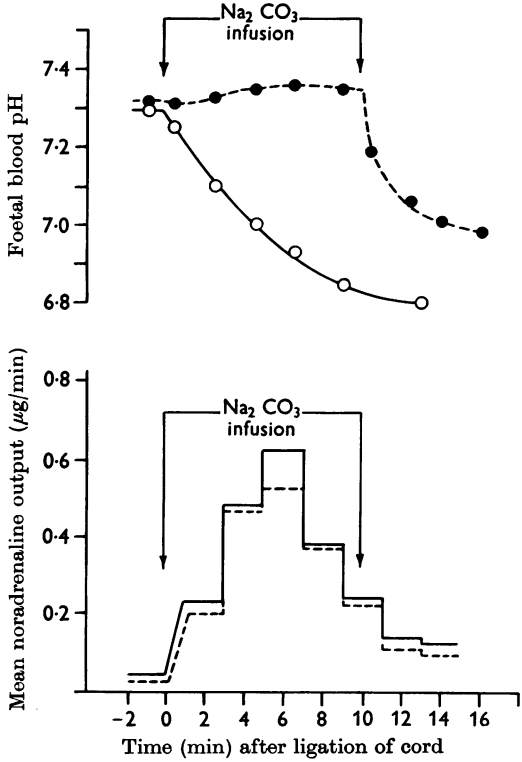

Fig. 6. Foetuses 115–130 days; splanchnic nerve discharge eliminated. Noradrenaline output during maintenance of a constant foetal blood pH for 10 min after ligation of the umbilical cord (– – –), compared with that after cord occlusion (—) alone. ○—○, pH of control group; ●– – –●, pH during infusion. Average results from seven pairs of foetuses.

Effect of foetal hypercapnia

In the first series of experiments the ewes were ventilated with a mixture of 15% CO_2 in O_2 for 6 min; this procedure increased the foetal blood P_{CO_2} from 40 to 70–95 mm Hg, with a concomitant fall in pH from 7·35 to 7·1–7·0. This rise in the foetal blood P_{CO_2} was as great, or greater, than that normally found after tying the umbilical cord (see Fig. 5) but the noradrenaline secretion was no more than that from the adrenal at rest, i.e. 0·03–0·08 μg/min. On the other hand, this gas mixture, with its high proportion of O_2, increased both the maternal and foetal arterial blood P_{O_2}. At the end of the 6 min period of forced maternal respiration the

foetal carotid P_{O_2} had risen from about 30–35 mm Hg to between 50 and 60 mm Hg. Since this rise in P_{O_2} might have obscured the effect of the increased P_{CO_2} it was eliminated by ventilation of the ewes with a mixture of 15% CO_2, 20% O_2 and 65% N_2. With this mixture only the foetal blood P_{CO_2} increased during the test, and the P_{O_2} remained within normal limits. Nevertheless, no change in output from the adrenal medulla was detected under these conditions. The results of the experiments in the last two series appeared to eliminate both the fall in blood pH and the rise in P_{CO_2} as the primary cause of the direct effect of asphyxia on the foetal adrenal medulla. Hypoxia remained as a possible stimulus.

Effect of foetal hypoxia

It was essential in these experiments to dissociate hypoxia as far as possible from the rise in P_{CO_2}, which is normally complementary to it during foetal asphyxia (Fig. 5), and which at other sites appears to potentiate stimulation by hypoxia (Nielsen & Smith, 1951; Joels & Neil, 1961).

The foetal blood P_{O_2} was lowered by ventilation of the ewes for 6 min with mixtures of 3, 6 or 10% O_2 in N_2. Positive pressure ventilation with these gas mixtures without CO_2 reduced the maternal arterial P_{CO_2} to between 25 and 30 mm Hg. This fall in P_{CO_2} was not, however, reflected in the foetal blood; in some foetuses the arterial P_{CO_2} fell at first by 2–3 mm Hg but had returned to normal by the end of the period. In others a slight rise occurred in the P_{CO_2} after 5 min of hypoxia. Typical changes in the foetal and maternal blood P_{O_2} during ventilation with a gas mixture of 6% O_2 in N_2 are shown in Fig. 1*b*.

In all these experiments, if the foetal P_{CO_2} rose by more than 3–4 mm Hg during the period of hypoxia the results were not included in this series. However, the extent of the discharge of noradrenaline from the denervated adrenal medulla appeared to depend solely on the level of oxygen tension, and changes in output could not be related to a rise or fall of a few mm P_{CO_2}.

The relation between noradrenaline output and the P_{O_2} level in the foetal carotid blood is shown as black circles in Fig. 7. Moderate falls from the normal level of 30 mm Hg to between 8 and 14 mm Hg resulted in only a slight increase in noradrenaline output; below a P_{O_2} of 4–5 mm Hg noradrenaline secretion increased sharply, and at about 2 mm Hg it was indistinguishable from that found after tying the cord (Fig. 7, ◑).

While these results showed that the direct response of the denervated foetal adrenal medulla to complete asphyxia was not dependent on the rise in P_{CO_2} normally associated with it, they did not eliminate the possibility that a high P_{CO_2} might potentiate the relatively small effect of less

severe hypoxia on noradrenaline secretion. This possibility was examined in the following experiments.

Effect of increased P_{CO_2} on different levels of foetal hypoxia

For these experiments 15 % CO_2 was included in the gas mixtures which also contained 3, 6 or 10 % O_2, the residual volume being made up with N_2. Positive pressure ventilation with these mixtures usually doubled the resting level of maternal blood P_{CO_2} and the foetal blood P_{CO_2} rose by 20–30 mm Hg. Figure 1*a* shows the fall in foetal and maternal P_{O_2} and the rise in foetal P_{CO_2} produced by ventilation with a gas mixture of 6 % O_2 and 15 % CO_2 in N_2. The increase in foetal P_{CO_2} attained during these tests was equivalent to that found during the maximal discharge from the adrenal medulla after tying the umbilical cord.

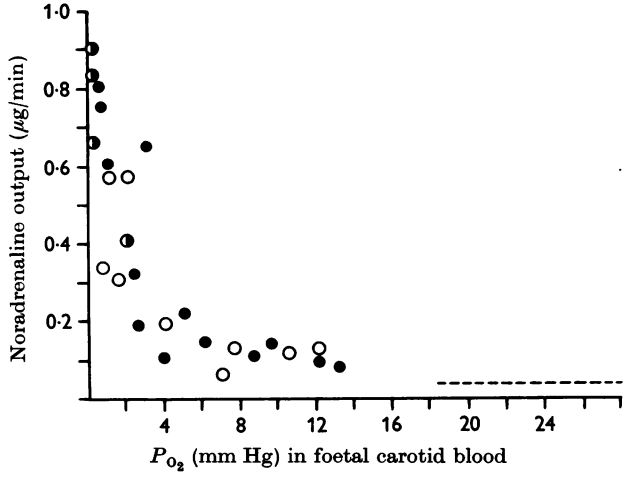

Fig. 7. The relation between rate of noradrenaline secretion from the adrenal medulla and carotid blood P_{O_2} level in 14 foetal lambs aged 115 days. ●, Hypoxia (mean foetal arterial P_{CO_2} = 49 ± 1·66 mm Hg, pH = 7·34 ± 0·013, n = 13); ○, hypoxia + hypercapnia (mean foetal arterial P_{CO_2} = 74 ± 3·75 mm Hg, pH = 7·18 ± 0·028, n = 9); ◑, maximum discharge after tying cord; – – –, mean resting output.

The noradrenaline output at various carotid P_{O_2} levels in the presence of increased P_{CO_2} is shown as open circles in Fig. 7 and can be compared with the output during hypoxia at normal P_{CO_2} levels. It is clear from these results that a foetal hypercapnia does not potentiate noradrenaline discharge during hypoxia.

It was impracticable in these experiments to extend the period of positive pressure ventilation and hypoxia to determine whether anoxia or other factors contribute to the prolonged direct response of the adrenal

medulla to asphyxia incurred after tying the cord; the following experiments with lactic acid and sodium cyanide throw some light on this problem.

Effects of intra-arterial injections of lactic acid or sodium cyanide

During total asphyxia the lactic-acid concentration in the foetal blood may rise to over 100 mg/100 ml. in addition to the increase in P_{CO_2}. In the experiments described above, the two effects were dissociated since the foetal P_{CO_2} was increased with only small changes (+12 mg/100 ml.) in the blood lactic-acid levels during the test period. The possibility that lactic acid could stimulate the adrenal medulla was investigated by testing the effect of intra-arterial injections of 4–10 mg on the output from the adrenal medulla. The results given in Table 3 show that a small increase in noradrenaline output was elicited by lactic acid; the mean rise of $0{\cdot}08 \pm 0{\cdot}03$ μg/min was statistically significant at the 5% level.

TABLE 3. Mean output of noradrenaline (*N*) and adrenaline (*A*) following the intra-arterial injection of lactic acid or sodium cyanide in foetal lambs aged 115 days

Mean pressor amine output μg/min (±S.E.)							
Resting control		Lactic acid (4–10 mg)		NaCN (2–5 mg)			
				Immediate response		3–6 min later	
A	*N*	*A*	*N*	*A*	*N*	*A*	*N*
0·05 (4)	$0{\cdot}09 \pm 0{\cdot}031$ (6)	0·06 (4)	$0{\cdot}17 \pm 0{\cdot}019$ (7)	0·24 (5)	0·77 (5)	0·26 (3)	0·67 (3)

No. of observations in parentheses.

The previous experiments demonstrated that hypoxia was probably the most effective direct stimulus to the foetal adrenal during asphyxia. This point was confirmed by the discharge which followed the intra-arterial injection of NaCN (2–5 mg) to the adrenal gland (Table 3). The response was equivalent to the maximal discharge found after tying the umbilical cord. The output from the gland remained high for 5–6 min after a single injection of NaCN (Table 3), but whether the foetal adrenal medulla would continue to respond to further injections for a period equivalent to the limits of the discharge during foetal asphyxia could not be ascertained, because some cyanide leaked into the general circulation even after a single injection, and caused an increase in the P_{O_2} of the foetal blood. Further injections or infusions would thus have resulted in more general anoxia of the foetus and would also probably have passed into the maternal circulation.

Factors involved in the response of the innervated adrenal medulla to asphyxia

Foetal lambs aged 140 days or more were used in all these experiments. During asphyxia at this age the contribution made by the direct response of the adrenal medulla is negligible, and the output is almost entirely dependent on the splanchnic nerves (see Fig. 4). In these older foetuses the experiments were confined to an examination of the effects of hypercapnia and hypoxia on the output of both adrenaline and noradrenaline from the adrenal medulla. The ewes were ventilated with the same gas mixtures used in the experiments on younger foetuses, to give the required changes in the gas tensions of the foetal blood.

Effect of foetal hypercapnia

Ventilation of the ewes with 15 % CO_2 in air or O_2 for 6–7 min generally increased the foetal P_{CO_2} to 65–86 mm Hg, although a level of 102 mm Hg was found in one foetus. The output of adrenaline did not increase in any of the experiments. Noradrenaline secretion did not change until the P_{CO_2} rose to between 70 and 80 mm Hg, whether the P_{O_2} increased or remained at the normal level. At all levels of P_{CO_2} the increase in noradrenaline output was very small; between 70 and 80 mm Hg the maximum output was only 0·1 μg/min and this increased to 0·17 μg/min at 85–102 mm Hg P_{CO_2}. This represents less than one-tenth of the maximum output during asphyxia.

Effect of foetal hypoxia

Ventilation of the ewes with 10, 6 or 3 % O_2 in N_2 for 6 min produced hypoxia with little or no increase in the P_{CO_2} of the foetal blood. The output of adrenaline and noradrenaline at different carotid P_{O_2} levels is shown in Fig. 8 (●). Both amines were secreted in increasing amounts as the P_{O_2} fell and when the P_{O_2} was reduced below 4 mm Hg the output of both amines was equivalent to that found after ligature of the umbilical cord.

However, in these older foetuses with an innervated adrenal medulla, a definite response was detectable at a higher P_{O_2} than in the 115-day foetuses. Thus, an increase in the rate of noradrenaline secretion was observed at 12–16 mm Hg, and by 8–10 mm Hg the discharge of this amine was 10 times that of the resting level. These values were well above those obtained for the direct response in the denervated gland of the 115-day foetus at similar P_{O_2} levels.

The increased output of adrenaline appeared to start at a lower P_{O_2} than that of noradrenaline and a detectable rise in output was not found above a carotid P_{O_2} of about 8 mm Hg; below this level a slight fall in P_{O_2} resulted

in an abrupt rise in adrenaline secretion. In view of these apparent differences in threshold and sensitivity to P_{O_2}, reflected in the rates of secretion of the two amines, further experiments were carried out to determine whether hypercapnia would potentiate the effects of hypoxia on the secretion of either amine at intermediate P_{O_2} levels.

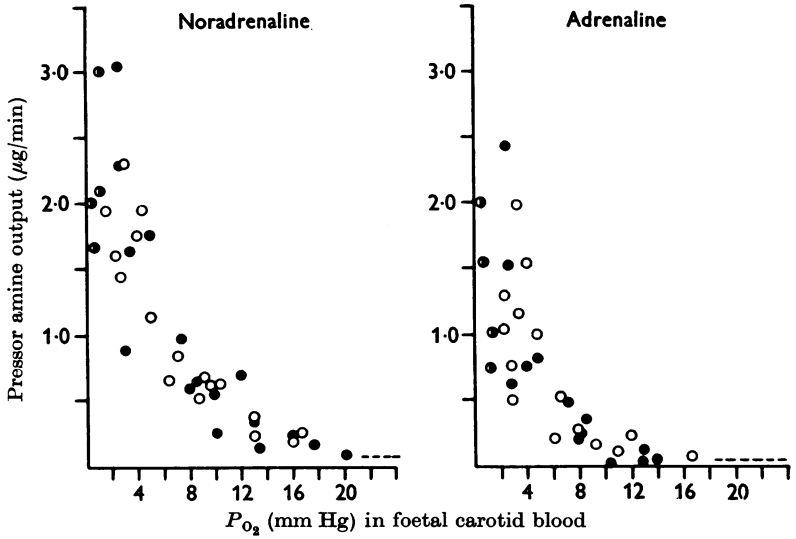

Fig. 8. Adrenaline and noradrenaline output from the innervated adrenal medulla of twenty-two foetal lambs aged 140 days at different levels of carotid blood P_{O_2}. ●, Hypoxia (mean foetal arterial $P_{CO_2} = 50 \pm 1{\cdot}23$ mm Hg, pH $= 7{\cdot}32 \pm 0{\cdot}01$, $n = 16$): ○, hypoxia+hypercapnia ($P_{CO_2} = 74{\cdot}2 \pm 2{\cdot}44$ mm Hg, pH $= 7{\cdot}12 \pm 0{\cdot}017$, $n = 17$). Other symbols as in Fig. 7.

The effect of increased P_{CO_2} on hypoxia

In these experiments the foetal-blood P_{CO_2} was increased by 20–30 mm Hg by the addition of 15 % CO_2 to the gas mixtures used to induce hypoxia. The outputs of adrenaline and noradrenaline under these conditions during different degrees of hypoxia are compared in Fig. 8 with those at normal levels of P_{CO_2}. The maximum response for both amines, which again occurred when the P_{O_2} fell below 4 mm Hg, was not increased by the high P_{CO_2} levels and did not differ significantly from the maximal discharge obtained after tying the umbilical cord. In addition, a high foetal P_{CO_2} did not appear to potentiate the output of either adrenaline or noradrenaline at any intermediate level of P_{O_2}. Thus, in Fig. 8, the curves for amine output at the two P_{CO_2} levels are virtually superimposed.

This point is also illustrated by the noradrenaline response in two experiments shown in Fig. 9. In one foetus (upper record) the P_{O_2} level was held at 11 mm Hg. In the other (lower record) the P_{O_2} was reduced

to 0–2 mm Hg to excite a maximal discharge. Both foetuses were subjected to two periods of hypoxia; in the first test the P_{CO_2} remained low, while in the second it was raised by including 15% CO_2 in the gas mixture. In each foetus the output of noradrenaline in the two tests was similar, i.e. it was independent of the CO_2 level, and the magnitude of the response was related to the degree of hypoxia. The close relation between noradrenaline output and P_{O_2} level was evident both during and immediately after hypoxia. Thus, three of the test periods shown in Fig. 9 were followed by

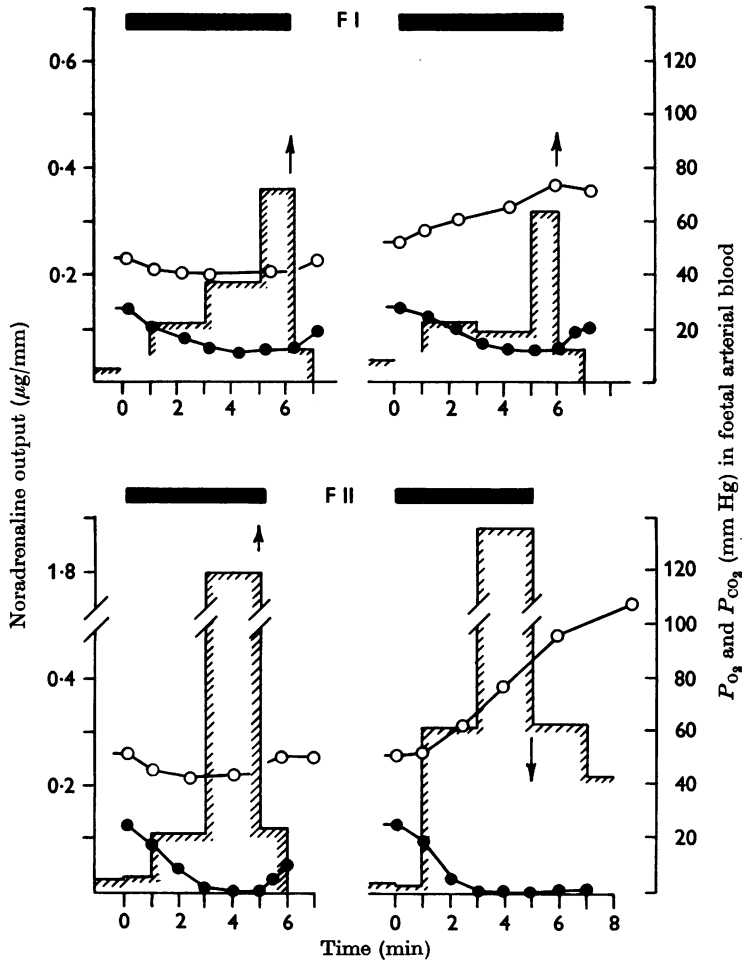

Fig. 9. Output of noradrenaline (▨) in two foetuses, F I and F II, aged 140 days, subjected to two successive periods of hypoxia, ▬; 1st period, normal P_{CO_2}; 2nd period, raised P_{CO_2}; further details in text. Re-oxygenation of ewe at end of each period of hypoxia (↑) except at end of 2nd experiment (↓) in F II where umbilical cord clamped. ●—●, P_{O_2} in carotid blood; ○—○, P_{CO_2} in abdominal aortic blood.

immediate increases in the foetal blood P_{O_2}, when the ewe breathed air or O_2, while P_{CO_2} showed no consistent change; in each case the output of noradrenaline fell dramatically at this point. In the fourth test, on the other hand, the cord was clamped at the end of the period, the P_{O_2} accordingly remained low, and the noradrenaline response then continued for several minutes, although no longer maximal.

DISCUSSION

In the present investigation of possible factors involved in the response of the foetal adrenal medulla to asphyxia, only a fall in the oxygen tension of the foetal arterial blood invariably caused a discharge from the gland. Other changes in the composition of the blood, such as the fall in pH, the rise in P_{CO_2} and the increase in lactic-acid concentration, which normally occur during asphyxia, had little or no effect on the rate of secretion. Hypoxia appeared to be the effective stimulus both for the direct response of the cells of the adrenal medulla to asphyxia in the young foetus and also for the much larger discharge from the innervated gland in foetuses near term, and, in fact, the same low foetal blood P_{O_2} of 2–4 mm Hg was required for a maximal discharge in both cases.

In nearly all other respects, however, the two mechanisms apparently responsible for the secretion by the adrenal medulla at different times were very different. The nervous response, which essentially reflects the effects of hypoxia on the central nervous system, either reflexly or directly, was more sensitive and more finely adjusted to variations in the oxygen tension than the direct effect on the adrenal medullary cells. This distinction between the two types of discharge was most clearly shown by the differences in threshold for each response. A definite direct effect of hypoxia on the gland of the 115-day foetus was not detectable until the carotid P_{O_2} fell to between 4 and 5 mm Hg: below this level the discharge from the gland rapidly became maximal and could be prolonged for a considerable time, even during complete anoxia. In the innervated gland of the older foetus an increase in the rate of secretion was observed when the carotid P_{O_2} was reduced to about 16 mm Hg; at this P_{O_2} level the discharge consisted largely of noradrenaline, and an increase in adrenaline output was not found until the oxygen tension fell to about 8 mm Hg.

The more detailed analysis in this paper of the output from the adrenal medulla after ligation of the umbilical cord has confirmed and extended the previous results on the composition of the secretion released by the two mechanisms (Comline & Silver, 1961). In the present series of experiments, a high proportion of noradrenaline was released during the direct

response throughout the entire period of asphyxia: both amines were secreted in very large amounts by the innervated gland, although the high initial rate of discharge was not maintained for more than 4 or 5 min.

The decline of the direct response with increasing maturity coincides, in the lamb, with the onset of the nervous mechanism and the secretion of significant amounts of adrenaline. In the calf, on the other hand, the response of the adrenal medulla to asphyxia appears to be independent of the nerve supply to the gland throughout gestation and, like the direct response in the lamb, the discharge consists almost entirely of noradrenaline (Comline & Silver, 1962). However, there is as yet insufficient evidence to show whether the development of innervation and the ability to secrete adrenaline are directly connected, or are merely part of a more general change in development.

In certain respects, the direct effect of hypoxia on the foetal adrenal medulla can be compared with that on chemoreceptor tissues such as the glomus cells of the carotid body. Both types of cell contain similar, but not identical, granules (Lever, Lewis & Boyd, 1959); the chemoreceptors also respond to a lowering of oxygen tension and to cyanide (Heymans & Neil, 1958) and continue to discharge over long periods of anoxia (Bogue & Stella, 1935). Both types of cell, therefore, share many common properties, but further examination shows great differences in their reactions. In the lamb, at least, the foetal adrenal medulla responded only to very low oxygen tensions and appeared to be much less sensitive to this stimulus than the glomus cells. Furthermore, CO_2 did not potentiate the effects of hypoxia, nor did hypercapnia stimulate the foetal adrenal medulla at normal P_{O_2} levels, although both these conditions are known to increase the rate of discharge from chemoreceptors of the carotid body (Joels & Neil, 1961). It might be argued that in the present experiments the resting P_{CO_2} levels of 45–50 mm Hg were sufficiently high to give maximum potentiation of the effect of hypoxia on the adrenal medulla and that a difference would only be found if the P_{CO_2} of the foetal blood were reduced to the low levels (10–19 mm Hg) at which M. Daly, J. Hazzledine & A. Howe (personal communication) found hypoxic blood (20–25 mm Hg) to be a comparatively ineffective stimulant of the isolated aortic body chemoreceptors of the dog. It was impracticable to test this point in the present study, and in any event such low P_{CO_2} levels will not normally occur in the intact foetus.

The finding that the effect of hypoxia on the discharge of the innervated adrenal gland was not potentiated by increased P_{CO_2} levels was, perhaps, more surprising than the absence of any direct action by CO_2 on the adrenal medullary cells. The behaviour of the gland after the development of its nervous supply can be compared with that of the adult, in which

a discharge from the adrenal medulla mediated by the splanchnic nerves forms part of the general sympathetic nervous activity during asphyxia. Even in the adult, however, the relative importance of increased P_{CO_2} and oxygen lack in the stimulation of the adrenal medulla during asphyxia, as distinct from the other parts of the sympathetic system, is not clear, nor, indeed, is it certain to what extent, if any, the peripheral chemoreceptors are involved in this mechanism (Heymans & Neil, 1958). Houssay & Molinelli (1926) first suggested, on indirect evidence, that hypoxia rather than hypercapnia is the primary stimulus to the adrenal medulla during asphyxia, and preliminary experiments have shown that oxygen lack is extremely effective in stimulating the intact adrenal medulla in the adult, whether it is accompanied by high or low CO_2 levels (R. S. Comline & M. Silver, unpublished observations). In this respect, at least, the foetal adrenal medulla of late pregnancy may not be very different from that of the adult. Nevertheless, there is some evidence that an increased P_{CO_2} alone can cause a rise in the catecholamine concentration of the blood, presumably derived from the adrenal medulla (Tenney, 1956; Millar, 1960). But in these experiments the level of P_{CO_2} required to elicit a discharge was far above that normally found, and was much higher than the levels tested in the present experiments. It is thus by no means certain that the effect of such severe hypercapnia can be ascribed to the usual stimulating property of CO_2, either on the central nervous system or the peripheral chemoreceptors; in view of the narcotic effect of excessively high levels of CO_2 on the central nervous system (Barcroft, 1934) it is possible that the effect may be indirect and resemble that of anoxia.

The present results show that, with the development of the nerve supply to the adrenal medulla towards the end of pregnancy, a graded response to different levels of oxygen tension can be elicited, in which the proportion of adrenaline to noradrenaline will vary according to the intensity of the stimulus. In all these experiments the resting secretory rate of the adrenal medulla was, at the most, extremely low, and the resting levels of carotid and aortic blood P_{O_2} were well above the threshold for excitation of the gland. M. Purves (personal communication) has also recently shown that the carotid body chemoreceptors of the foetal lamb are active and respond to even smaller falls in arterial P_{O_2}. The presence of mechanisms which will activate the sympathetic system and the adrenal medulla, either directly or reflexly, should the foetal oxygen tension fall below critical levels, raises the controversial issue of the range of P_{O_2} values which normally occur in the foetal blood at different stages in pregnancy.

In the past much useful information on foetal blood oxygenation has been obtained from measurements of O_2 saturation, oxygen content and

capacity (Barcroft, 1946; Barron, 1951; Dawes *et al.* 1954). But, probably because of the lack of a direct method for measuring blood P_{O_2}, data on levels of oxygen tension in foetal blood are scarce, and the only comprehensive study is that of Barron (1951). However, the recent development of the membrane-covered oxygen electrode (Clark, 1956) has led to a number of investigations involving direct measurements of resting foetal and maternal blood P_{O_2} levels (Assali, Manson, Holm & Ross, 1963; Dawes, 1964; M. Purves, personal communication). Despite the use, in all these experiments, of different anaesthetic and operative procedures and a variety of oxygen electrodes, the values for both carotid and aortic foetal P_{O_2} reported in these later investigations are in good agreement, and correspond with those given in the present paper. Nevertheless, the measurements were all made in preparations in which the uterus was open and the foetus wholly or partially removed. They are, therefore, not necessarily comparable with conditions in the intact foetus *in utero*.

A different approach has recently been made by Barron and his colleagues, who have now extended their studies on the relation between foetal and maternal blood gases in sheep under spinal anaesthesia (Barron, 1951; Barron & Meschia, 1957), to unanaesthetized ewes and foetuses by the use of indwelling catheters (Blechner, Meschia & Barron, 1960; D. H. Barron, personal communication). In all their experiments, however, whether the foetuses were undisturbed or removed from the uterus, they have found low levels of foetal arterial oxygen saturation and their estimates of foetal P_{O_2} are considerably below those recently recorded here and elsewhere. On the other hand, the levels observed for foetal and maternal blood P_{CO_2} and pH in the two series of investigations by this group are very similar to those reported by others, and the arterial oxygen saturation and P_{O_2} of their ewes was satisfactory in both acute and chronic preparations.

In the present experiments, in which sensitive oxygen electrodes responded rapidly to variations in foetal and maternal P_{O_2}, the placental exchange of oxygen appeared to be influenced by many factors in addition to the maternal arterial blood pressure and P_{O_2}, such as the state of uterine contraction and vasoconstriction, the uterine venous P_{O_2} and the foetal arterial blood pressure. It is possible that removal of the foetus from the uterus, or even an abnormal position of the ewe, may lead to alterations in uterine and placental blood supply, and thereby result in P_{O_2} levels which differ from those of the normal undisturbed foetus. If this is the case, such manipulations would appear to affect the foetal arterial P_{O_2} almost specifically, for there appears to be little discrepancy between the foetal P_{CO_2} and pH levels obtained by a number of different experimental techniques.

Further work is clearly required to elucidate the problem of the factors which may affect the exchange of oxygen across the placenta. Whatever the final outcome, there is no doubt that, in the earlier part of pregnancy in the sheep, the foetal P_{O_2} is unlikely to fall to levels at which the direct response of the adrenal medulla would become important, except under abnormal circumstances, such as disease, premature birth, or severe asphyxia of the ewe. Towards the end of pregnancy, an increased adrenal discharge becomes more probable, with the combination of increased sensitivity to low P_{O_2} and the possibility of wide variations in the foetal blood oxygen tension such as might occur during increased uterine motility. The critical level in the older foetuses appears to be a carotid P_{O_2} of about 16 mm Hg (abdominal aortic P_{O_2}, 14–15 mm Hg). This is within the range of values reported for umbilical arterial blood from foetuses *in utero* towards the end of pregnancy (D. H. Barron, personal communication), and if such levels are characteristic of the intact foetus, then almost continuous activity of the sympathetic system and a low level of discharge from the adrenal medulla could be expected at this time. If, however, the resting P_{O_2} values are higher, then excitation of the sympathetic system and the adrenal medulla would be, at the most, intermittent.

The possible effects of a raised catecholamine content of the foetal blood are, as yet, relatively unknown. It is also difficult to assess the contribution of other parts of the sympathetic nervous system which may be excited at P_{O_2} levels higher than that of the adrenal medulla. But in the lamb, at least, it would appear that the margin near parturition for the excitation of the sympathetic system, including the adrenal medulla, is probably small.

SUMMARY

1. The relation between the changes in the composition of the foetal blood during asphyxia and the release of catecholamines from the adrenal medulla have been investigated in the foetal lamb.

2. The lambs were tested either at 115 days gestational age, when the cells of the adrenal medulla can respond directly, or at 140 days, immediately before term, when the nerve supply to the adrenal gland has developed and has become the main controlling agent.

3. Resting values for foetal blood P_{O_2}, P_{CO_2}, lactic acid, glucose and pH were recorded; under the conditions of the experiments the foetal arterial P_{O_2} was higher and the foetal blood P_{CO_2} lower at 115 days than at 140 days gestation. Only small changes in the composition of the foetal blood occurred in the absence of asphyxia. Under conditions of good placental exchange foetal arterial P_{O_2} rapidly followed changes in maternal arterial P_{O_2}; the foetal P_{O_2} levels were predictable at any given maternal level provided these lay between 30 and 150 mm Hg.

4. Large changes in the foetal blood P_{O_2}, P_{CO_2}, pH and lactic acid concentration occurred after ligature of the umbilical cord; each of these factors was investigated for its possible effect, whether direct or indirect, upon the adrenal medulla.

5. Severe hypoxia appeared to be the specific stimulus for both types of response. The level of hypoxia which elicited an adrenal discharge varied with age, and a much smaller fall in P_{O_2} was necessary to stimulate the innervated gland at 140 days. In both groups, a response equivalent to that found after occlusion of the umbilical cord was obtained below a P_{O_2} of 4 mm Hg.

6. The changes in P_{CO_2}, pH and lactic acid during asphyxia appeared to have little or no effect on the adrenal medulla. In the younger foetuses prevention of the fall in pH after umbilical cord occlusion did not reduce the discharge from the gland, nor did hypercapnia cause any increase in secretion.

7. At 115 days the direct effect of anoxia could be mimicked by intra-arterial injection of NaCN. Lactic acid injected in a similar manner produced only a very small response.

8. At 140 days a discharge which consisted primarily of noradrenaline was obtained when the carotid P_{O_2} was reduced to 12–16 mm Hg; at about 8 mm Hg adrenaline was also secreted, and below 4 mm Hg the output of both amines was maximal.

9. An increase in the P_{CO_2} of the foetal blood did not potentiate the effect of hypoxia on the discharge of the adrenal medulla either at 115 days or at 140 days; hypercapnia alone (70–100 mm Hg P_{CO_2}) produced only a very slight increase in the output from the adrenal gland in the older foetuses.

10. A comparison of the P_{O_2} recorded for foetal arterial blood in these experiments with values obtained by others showed that in the lamb only a slight margin was present between the resting P_{O_2} levels at the end of pregnancy and the threshold for the discharge from the innervated adrenal medulla.

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