J. Physiol. (1978), **277**, *pp.* 341–357
With 8 text-figures
Printed in Great Britain

PLASMA VASOPRESSIN LEVELS DURING HYPOXAEMIA AND THE CARDIOVASCULAR EFFECTS OF EXOGENOUS VASOPRESSIN IN FOETAL AND ADULT SHEEP

BY D. W. RURAK*

From the Nuffield Institute for Medical Research, University of Oxford, Headley Way, Oxford

(*Received 17 August 1977*)

SUMMARY

1. Foetal plasma vasopressin levels were measured by bio-assay in chronically catheterized sheep from 110 to 145 days gestation.

2. In foetuses in good condition resting circulating vasopressin concentrations were generally undetectable (< 5 μu./ml.). In 15% of the samples low concentrations (5–10 μu./ml.) were observed.

3. Hypoxaemia in the foetus was caused by allowing the ewe to breathe 9% O_2–3% CO_2 in N_2 for 1 hr. Plasma vasopressin levels rose in the foetus to 119 ± 32 μu./ml., whereas the hormone levels in the ewe were not routinely increased. In the foetus, the rise in plasma vasopressin levels was significantly related to the fall in pH and P_{a,O_2} during the hypoxia.

4. In foetuses in which the cervical vagosympathetic trunks were cut, the rise in plasma vasopressin levels (to 48 ± 25 μu./ml.) during hypoxaemia was less than in intact foetuses. The increase was related only to the fall in arterial pH and the regression coefficient was less than in intact foetuses.

5. During hypoxaemia arterial pressure rose and heart rate fell in the normal foetuses. The rise in arterial pressure was greatest when the plasma vasopressin concentration was highest.

6. Spontaneous episodes of hypoxaemia ($P_{a,O_2} < 15$ mmHg) and/or acidaemia (pH < 7·30) occurred in four intact foetuses and four foetuses in which the cervical vagosympathetic trunks were cut; all of the latter lambs died *in utero*. Plasma vasopressin levels were elevated and the concentrations were inversely related to arterial pH.

7. Intravenous infusions of vasopressin to foetuses increased plasma vasopressin levels to 6–202 μu./ml.; the rate of clearance of the hormone was three times that in adult ewes. There was a large increase in arterial pressure and bradycardia. The hypertensive effects of vasopressin were relatively much greater in the foetus than in adult ewes.

* Present address: Department of Zoology, University of British Columbia, Vancouver, B.C., Canada.

INTRODUCTION

The neurohypophysis of the sheep foetus contains measurable quantities of arginine vasopressin during the last half of gestation (Alexander, Britton, Forsling, Nixon & Ratcliffe, 1973). The hormone is also present in the plasma of exteriorized foetal lambs over this period of gestation and is increased by foetal hypoxaemia, haemorrhage or the intracarotid infusion of hypertonic saline (Alexander *et al.* 1973; Alexander, Bashore, Britton & Forsling, 1976). In undisturbed foetal lambs *in utero* with chronically implanted catheters, plasma vasopressin levels are in the same range as maternal values, although the ovine placenta appears effectively impermeable to the hormone (Alexander *et al.* 1976; Stark, Hussain, Daniel, Milliez, Morishima & James, 1977; Leake, Weitzman & Fisher, 1977).

These data indicate that the foetal sheep neurohypophysis has the capacity to synthesize vasopressin and release it in response to appropriate stimuli. But there is little information on the factors controlling foetal vasopressin secretion *in utero*, and on the physiological effects of the hormone. In the present investigation, plasma vasopressin levels have been measured in chronically catheterized foetal lambs under undisturbed conditions and during periods of experimental and spontaneous hypoxaemia. Intravenous infusion of vasopressin was carried out on normoxic foetuses to assess the cardiovascular effects of the hormone, and similar experiments were performed on non-pregnant ewes to compare the circulatory effects in foetus and adult. Some of these observations have been reported elsewhere (Rurak, 1976).

METHODS

Twenty-eight foetuses of 110–136 days gestation from twenty-one pregnant ewes of mixed breeds were used. Employing aseptic techniques, catheters were implanted in the amniotic cavity, a foetal carotid artery and jugular vein, and in some cases in the trachea, and in a maternal carotid artery and jugular vein (Dawes, Fox, Leduc, Liggins & Richards, 1972). In eight foetuses a 1 cm segment of each vagosympathetic trunk was removed at about the level of the larynx. Continuous recordings of foetal heart rate and carotid arterial and amniotic pressures and in some cases intra-tracheal pressure were commenced within 48 hr of surgery (Dawes *et al.* 1972), but the animals were allowed to recover for 3–9 days before experimentation. In three non-pregnant ewes, under halothane anaesthesia, catheters were implanted in a jugular vein and a carotid or femoral artery. Experiments were begun from two to four days later.

Hypoxia experiments

In sixteen pregnant ewes a 9% O_2, 3% CO_2 in N_2 gas mixture was delivered for 1 hr at 40 l./min into a large polyethylene bag fitted over the ewe's head with the outflow around the neck (Boddy, Dawes, Fisher, Pinter & Robinson, 1974). Carotid arterial blood samples for vasopressin assay and blood gas determination were collected from the ewe and foetus at 60 and 10 min before, 10, 25, 45 (blood gas sample only) and 60 min during and 10 and 70 min after the hypoxic episode. Three of the ewes were also used in control experiments in which air was delivered into the bag for 1 hr.

Vasopressin infusions

Vasopressin (Pitressin, Parke-Davis Co.) was infused intravenously in nine foetal lambs in eight ewes and in three non-pregnant ewes. Pitressin was added to sterile, isotonic sodium chloride solution immediately before each experiment and was infused for 1 hr at rates of 9, 4·52 and 2·25 m-u./min (1·5 ml./hr) to the foetuses and at 185, 92·7, 46·3 and 23·2 m-u./min (27·8 ml./hr) to the non-pregnant ewes. Blood for the assay of vasopressin and for blood gas

determination was collected at 60 and 10 min before, 10, 20, 40 and 60 min during and 10 and 70 min after the infusion. In one further experiment, chlorbutol (1,1,1-trichloro-2-methylpropan-2-ol, B.D.H. Chemicals), the preservative present in Pitressin, was infused to the foetus at a rate equal to that in the highest Pitressin infusions.

Blood samples (3–5 ml.) for vasopressin determination were collected in chilled plastic syringes containing 15–20 u. heparin (Boots Pure Drug Co., Nottingham). They were immediately transferred to chilled polystyrene tubes (Henleys Medical Supplies, London) in ice. The samples were centrifuged at 1500 *g* and 2 °C for 15 min and the supernatants were stored in stoppered polystyrene tubes at − 20 °C. No significant loss of hormone occurred during centrifugation or storage at − 20 °C. Blood samples (1 ml.) were collected in siliconized, glass syringes and immediately analysed for packed cell volume and for P_{O_2}, P_{CO_2} and pH at 39·5 °C using a Model 27 Acid-Base Analyzer (Radiometer, Copenhagen).

Extraction of vasopressin

Vasopressin in plasma was extracted by a modification of the method of Bisset, Hilton & Poissner (1967). The plasma was mixed with 3 volumes of absolute ethanol (redistilled A.R. quality, James Boroughs Ltd, London) and centrifuged at 1600 *g* and 0 °C for 10 min. After acidification to pH 4–5 with N-HCl, the supernatant was evaporated to dryness at 50 °C and 25 mmHg. The residue was washed with 1 ml. and then 0·5 ml. ethanol and the combined washings were centrifuged at 1600 *g* or 5 min. Then the supernatant was evaporated to dryness at 45 °C under a stream of air, and the residue was suspended in 0·45 ml. 0·01 N-NaOH. The pH of the resulting solution was 2–3. Recovery of Pitressin (75–1200 μu.) added to plasma lacking endogenous vasopressin averaged 53·7 % (± 10·50, n = 114). Plasma hormone levels have been corrected for losses incurred during extraction.

Assay of vasopressin

Vasopressin was assayed by a modification of the method of Dicker (1953), using female Wistar rats (125–200 g) anaesthetized and hydrated with 13 % ethanol solution (5 ml./100 g) given by stomach tube, followed 15 min later by sodium thiopentone 20 mg/kg (Pentothal, Abbott Laboratories, Queenborough, Kent) given intraperitoneally. Urine flow was measured by an electronic drop counter connected to a catheter in the urinary bladder, and urine conductivity was measured between two stainless-steel inserts in the bladder cannula. Injections were given via a catheter implanted in a saphenous vein, which was infused continuously (50–250 μl./min) with a solution containing NaCl (0·05 M), glucose (0·09 M) and ethanol (4–8 %, v/v). Responses were calculated as the percentage fall in urine flow from the value during the 5 min before the injection to that during the second to the seventh minute after injection. Assays were performed by the four-point method (Holton, 1948); normally only one group of four injections was carried out for each sample. Pitressing was used as the standard and each batch used was checked for its antidiuretic and vasopressor (Dekanski, 1952) potencies against the Third International Standard for the Posterior Pituitary (Bangham & Mussett, 1958). In no case did the vasopressin potency differ from the stated value (20 u./ml.), nor did the ratio of vasopressor and antidiuretic activities differ from unity.

To check that the endogenous antidiuretic activity measured in extracts of foetal and maternal plasma was due to vasopressin, aliquots from some extracts were incubated with sodium thioglycollate (0·1 M, n = 39) or tyrosinase (1 mg/ml., n = 24), both of which inactivate neurohypophysial principles (Vogt, 1953; Bisset, 1962). In every case the endogenous antidiuretic activity was destroyed.

Statistics

Changes during an experiment were tested for significance using the paired t test, while differences between different groups of foetuses or between foetus and ewe were tested using the t test for small samples. Linear regression analysis with one or two independent variables was carried out as described by Bailey (1959). Unless otherwise stated mean values are given with ± S.E.

RESULTS

Resting-foetal plasma vasopressin concentrations

In seven normal foetuses, all twenty-one samples collected before labour lacked detectable levels of vasopressin. The mean threshold sensitivity of the assay was 5 μu./ml., but some samples tested negative on assay preparations with thresholds as low as 2 μu./ml. In a further nine foetuses, twenty-nine of the forty-three samples collected had undetectable amounts of vasopressin, while in the remaining fourteen low levels (10 ± 2 μu./ml.) were present. All sixteen foetuses appeared normal on the bases of blood gases ($P_{a,O_2} = 20 \pm 0{\cdot}6$ mmHg, $P_{a,CO_2} = 44 \pm 0{\cdot}6$ mmHg) and pH ($7{\cdot}32 \pm 0{\cdot}005$), blood pressure, heart rate and, in some foetuses, breathing patterns.

In four foetuses in good condition in which the cervical vago-sympathetic trunks were cut plasma vasopressin concentrations were similar to those in the intact foetuses. In two of these, all seven samples lacked vasopressin while in the other two the levels were undetectable or low (5 ± 1 μu./ml.).

Of the thirty samples collected from seven pregnant ewes, twenty-six lacked detectable vasopressin while low levels (10 ± 2) were present in the remaining four. There was no relationship between foetal and maternal plasma vasopressin concentrations in thirty paired samples collected from seven preparations. Nor was there any correlation between the hormone concentration in twenty paired samples collected from four twin preparations.

Hypoxaemia

The effect of hypoxaemia on foetal plasma vasopressin levels was examined in twenty experiments on fourteen foetuses from 114 to 141 days of gestation. Nine of the experiments were carried out on four foetuses with severed vago-sympathetic trunks and one was performed on a twin preparation containing catheterized intact and vagotomized foetuses. The changes in maternal and foetal blood gas values during the experiment were similar to published results (Boddy *et al.* 1974). Maternal arterial P_{O_2} fell from a mean of 97 to 45 mmHg; P_{a,CO_2} and pH remained unchanged at 31 mmHg and 7·47 respectively. In the foetuses carotid arterial P_{O_2} fell on average from 21 mmHg to between 12 and 13, while P_{a,CO_2} was maintained at 45 mmHg. Arterial pH averaged 7·37 in the control period and then declined progressively during the hypoxia to 7·32 in the intact foetuses and 7·28 in the vagotomized foetuses. It was only partially restored 1 hr after the end of hypoxia.

Fig. 1 illustrates the changes in foetal plasma vasopressin concentrations during the experiment. The levels were generally undetectable (< 5 μu./ml.) before the hypoxaemia in both the intact and vagotomized foetuses; the mean control values given in the figure were calculated by assigning samples lacking measurable quantities of vasopressin a value equal to one half the threshold sensitivity of the assay. The vasopressin concentrations rose steadily during hypoxaemia in all eleven experiments on intact foetuses, to a mean value at 60 min of 119 μu./ml. $\pm$ 32. In the vagotomized foetuses the hormone levels remained unchanged or undetectable in three of the experiments, while the rise in the others was generally less than that observed in the intact lambs. The mean concentration after 60 min of hypoxaemia was 48 μu./ml. $\pm$ 25, which was not significantly different from the value found in the

intact foetuses ($P < 0{\cdot}10$). In both groups of foetuses, the plasma vasopressin concentrations declined after the hypoxaemia, but at the end of the experiment were still somewhat elevated, the mean values being 14 ± 4 and 9 ± 3 respectively for the intact and vagotomized foetuses.

The maximum plasma vasopressin concentrations observed in the foetus during hypoxaemia varied greatly in the different experiments, ranging from 7 to 348 μu./ml. in the intact animals and from undetectable to 243 μu./ml. in the vagotomized

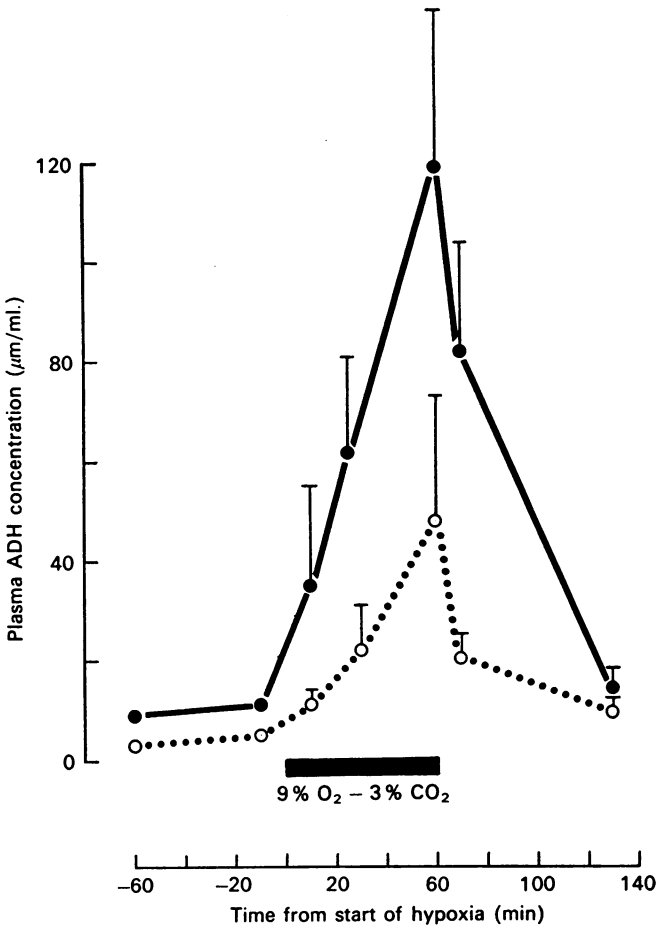

Fig. 1. Plasma vasopressin concentrations before, during and after hypoxia. The results are expressed as mean ± S.E.of eleven experiments on ten normal foetuses (●) of gestational age 114–141 days and of nine experiments on four foetuses (○) of gestational age 122–136 days and in which the cervical vagosympathetic trunks were cut.

foetuses. In Fig. 2 the vasopressin concentrations in individual foetal plasma samples collected during the period of hypoxaemia are plotted against the changes in carotid arterial P_{O_2} and pH from control values. Multiple regression analysis of the data from intact foetuses indicated that a statistically significant relationship existed between plasma vasopressin concentration and the fall in both P_{O_2} and pH (Table 1). In the vagotomized lambs only the relationship between vasopressin level and change in

arterial pH was significant (Table 1) and the regression coefficient (421·10 ± 130·4) was significantly different ($P < 0.001$) from the corresponding value (1404·3 ± 134·0) calculated for the intact lambs.

Maternal blood samples for vasopressin assay were collected in five of the hypoxia experiments. Plasma vasopressin levels were low or undetectable in all five ewes

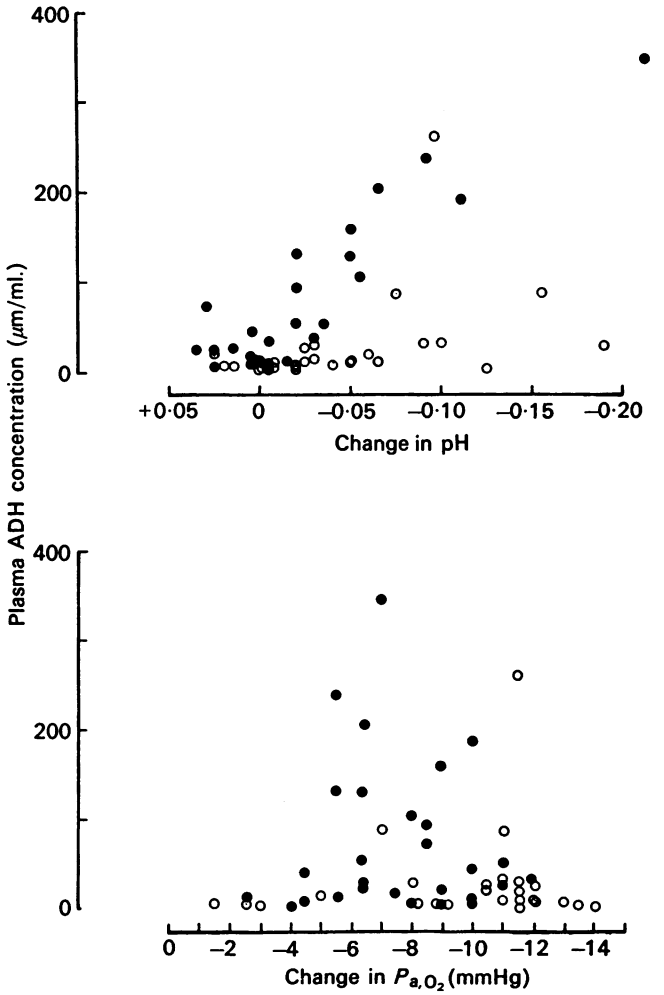

Fig. 2. Relationship between plasma vasopressin concentration and arterial pH and P_{O_2} during hypoxia in eleven experiments on ten normal foetuses (●) and in nine experiments on four foetuses (○) in which the cervical vagosympathetic trunks were cut.

before breathing the 9% mixture, and in four the concentrations were unchanged throughout the rest of the experiment. In the other ewe, the concentration rose to 300 μu./ml. at 10 min during the hypoxaemia, declined to 33 μu./ml. at 25 min and was undetectable by 60 min.

In three control experiments, air was delivered for 1 hr to the polyethylene bag over the ewe's head. Plasma vasopressin levels were measured in one of the ewes and

TABLE 1. Linear regression analysis of the relationship between plasma vasopressin concentrations and arterial P_{O_2} and pH during hypoxia experiments in intact and vagotomized fetal lambs

	N	a	b_1	P	b_2	P
Intact foetuses*	47	10·2	$-4{\cdot}00 \pm 1{\cdot}32$	$< 0{\cdot}01$	-1404 ± 134	$< 0{\cdot}001$
Vagotomized foetuses†	42	7·2	-421 ± 130	$< 0{\cdot}01$	—	—

* Equation – plasma ADH $= a + b_1 (\Delta P_{O_2}) + b_2 (\Delta \text{ pH})$.
† Equation – plasma ADH $= a + b_1 (\Delta \text{ pH})$.

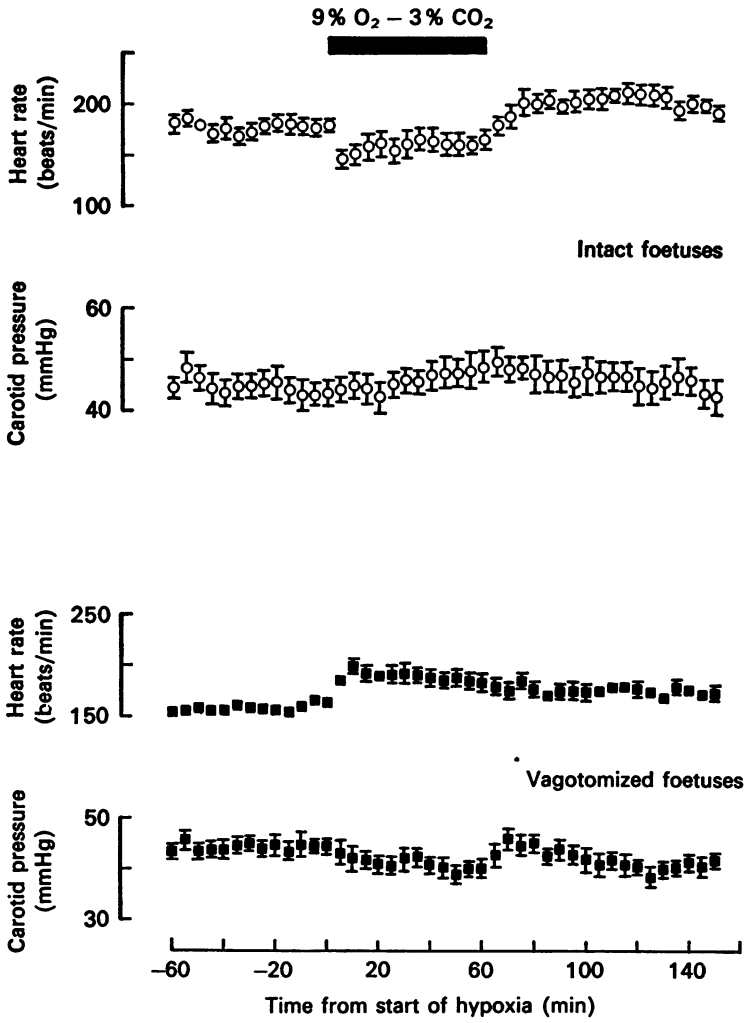

Fig. 3. Foetal heart rate and arterial pressure before, during and after hypoxia. The results are expressed as mean ± S.E. for eleven experiments on ten normal foetuses with gestation ages 114–141 days and for nine experiments on four vagotomized foetuses 122–136 days gestational age.

four of the foetuses, one of which was vagotomized. In every case, plasma vasopressin levels remained undetectable throughout the experiment.

In the intact foetuses, heart rate fell shortly after the onset of hypoxaemia and remained low throughout (Fig. 3). Arterial pressure rose in eight of the experiments (3–16 mmHg rise) and fell slightly (2 and 4 mmHg) in the remaining 2. The greatest hypertension occurred when the plasma vasopressin levels were highest. This is illustrated in Fig. 4, where the plasma vasopressin concentrations in individual

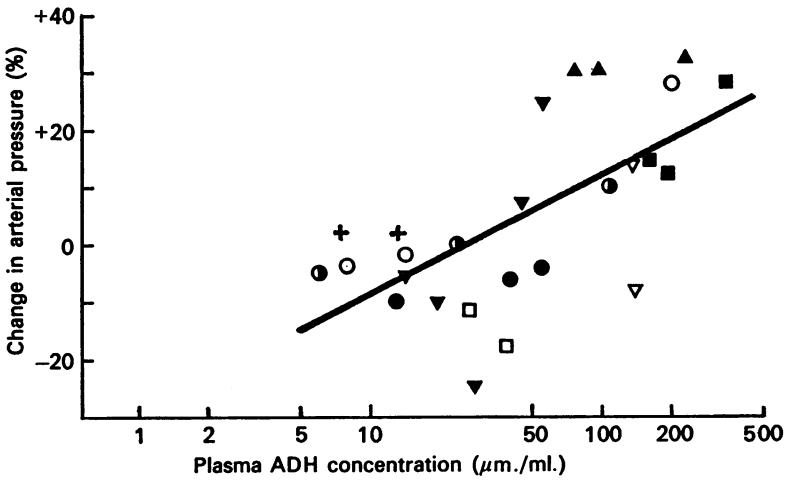

Fig. 4. The relationship between change in arterial pressure and plasma vasopressin concentration during hypoxia in eleven experiments on ten foetuses. Vasopressin concentration is plotted on a logarithmic scale; the different symbols refer to individual experiments. The line was fitted by regression analysis and conforms to the equation: percentage change in arterial pressure = $-28{\cdot}6+(20{\cdot}4\pm4{\cdot}9)$ (log plasma ADH concentration) ($P < 0{\cdot}001$).

samples are plotted against the percentage change in arterial pressure. In the foetuses with severed vago-sympathetic trunks, hypotension and tachycardia were present throughout the period of hypoxaemia (Fig. 3). There was no obvious relationship between the plasma vasopressin levels and the change in arterial pressure.

Spontaneous hypoxaemia and acidaemia

In four foetal lambs with intact vago-sympathetic trunks, spontaneous episodes of hypoxaemia ($P_{a,O_2} < 15$ mmHg) and/or acidaemia (pH < 7·30) were observed. They occurred 3–5 days before the onset of premature labour and lasted 1–3 days, after which the blood gas status improved (in two instances after the death *in utero* of an hypoxaemic, vagotomized twin). In all four lambs plasma vasopressin levels were raised (44 ± 16, $n = 15$) during the period of blood gas deterioration.

Similar observations were made in four vagotomized foetuses which died *in utero* after 1–8 days of hypoxaemia and acidaemia due to unknown causes (mean plasma vasopressin concentration 57 ± 16, $n = 17$), and five foetuses which developed asphyxia in labour. In these latter animals, plasma vasopressin levels were low or undetectable until 24 hr before birth. During early labour the mean hormone

concentration of 8 μu./ml. (n = 5) was associated with slight hypoxaemia (P_{a,O_2} = 17 mmHg) but normal pH (7·38). As labour progressed the vasopressin levels became higher (147 μu./ml., n = 8) and were accompanied by a low arterial P_{O_2} (15 mmHg) and pH (7·25). All five foetuses were born alive, but one was in poor condition and died shortly afterwards.

These various observations show that high foetal vasopressin concentrations can be maintained for hours and days during asphyxia. Fig. 5 shows that in the foetus plasma vasopressin concentrations are inversely related to arterial pH.

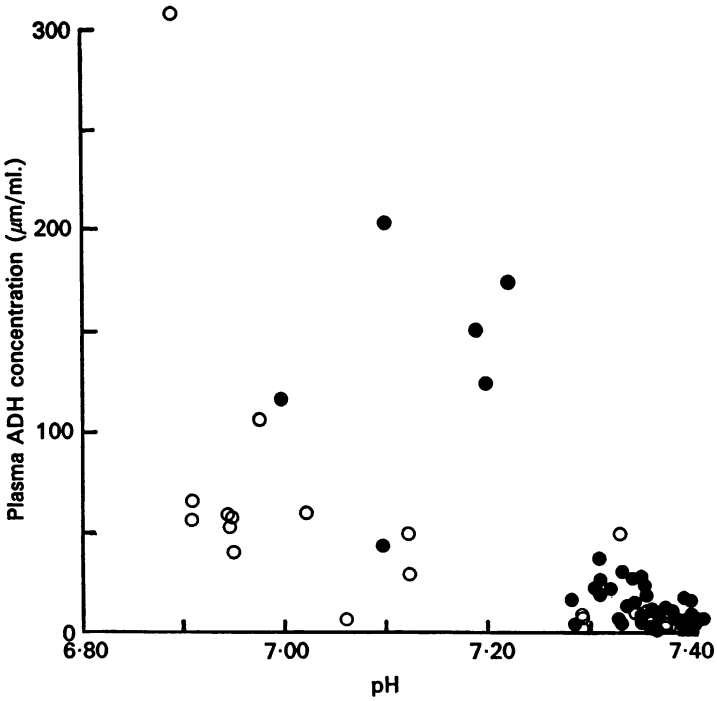

Fig. 5. Relationship between plasma vasopressin concentration and arterial pH in thirteen intact foetal lambs (●) and in six vagotomized foetuses (○). Blood samples were collected when blood gas values were in the normal range and during spontaneous episodes of hypoxaemia and/or acidaemia.

Vasopressin infusions

Vasopressin (Pitressin) was infused intravenously for 1 hr on thirteen occasions in nine foetal lambs from eight ewes. Two of the ewes had catheterized twins, with one of each pair being vagotomized. Three rates of infusion were used, 9 (n = 5), 4·5 (n = 4) and 2·25 (n = 2) m-u./min. Upon delivery of the foetuses, lamb weights were obtained, and, using the formula derived by Gresham, Rankin, Makowski, Meschia & Battaglia (1972), the foetal weight at the time of experimentation was estimated. Using these data, the rates of infusion ranged from 0·45 to 4·09 m-u./kg.min.

The mean foetal plasma vasopressin concentrations before, during and after the infusions are illustrated in Fig. 6. The levels rose steadily during the first 20 to 40 min

of the infusion and then reached a plateau. The mean difference between the hormone concentrations in the 40 and 60 min sample was only 0·6%. To calculate the steady-state plasma clearance of vasopressin in the individual experiments, the rate of hormone infusion was divided by the average of the 40 and 60 min concentrations. The clearance rate ranged from 15·4 to 33·3 ml./min.kg with a mean of 23·3 ± 1·6 (n = 13). After stopping the infusion, plasma vasopressin levels fell, but when the infusion rate was 9 m-u./min, the decline was not complete by the end of the experiment.

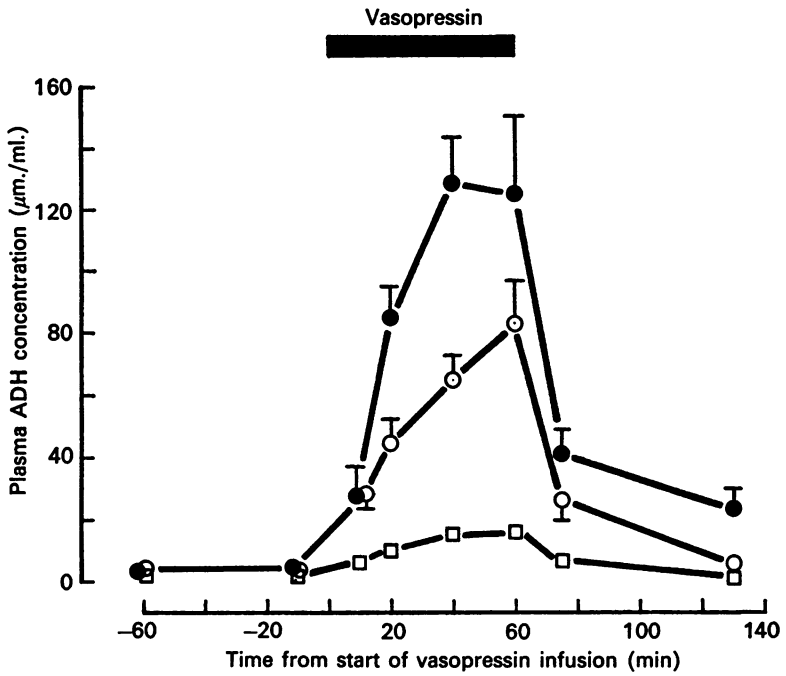

Fig. 6. Plasma vasopressin levels before, during and after the I.V. infusion of vasopressin to foetal lambs. The results are given as mean ± S.E. for five infusions at 9·0 m-u./min (●), four infusions at 4·5 m-u./min (○), and two at 2·25 m-u./min (□).

In one experiment, maternal plasma vasopressin concentrations were measured before, during and after the infusion of vasopressin to the foetus. The maternal levels remained undetectable (< 5 μu./ml.) throughout the experiment. Similarly, in two experiments in which vasopressin was infused to one foetus of twin preparations, plasma vasopressin levels remained undetectable in the uninfused twin.

In three non-pregnant ewes vasopressin was infused intravenously on seven occasions at rates of 185·3 (n = 2), 92·7 (n = 3), 46·3 (n = 1) and 23·1 (n = 1) m-u./min or from 0·40 to 2·90 m-u./kg. min. The changes in plasma vasopressin concentrations were similar to those observed during infusions to the foetal lambs, except that the maximum concentrations achieved were somewhat higher (40–468 μu./ml.). The rate of plasma clearance of the hormone varied from 6·2 to 10·1 ml./min.kg with a mean of 8·1 ml./min.kg ± 0·5 (n = 7). The value was significantly lower (P < 0·001) than the mean value determined for the foetus.

In one experiment, chlorbutol (100 mg/ml. in 0·9% NaCl), the preservative agent

in Pitressin, was infused intravenously to a foetus at the same rate as was given during the highest rate of Pitressin infusion. There were no changes in foetal carotid arterial pressure or heart rate. In contrast, the infusion of vasopressin consistently resulted in hypertension and bradycardia (Fig. 7). The rise in arterial pressure (27–43 %) was similar at the three rates of infusion, while the fall in heart rate (14–41 %) was greater with increasing rates of hormone administration. In the two experiments in which vasopressin was infused to a vagotomized foetus, arterial pressure rose with no change in heart rate.

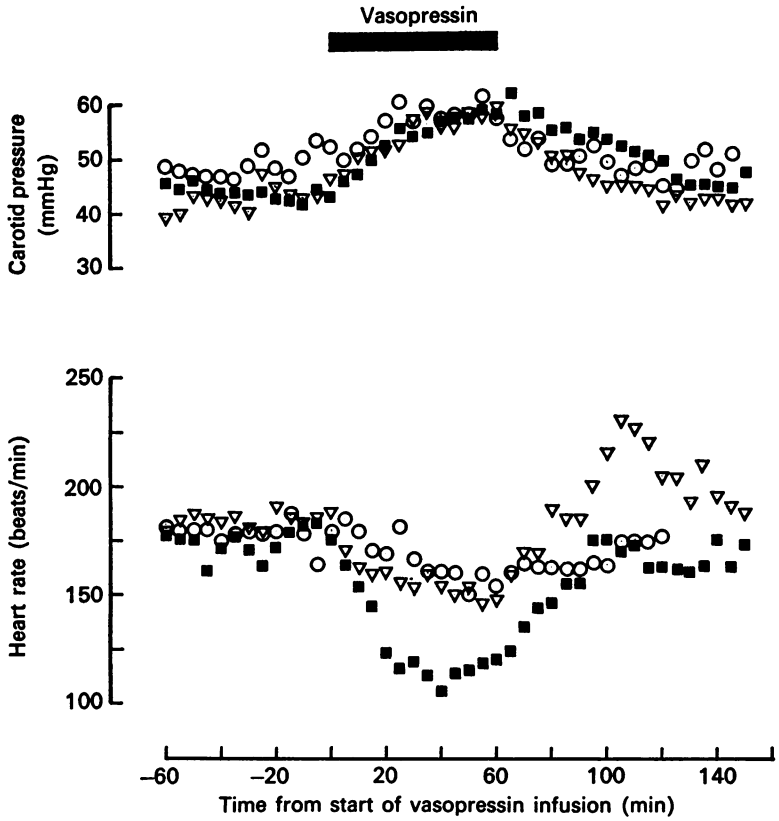

Fig. 7. Arterial pressure and heart rate before, during and after the intravenous infusion of vasopressin to foetal lambs at 9·0 m-u./min (■, n = 5), 4·5 m-u./min (▽, n = 4) and 2·25 m-u./min (○, n = 2).

Infusion of vasopressin to the three non-pregnant ewes also was accompanied by an increase in arterial pressure (9–16 %) and a fall in heart rate (20–45 %). However, the hypertension was relatively much less than in the foetus. This is illustrated in Fig. 8, where the plasma vasopressin concentration in individual foetal and adult plasma samples collected during the infusions are plotted on a logarithmic scale against the percentage change in mean arterial pressure from control values. In the foetus a 10 % increase in arterial pressure occurred when plasma vasopressin levels were relatively low (5 μu./ml.), while in the ewe, a similar hypertension did not occur until the hormone levels were much higher ($\sim$ 200 μu./ml.). Linear regression analysis of the

data present in Fig. 8 yielded coefficients of $16{\cdot}0 \pm 4{\cdot}9$ ($n = 48$) for the foetus and $6{\cdot}7 \pm 3{\cdot}0$ ($n = 32$) for the ewe. These were statistically different ($P < 0{\cdot}05$).

The changes in arterial P_{O_2}, P_{CO_2} and pH during the infusions of vasopressin to the ewes were slight and inconsistent. Packed cell volume fell continuously, from a mean of $24{\cdot}6 \pm 1{\cdot}1\%$ ($n = 7$) to a low value at $20{\cdot}0 \pm 0{\cdot}7\%$ 15 min after stopping the infusion. By 70 min the mean value had risen to $21{\cdot}5\% \pm 0{\cdot}4\%$. During the infusions to the foetal lambs, carotid arterial oxygen tension rose from 20 to 23 mmHg; the mean rise in P_{O_2} ($2{\cdot}4 \pm 0{\cdot}9$ mmHg) was significantly different from zero ($P < 0{\cdot}05$).

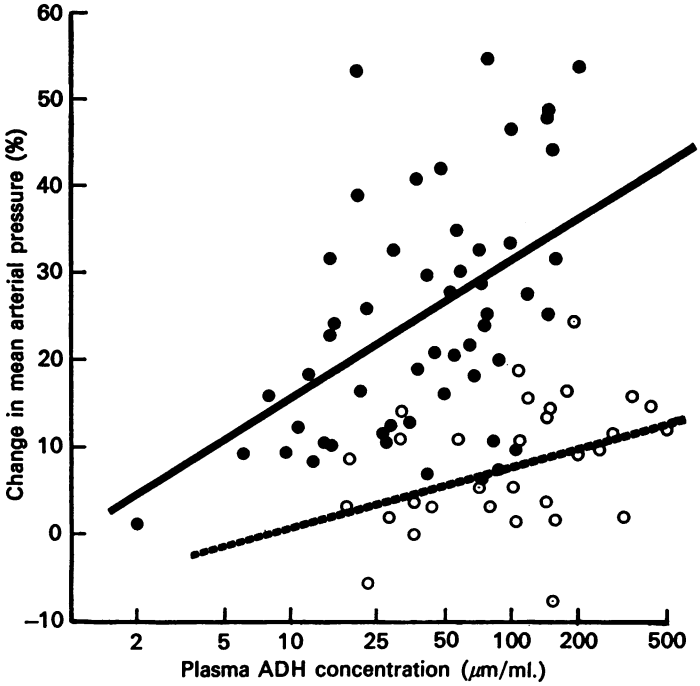

Fig. 8. Relationship between percentage change in arterial pressure and plasma vasopressin concentration during the I.V. infusion of vasopressin in eleven experiments on nine foetal lambs (— ● —) and in seven experiments on four non-pregnant ewes (- - - - ○ - - - -). The lines were fitted by regression analysis; The equation for the foetal experiments is: percentage change in arterial pressure $= -0{\cdot}4 + (16{\cdot}0 \pm 4{\cdot}9)$ (log plasma ADH concentration), ($P < 0{\cdot}002$) and for the adult experiments is: percentage change in arterial pressure $= -5{\cdot}4 + (6{\cdot}7 \pm 2{\cdot}9)$ (log plasma ADH concentration) ($P < 0{\cdot}05$).

The P_{CO_2} declined slightly from 45 to 43 mmHg, and there was a significant decline ($P < 0{\cdot}05$) in arterial pH from 7·36 to a low value at 7·34, 15 min after the end of the infusion. While these changes in foetal blood gas values and pH were slight, they were consistent and were not accompanied by similar changes in the ewe. Foetal arterial P_{O_2} and P_{CO_2} were restored to control levels by the end of the experiment, but pH remained low (7·34) for several hours.

DISCUSSION

Resting plasma concentrations of vasopressin in the foetus

The findings of this investigation are in accord with the results from other studies employing bio-assay or radio-immunoassay techniques, all of which report plasma vasopressin levels in the foetal lamb between 0·5 and less than 3·5 μu./ml. (Alexander *et al.* 1976; Stark *et al.* 1977; Leake *et al.* 1977). These concentrations are similar to those found in the ewe and in other adult mammals. In the present and previous investigations, maternal and foetal plasma vasopressin concentrations were found to vary independently, suggesting that the ovine placenta is effectively impermeable to the hormone. Thus the similarity in circulating ADH levels in the ewe and foetus is unlikely to be due to equilibration across the placenta. Modest elevations (mean 10 μu./ml.) in plasma vasopressin levels were observed in 15% of the samples collected from the intact foetuses. Such increases were observed only very occasionally in any one foetus and were not associated with deviations from normal values of arterial blood gases, blood pressure or heart rate. They may have been the result of periodic increases in foetal plasma osmolality as suggested by Mellor & Slater (1973). As the rate of clearance of vasopressin from the foetal circulation is nearly three times that in adult ewes, the rate of secretion of vasopressin in the foetus must be relatively greater than during adult life. Other observations on exteriorized foetal lambs suggest that the placenta is a major site for clearance of vasopressin in the foetus (Jones & Rurak 1976*a*), so that the presence of this organ may account for the high clearance rate.

Hypoxaemia and acidaemia

Plasma vasopressin levels were elevated in foetuses made hypoxic for 1 hr and during spontaneous asphyxial episodes; in the latter situation the levels were maintained high for hours and days. Reduction in foetal oxygen supply thus appears to be a potent stimulus for vasopressin release. The results from vagotomized lambs suggest that this increase in vasopressin release involves peripheral receptors whose afferent fibres run in the vagus nerves. Aortic chemoreceptors may play a role, since they are important in the cardiovascular responses to hypoxaemia in the lamb foetus (Dawes, Lewis, Milligan, Roach & Talner, 1968; Dawes, Duncan, Lewis, Merlet, Owen-Thomas & Reeves, 1969*a*, *b*). In the adult, the carotid chemoreceptors appear to be able to stimulate vasopressin release, since in anaesthetized dogs, perfusion of one carotid sinus region with deoxygenated blood results in increased circulating vasopressin levels (Share & Levy, 1966). The involvement of the carotid bodies in the foetal vasopressin rise is unlikely, since these receptors appear to be relatively insensitive to hypoxaemia in the foetal lamb (Dawes *et al.* 1969*a*, *b*; Biscoe, Purves & Sampson, 1969). The failure of vagal nerve section completely to block the rise in foetal vasopressin levels during hypoxaemia indicates that other peripheral or central mechanisms play a role in the response. In the lamb foetus, ligation of both carotid arteries at 70–80 days gestation dramatically reduces forebrain development in a manner similar to that described for foetal rhesus monkeys (Myers, 1969). In such preparations low (1–2 μu./ml.) circulating levels of vasopressin can be measured, but these are not increased during hypoxaemia (J. S. Robinson, D. W. Rurak & G. D. Thorburn,

unpublished data). The lack of response could be due to the disruption of ascending afferent pathways from lower brain-stem centres or peripheral receptors, or could result from the absence of certain forebrain structures.

The pathways involved in vasopressin release during foetal hypoxaemia may be fairly specific since oxytocin secretion does not appear increased in this situation (Alexander *et al.* 1973). Moreover, while foetal plasma ACTH levels are increased during hypoxaemia, the concentrations do not relate to the fall in arterial pH (Boddy, Jones, Mantell, Ratcliffe & Robinson, 1974), and the rise is not reduced in foetuses subject to section of the cervical vagosympathetic trunks (Jones & Rurak, 1976*b*). The foetal plasma concentration of prolactin is not affected by hypoxaemia (McMillen, Jenkin, Robinson, Kingston & Thorburn, 1978).

Hypoxaemia in the ewe had little effect on her plasma vasopressin levels. In only one of five experiments did these rise, and the largest increase occurred early and was not maintained for the hour's hypoxia. This increase may have resulted from emotional disturbance, rather than the reduced blood oxygen tension. Ryden & Verney (1938) observed inhibition of a water diuresis in conscious dogs exposed to disturbances such as sounding an automobile horn. Plasma vasopressin levels rise in anaesthetized dogs made hypoxaemic (P_{a,O_2} circa 40 mmHg, Forsling & Ullman, 1974) and perfusion of the carotid sinus region with deoxygenated blood also results in increased hormone concentrations, but only when aortic blood pressure is held constant and hyperpnea is minimized (Share & Levy, 1966). In conscious men acclimatized to high altitude, breathing 10% oxygen for 4 hr does not result in increased plasma ADH concentration (Forsling & Milledge, 1977). It would appear that, in the conscious adult, hypoxaemia is a relatively ineffective stimulus for vasopressin release, in contrast to the situation in the foetus.

Cardiovascular effects of vasopressin

Intravenous infusion of vasopressin to adult ewes resulted in hypertension and bradycardia. The threshold plasma vasopressin level for the hypertensive response of 25 μu./ml. is in the range of concentrations observed in normal or pathological states. These findings are in accord with other recent studies, which demonstrate that in conscious adults modest elevations in circulating vasopressin levels can increase systemic arterial pressure (Szczepánska-Sadowska, 1973; Cowley, Monos & Guyton, 1974; Schmid, Abboud, Wendling, Ramberg, Mack, Heistad & Eckstein, 1974; Möhring, Möhring, Petrie & Haack, 1977). Thus there is increasing evidence for a role of vasopressin in normal cardiovascular control.

Of greater interest in the present investigation are the much larger cardiovascular effects in the foetal lamb. In comparison to the adult the dose–response curve for the hypertensive effects of vasopressin was shifted to the left and increased in slope. Thus measurable increases in foetal arterial pressure occurred with plasma vasopressin concentrations as low as 6 μu./ml. which is in the range of spontaneous elevations of plasma vasopressin levels observed in foetuses in good condition. Pressures up to 160% of control values were observed, which is unusual in the foetus, due to the presence of the low resistance, relatively unreactive placental vascular bed (Dawes, 1968). It is unlikely that placental vasoconstriction contributed to the hypertension, since during vasopressin infusions in exteriorized foetal lambs umbilical blood flow,

measured by an electromagnetic flowmeter on the common umbilical vein, increased in parallel with the increase in arterial pressure (unpublished observations). The degree of vasoconstriction in other foetal vascular beds must be intense to account for the large hypertension. Foetal arterial P_{O_2} increased and P_{CO_2} fell slightly during the infusions while pH exhibited a progressive decline which was greatest after stopping the infusion. The change in P_{O_2} and P_{CO_2} could have resulted from a decreased oxygen consumption (Dawes, 1968) and the acidaemia could be due to increased plasma lactate levels resulting from a rise in tissue anaerobic respiration. Corliss, McKenna, Sialer, O'Brien & Rowe (1968) observed a reduction in oxygen consumption and increase in blood lactate concentration in anaesthetized dogs infused with vasopressin at relatively high rates.

The increased hypertensive effects of vasopressin in the foetus could be the result of relatively ineffective baroreflex mechanisms, since these appear to be less powerful than in the adult (Shinebourne, Vapaarouri, Williams, Heymann & Rudolph, 1972; Faber, Green & Thornburg, 1974). In adults suppression of autonomic depressor mechanisms by pharmacological or surgical means potentiates the vasopressor actions of ADH (Nakano, 1967; Rocha e Silva & Rosenburg, 1969; Cowley *et al.* 1974). Alternatively vascular smooth muscle in the foetus could be more sensitive to vasopressin than in post-natal life. There are few comparisons of the vasopressor effects of other vasoactive substances in the foetus and adult. Jones & Robinson (1975) observed an increase in arterial pressure during adrenaline infusions to foetal lambs, while in adult ewes, infusions to achieve the same range of circulating adrenaline concentrations had no effect on blood pressure. And the cardiovascular effects of vasopressin and adrenaline, but not angiotensin, are greater in the new-born rabbit than in adults (Broughton-Pipkin, 1971).

In the present investigation, spontaneous episodes of hypoxaemia and acidaemia occurred in several foetuses, some of which died *in utero*. While it is likely that some of these hypoxic episodes resulted from surgery or post-operative infection, a reduction in oxygen supply is a serious danger faced by the foetus both during the course of gestation and during labour. As the plasma levels of vasopressin in the foetal lamb during hypoxaemia were in the range of those achieved during the vasopressin infusions, it is likely that the hormone contributes to the increase in arterial pressure that occurs in this situation. Vasopressin may act in concert with altered autonomic tone (Dawes *et al.* 1968, 1969*a*, *b*) and increased circulating catecholamine concentrations (Jones & Robinson, 1975) to reduce blood flow to peripheral vascular beds, thereby maintaining an adequate oxygen supply to the heart and brain. Moreover, as a consequence of the enhanced cardiovascular effects of ADH in the foetus, even modest elevations in circulating hormone levels, perhaps resulting from minor perturbations in blood oxygen tension or increases in plasma osmolality, could exert circulatory actions. In the adult mammal, the most important function of vasopressin is to maintain the tonicity of body fluids by controlling renal water excretion. While vasopressin can promote solute free water reabsorption in the foetal lamb kidney (D. W. Rurak & D. Walker, unpublished data), the cardiovascular effects of the hormone may be of greater importance in prenatal life.

This work was supported by the Medical Research Council; personal support was provided by the Association of Commonwealth Universities and the Medical Research Council of Canada. I am grateful to Professor G. S. Dawes for his advice and to A. Stevens and N. Green for expert technical assistance.

REFERENCES

ALEXANDER, D. P., BASHORE, R. A., BRITTON, H. G. & FORSLING, M. L. (1976). Antidiuretic hormone and oxytocin release and antidiuretic hormone turnover in the fetus, lamb and ewe. *Biologia neonat.* **30**, 80–87.

ALEXANDER, D. P., BRITTON, H. G., FORSLING, M. L., NIXON, D. A. & RATCLIFFE, J. G. (1973). Adrenocorticotrophin and vasopressin in foetal sheep and the response to stress. In *The Endocrinology of Pregnancy and Parturition, Experimental Studies in the Sheep*, ed. PIERREPOINT, C. G., pp. 112–125. Cardiff: Alpha Omega.

BAILEY, N. T. J. (1959). *Statistical Methods in Biology*. London: English Universities Press.

BANGHAM, D. R. & MUSSETT, M. V. (1958). Third international standard for posterior pituitary. *Bull. Wld Hlth Org.* **19**, 325–340.

BISCOE, T. J., PURVES, M. J. & SAMPSON, S. R. (1969). Types of nervous activity which may be recorded from the carotid sinus nerve in the sheep foetus *J. Physiol.* **202**, 1–23.

BISSET, G. W. (1962). Effect of tyrosinase preparations on oxytocin, vasopressin and bradykinin. *Br. J. Pharmac. Chemother.* **18**, 405–420.

BISSET, G. W., HILTON, S. M. & POISNER, A. M. (1967). Hypothalamic pathways for independent release of vasopressin and oxytocin. *Proc. R. Soc.* B **166**, 422–442.

BODDY, K., DAWES, G. S., FISHER, R., PINTER, S. & ROBINSON, J. S. (1974). Foetal respiratory movements, electrocortical and cardiovascular responses to hypoxaemia and hypercapnia in sheep. *J. Physiol.* **243**, 599–618.

BODDY, K., JONES, C. T., MANTELL, C., RATCLIFFE, J. G. & ROBINSON, J. S. (1974). Changes in plasma ACTH and corticosteroid concentrations of the maternal and fetal sheep during hypoxia. *Endocrinology* **94**, 588–591.

BROUGHTON-PIPKIN, F. B. (1971). Cardiovascular responses in rabbits of different ages to hypertensin and adrenaline. *Q. Jl exp. Physiol.* **56**, 210–220.

CORLISS, R. J., MCKENNA, D. H., SIALER, S., O'BRIEN, G. S. & ROWE, G. G. (1968). Systemic and coronary haemodynamic effects of vasopressin. *Am. J. med. Sci.* **256**, 293–299.

COWLEY, A. W. JR, MONOS, E. & GUYTON, A. C. (1974). Interaction of vasopressin and the baroreceptor reflex system in the regulation of arterial blood pressure in the dog. *Circulation Res.* **34**, 505–514.

DAWES, G. S. (1968). *Foetal and Neonatal Physiology*. Chicago: Year Book Medical.

DAWES, G. S., DUNCAN, J. L. B., LEWIS, B. V., MERLET, C. L., OWEN-THOMAS, J. B. & REEVES, J. T. (1969*a*). Hypoxaemia and aortic chemoreceptor function in foetal lambs. *J. Physiol.* **201**, 105–116.

DAWES, G. S., DUNCAN, S. L. B., LEWIS, B. V., MERLET, C. L., OWEN-THOMAS, J. B. & REEVES, J. T. (1969*b*). Cyanide stimulation of the systemic arterial chemoreceptors in foetal lambs. *J. Physiol.* **201**, 117–128.

DAWES, G. S., FOX, H. E., LEDUC, B. M., LIGGINS, G. C. & RICHARDS, R. T. (1972). Respiratory movements and rapid eye movement sleep in the foetal lamb. *J. Physiol.* **220**, 119–143.

DAWES, G. S., LEWIS, B. V., MILLIGAN, J. E., ROACH, M. R. & TALNER, N. S. (1968). Vasomotor responses in hind limbs of foetal and new-born lambs to asphyxia and aortic chemoreceptor stimulation. *J. Physiol.* **195**, 55–81.

DEKANSKI, J. (1952). The quantitative assay of vasopressin. *Br. J. Pharmac. Chemother.* **7**, 567–572.

DICKER, S. E. (1953). A method for the assay of very small amounts of antidiuretic activity with a note on the antidiuretic titre of rats' blood. *J. Physiol.* **122**, 149–157.

FABER, J. J., GREEN, T. J. & THORBURG, K. L. (1974). Arterial blood pressure in the unanaesthetized fetal lamb after changes in fetal blood volume and haematocrit. *Q. Jl exp. Physiol.* **59**, 241–255.

FORSLING, M. L. & MILLEDGE, J. S. (1977). Effect of hypoxia on vasopressin release in man. *J. Physiol.* **267**, 22–23*P*.

FORSLING, M. L. & ULLMANN, E. (1974). Release of vasopressin during hypoxia. *J. Physiol.* **241**, 35–36*P*.

GRESHAM, E. L., RANKIN, J. C., MAKOWSKI, E. L., MESCHIA, G. & BATTAGLIA, F. C. (1972). An evaluation of fetal renal function in a chronic sheep preparation. *J. clin. Invest.* **51**, 149–156.

HOLTON, P. (1948). A modification of the method of Dale and Laidlaw for standardization of posterior pituitary extract. *Br. J. Pharmac. Chemother.* **3**, 328–334.

JONES, C. T. & ROBINSON, R. O. (1975). Plasma catecholamines in fetal and adult sheep. *J. Physiol.* **248**, 15–33.

JONES, C. T., & RURAK, D. (1976*a*). The distribution and clearances of hormones and metabolites in the circulation of the foetal sheep. *Q. Jl exp. Physiol.* **61**, 287–295.

JONES, C. T. & RURAK, D. (1976*b*). Absence of a correlation between the secretion of adrenocorticotrophin and vassopressin in foetal sheep. *J. Endocr.* **70**, 327–328.

LEAKE, R. D., WEITZMAN, R. E. & FISHER, D. E. (1977). Maternal fetal osmolar homeostasis: fetal posterior pituitary autonomy. *Pediatric Res.* **11** (4), 408.

MCMILLEN, I. C., JENKIN, G., ROBINSON, J. S., KINGSTON, E. J. & THORBURN, G. D. (1978). Prolactin concentrations in foetal and neonatal sheep; the effect of thyrotrophin releasing factor, hypoxia and chlorpromazine. *J. Endocr.* (In the Press.)

MELLOR, D. J. & SLATER, J. S. (1973). The composition of maternal plasma and foetal urine after feeding and drinking in chronically catheterized ewes during the last two months of pregnancy. *J. Physiol.* **234**, 519–531

MÖHRING, J., MÖHRING, B., PETRI, M. & HAACK, D. (1977). Vasopressor role of ADH in the pathogenesis of malignant DOC hypertension. *Am. J. Physiol.* **232**, F260–F269.

MYERS, R. E. (1969). Brain pathology following fetal vascular occlusion: an experimental study. *Investve Ophth.* **8**, 41–50.

NAKANO, J. (1967). Studies on the cardiovascular effects of synthetic vasopressin. *J. Pharmac. exp. Ther.* **157**, 19–31.

ROCHA E SILVA, M. JR & ROSENBURG, M. (1969). The release of vasopressin in response to haemorrhage and its role in the mechanism of blood pressure regulation. *J. Physiol.* **202**, 535–557.

RURAK, D. W. (1976). Plasma vasopressin in foetal lambs. *J. Physiol.* **256**, 36–37*P*.

RYDEN, A. & VERNEY, E. B. (1938). The inhibition of water diuresis by emotional stress and muscular exercise. *Q. Jl exp. Physiol.* **27**, 343–374.

SCHMID, P. G., ABBOUD, F. M., WENDLING, M. G., RAMBERG, E. S., MACK, A. L., HEISTAD, D. D. & ECKSTEIN, J. W. (1974). Regional vascular effects of vasopressin: plasma levels and circulatory responses. *Am. J. Physiol.* **277**, 998–1004.

SHARE, L. & LEVY, M. N. (1966). Effects of carotid chemoreceptor stimulation on plasma antidiuretic hormone. *Am. J. Physiol.* **210**, 157–161.

SHINEBOURNE, E. A., VAPAAROURI, E. K., WILLIAMS, R. L., HEYMANN, M. A. & RUDOLPH, A. M. (1972). Development of baroreflex activity in unanaesthetized fetal and neonatal lambs. *Circulation Res.* **31**, 710–718.

STARK, R., HUSSAIN, K., DANIEL, S., MILLIEZ, J., MORISHIMA, L. & JAMES, L. S. (1977). Characteristics of vasopressin (AVP) release during adrenocorticotrophin (ACTH) induced parturition in the lamb. *Pediatric Res.* **11**, 412.

SZCZEPÁNSKA-SADOWSKA, E. (1973). Hemodynamic effects of a moderate increase of plasma vasopressin level in conscious dogs. *Pflügers Arch.* **338**, 313–322.

VOGT, M. (1953). Vasopressor, antidiuretic and oxytocic activities of extracts of the dogs hypothalamus. *Br. J. Pharmac. Chemother.* **8**, 193–196.