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CYANIDE STIMULATION OF THE SYSTEMIC ARTERIAL CHEMORECEPTORS IN FOETAL LAMBS

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SUMMARY

1. In foetal lambs, delivered by Caesarean section under light chloralose anaesthesia, injection of sodium cyanide into the left atrium or ascending aorta caused a rise of arterial pressure and femoral vasoconstriction. The response to 0·77 mg/kg was barely present at 0·6 of term; by 0·8 of term there was a large response to one third of this dose.

2. The cardiovascular response to cyanide injection into the left atrium or ascending aorta was diminished either by section of the vagi or by carotid denervation, and was abolished by cutting both sets of nerves.

3. Injection of sodium cyanide into both common carotids simultaneously caused a substantial cardiovascular response and often a respiratory effort, abolished by carotid denervation, whereas injection into a single carotid rarely caused an effect.

4. It is concluded that the carotid chemoreceptors in mature foetal lambs can be excited by a stimulus of sufficient intensity, even though they do not respond to moderate hypoxaemia.

INTRODUCTION

In a previous paper (Dawes, Lewis, Milligan, Roach & Talner, 1968) it was concluded that the cardiovascular response on injection of sodium cyanide into the left atrium of mature foetal lambs was largely due to stimulation of the aortic chemoreceptors. There was no evidence of stimulation of the carotid chemoreceptors even on injection of a relatively large

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dose into one common carotid artery. However, subsequent experiments have shown that the effect of injection of cyanide into the left atrium or ascending aorta is much decreased by carotid denervation, and that injection of cyanide into both common carotids simultaneously excites a large cardiovascular response and, if anaesthesia is light, a respiratory effort or efforts. Hence the carotid bodies of the foetal lamb can be shown to be excited by cyanide under appropriate conditions even though, as the preceding paper (Dawes, Duncan, Lewis, Merlet, Owen-Thomas & Reeves, 1969) shows, they are less sensitive to hypoxaemia than the aortic bodies.

METHODS

New observations were made on thirty-two foetal lambs of 89–140 days gestation age. (term is ~ 147 days), weighing 0·56–4·8 kg under light chloralose anaesthesia administered to the maternal ewe (British Drug Houses; 15–30 mg/kg I.V. initially). Further small doses of chloralose were given at intervals of 1–2 hr. The lambs were wholly delivered from the uterus, and were placed on a warmed table alongside the mother with intact umbilical cords. Exposed surfaces were covered and a long saline-filled open-ended cannula was inserted into the trachea. The results also include observations on twelve foetal lambs, studied previously under similar conditions (Dawes *et al.* 1968).

The external flow circuits, recording and other methods were as described in the preceding paper (Dawes *et al.* 1969). Blood samples were taken from a brachial artery of the foetus and were analysed as already described. For recording respiratory movements a catheter was either passed into the thoracic oesophagus or tied into the pleural cavity. The movement of fluid in the tracheal cannula was displayed from a light weight volume recorder (Campbell, Dawes, Fishman, Hyman & James, 1966).

Injections of sodium cyanide (4 mg/ml. NaCl solution, 0·9 %, w/v) were made into the left atrium either through a polyethylene catheter introduced via a femoral vein and the foramen ovale, or directly through the atrial wall, after opening the left side of the chest between the third and fourth ribs. Injections were made into the ascending aorta through a fine catheter introduced via a lingual artery. This was advanced until it reached the aortic valves (as indicated by the appearance of cardiac pulsations) and then withdrawn 1–1·5 cm; its position was verified at autopsy. The end of this aortic catheter was blocked and several fine side holes were made near the tip. For injections into common carotid arteries a fine catheter was either introduced through a thyroid branch or was passed down from a lingual artery. Injections of saline solution alone (NaCl, 0·9 %, w/v) in volumes of 0·2–0·4 ml. caused no cardiovascular or respiratory effect.

RESULTS

The effect on the cardiovascular system of injecting sodium cyanide into the ascending aorta, just above the valves, was similar to that of left atrial injections (Table 1) and therefore, in subsequent analyses, the results have been combined. The former procedure has the advantage that the chest need not be opened for insertion of a catheter, or for verification of its position until the end of the experiment.

Table 2 shows the effects of cyanide injections into the left atrium or

ascending aorta in lambs of different ages. The foetal blood gas values were approximately the same. The dose of cyanide was also about the same for the first three groups of lambs (age range 105–140 days gestation). But for the youngest group (89–91 days gestation) the dose had to be increased about threefold in order to obtain a response; even so the rise in arterial pressure and fall in femoral flow were much reduced, but the fall in heart rate was relatively large.

TABLE 1. Effects of cyanide injection into the ascending aorta or left atrium in lambs of 120–140 days gestation

Sodium cyanide injection into	Dose of cyanide (mg/kg)	No. of lambs	Initial arterial pressure (mm Hg)	Change	Initial femoral flow (ml./ 100 g.min)	Change
Ascending aorta	0·30 ± 0·03	12	49·7 ± 2·6	+8·3 ± 0·7	4·3 ± 0·5	−1·6 ± 0·3
Left atrium	0·28 ± 0·03	20	47·0 ± 1·6	+9·7 ± 1·2	4·1 ± 0·5	−1·5 ± 0·2

Means ± S.E.

The arterial pressure–femoral flow results of Table 2 are illustrated in Fig. 1 on a pressure–flow diagram, in which observations on lambs of 120–140 days gestational age are combined. For orientation three pressure–flow curves are shown, one on a single lamb at 95 days gestational age, and the other two mean pressure–flow curves on twelve lambs of 132–141 days gestational age at P_{O_2} values of 35 and 15 mm Hg. It is evident that the effect of cyanide injection increases with gestational age from 90 to 120 days gestation. Also after 120 days the cardiovascular effects of injection of 0·28 mg/kg cyanide into the left atrium or ascending aorta are approximately equal to those caused by relative foetal hypoxaemia (P_{O_2} 35–15 mm Hg) induced by giving the maternal ewe low oxygen mixtures.

The effect of carotid denervation and vagotomy. In a previous paper (Dawes *et al.* 1968) it was concluded that none of the cardiovascular effects of cyanide, injected into the left atrium, were likely to result from excitation of carotid chemoreceptors. However, early in the course of the present experiments, observations on two foetal lambs of 137–138 days gestation showed that the not inconsiderable cardiovascular response remaining after bilateral section of the cervical vago-sympathetic trunks was wholly abolished by carotid denervation. The results of one of these experiments are illustrated in Fig. 2. Section of the cervical vagi abolished the bradycardia on cyanide injection, probably due to interruption of efferent cardioinhibitory fibres, but there was still a rise of arterial pressure and femoral vasoconstriction. After carotid denervation, injection of cyanide into the ascending aorta caused no alteration in arterial pressure and the fall in femoral flow was abolished. There was a late increase in femoral flow,

TABLE 2. Effects of injection of cyanide into foetal lambs of different gestational ages

Gestation age (days)	No. of lambs	Foetal arterial P_{O_2} (mm Hg)	Foetal arterial P_{CO_2} (mm Hg)	Foetal arterial pH	Initial values of: Arterial pressure (mm Hg)	Initial values of: Femoral flow (ml./100 g.min)	Initial values of: Heart rate (beats/min)	Dose of NaCN (mg/kg)	Consequent change in: Arterial pressure (mm Hg)	Consequent change in: Femoral flow (ml./100 g.min)	Consequent change in: Heart rate (beats/min)
130–140	16	30 ±1·7	48 ±1·9	7·32 ±0·01	49 ±1·7	4·4 ±0·4	193 ±10	0·29* ±0·03	+8·3† ±1·1	−1·7 ±0·2	−45 ±10
120–128	16	28 ±1·3	48 ±1·1	7·35 ±0·01	47 ±2·2	4·0 ±0·5	197 ± 8	0·28* ±0·03	+10·1† ±1·1	−1·3 ±0·2	−36 ±12
105–114	7	29 ±2·2	43 ±3·5	7·29 ±0·02	38 ±0·9	5·9 ±1·0	203 ±15	0·25† ±0·04	+5·3 ±0·9	−1·3 ±0·2	−15 ±17
89–91	5	29 ±4·3	48 ±3·2	7·34 ±0·04	33 ±1·9	5·7 ±0·9	211 ±22	0·77† ±0·05	+1·4 ±0·6	−0·3 ±0·4	−27 ±11

Means ±S.E. † Sodium cyanide was injected into the left atrium only, * or into either left atrium or ascending aorta.

attributed to a direct effect of cyanide on the femoral vascular bed (similar to that which was observed on close arterial injection).

The effects of bilateral section of the cervical sympathetic nerves and/or

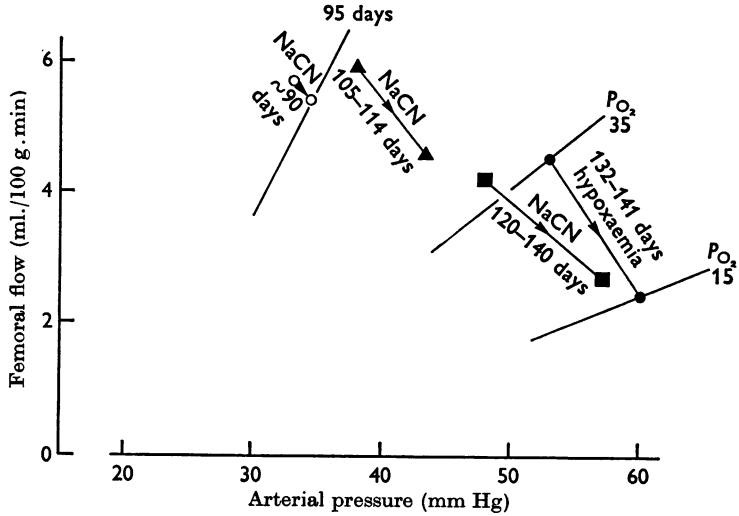

Fig. 1. Arterial pressure–femoral blood flow (per 100 g leg weight) diagram in foetal lambs to show the mean effects of injection into the left atrium or ascending aorta of sodium cyanide 0·25–0·28 mg/kg at 120–140 days gestation (■) or 105–114 days (▲) and of 0·77 mg/kg at 89–91 days (○). Pressure–flow curves are shown for a lamb at 95 days, and for twelve lambs at 132–141 days at a P_{O_2} of 35 and 15 mm Hg. The arrows indicate the direction of change on injection of cyanide or hypoxaemia.

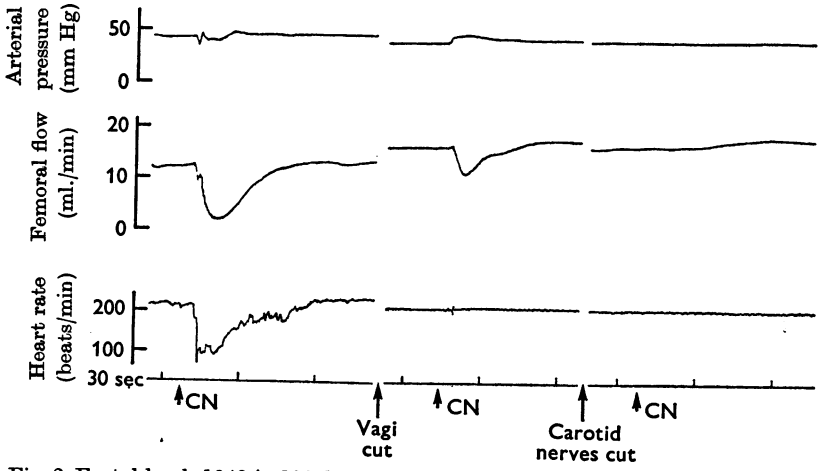

Fig. 2. Foetal lamb 1842A, 138 days gestation, 3·8 kg. At each small arrow 0·8 mg sodium cyanide solution (0·2 ml.) was injected into the ascending aorta before and after section of the cervical vagi and carotid nerves.

of carotid denervation were then examined in lambs whose vagi were intact. Figure 3 illustrates an experiment on the twin lamb to that used for illustration in Fig. 2. Section of the cervical sympathetics in the lower part of the neck (before they joined the vagi) did not reduce the rise in arterial pressure on cyanide injection. The fall in femoral flow at first sight seems less, but when examined on a pressure–flow diagram the change is insignificant. Similar results were observed in two other lambs of 138–140

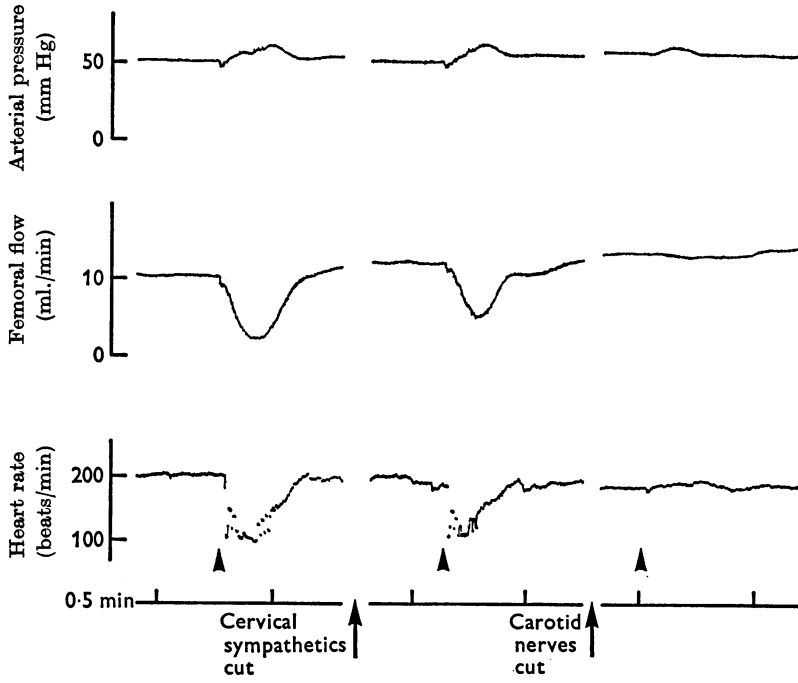

Fig. 3. Foetal lamb 1842B, 138 days gestation, 3·2 kg, twin of that used for Fig. 2. At each small arrow 0·8 mg. sodium cyanide (0·2 ml.) was injected into the ascending aorta before and after section of the cervical sympathetic and carotid nerves; the vagi were intact.

days gestation. Section of the carotid nerves much reduced all the cardiovascular effects of cyanide injection into the ascending aorta, either after cutting the cervical sympathetics (as in Fig. 3) or when they were intact.

The mean results of carotid denervation, as compared with section of the cervical vago-sympathetic trunks or vagi (combined) in lambs of 120–140 days gestation are shown in Table 3. Measurements on lambs with intact nerves were not significantly different, and so these control observations have been combined. Either section of the vagi or of the carotid nerves (separately) virtually abolished the bradycardia on cyanide injection, and much reduced the rise in arterial pressure and the fall in

femoral flow. However, when the results are inspected on a pressure–flow diagram (Fig. 4) there is a difference. Two pressure–flow lines (from twelve lambs of 132–141 days gestation at mean P_{O_2} values of 41 and 15 mm Hg) have been included for orientation. Section of the cervical vagi caused a small fall of arterial pressure and femoral vasodilatation (○⟶△); subsequent injection of cyanide into the left atrium or ascending aorta caused a smaller change (△⟶▲) than previously (○⟶●). After section of the carotid nerves there was a rise of arterial pressure and

TABLE 3. Effects of injection of sodium cyanide (0·27 ± 0·03 mg/kg) into left atrium or ascending aorta in lambs of 120–140 days gestation before nerve section and after either vagotomy or carotid denervation

	No. of lambs	Arterial pressure (mm Hg)	Femoral flow (ml./100 g.min)	Heart rate (beats/min)
Before nerve section	21	48·6 ± 1·8	4·06 ± 0·39	194 ± 7
Change on NaCN injection	—	+7·8 ± 0·9	−1·60 ± 0·21	−45 ± 9
After vagotomy	14	43·9 ± 2·1	4·18 ± 0·51	186 ± 9
Change on NaCN injection	—	+3·1 ± 0·7	−0·30 ± 0·15	+6 ± 3
After carotid denervation	7	59·1 ± 3·1	3·04 ± 0·41	196 ± 12
Change on NaCN injection	—	+2·5 ± 1·4	−0·45 ± 0·21	−4 ± 10

Means ± S.E. Either the cervical vago-sympathetic nerves or the carotid nerves were cut in each lamb.

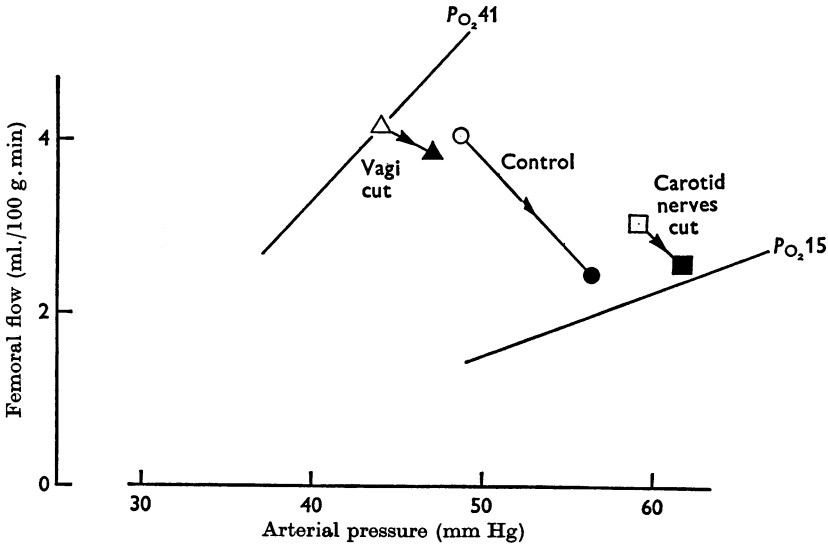

Fig. 4. Arterial pressure–femoral blood flow (per 100 g leg weight) diagram in foetal lambs of 120–140 days gestation to show the mean effects of injection into the left atrium or ascending aorta of sodium cyanide 0·27 mg/kg before nerve section (○, ●) and after cutting the carotid nerves (□, ■) or vagi (△, ▲), from the data of Table 3. Two pressure–flow lines are shown at mean arterial P_{O_2} values of 41 and 15 mm Hg.

femoral vasoconstriction (○⟶□), so that the subsequent response to cyanide injection (□⟶■) is difficult to interpret.

After carotid denervation there was still a considerable, but reduced, rise of arterial pressure and fall of femoral flow on injection of sodium cyanide (0·16–0·19 mg/kg) in two of seven lambs, and in the remainder also on doubling the dose previously used (to 0·4–0·8 mg/kg). But whereas before carotid denervation, injection of cyanide into the left atrium or

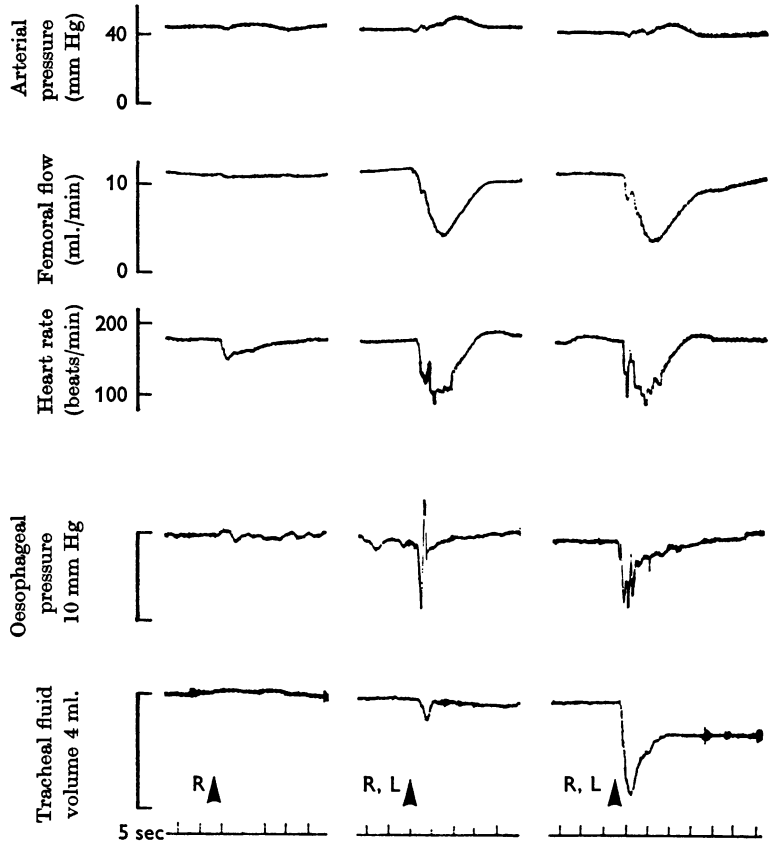

Fig. 5. Foetal lamb, 137 days gestation, 2·2 kg. The effects are shown of the injection of 0·4 mg sodium cyanide either into the right common carotid only (R) or into both carotids simultaneously (R, L).

ascending aorta caused predominantly a fall of heart rate, afterwards it usually caused a small rise. This rise of heart rate, together with the accompanying rise of arterial pressure and femoral vasoconstriction, was abolished by cutting the vagi.

The effects of carotid injection of cyanide. Injection of cyanide into either carotid artery alone rarely caused any substantial cardiovascular effect.

But when the dose which was effective on left atrial or ascending aorta injection was divided into two, and was injected simultaneously into both carotids, this caused a rise of arterial pressure, and a fall in femoral flow and heart rate (Fig. 5). The effects on the arterial pressure and femoral flow were on average less than on injection of the same total dose into the left atrium or ascending aorta (compare Tables 3 and 4). The effective concentration of cyanide on intracarotid injection must have been greater than on atrial injection by about fivefold (since carotid flow is only about a fifth of left ventricular output in mature foetal lambs). Section of the carotid nerves abolished the response to bilateral simultaneous carotid injection of cyanide.

TABLE 4. Effects of injection of equal quantities of sodium cyanide ($0{\cdot}30 \pm 0{\cdot}04$ mg/kg; total dose) simultaneously into both common carotid arteries in eight lambs of 123–140 days gestation

	Arterial pressure (mm Hg)	Femoral flow (ml./100 g.min)	Heart rate (beats/min)
	$46{\cdot}4 \pm 2{\cdot}9$	$4{\cdot}44 \pm 0{\cdot}55$	184 ± 15
Change on NaCN injection	$+5{\cdot}6 \pm 1{\cdot}2$	$-1{\cdot}37 \pm 0{\cdot}25$	-48 ± 9

Means ± S.E.

Respiratory movements. In previous experiments, in which injection of cyanide was rarely seen to cause respiratory effects, the dose of chloralose used was 30 mg/kg (Dawes *et al.* 1968). In the present series it was less ($20 \pm 1{\cdot}4$, S.E., mg/kg), the cardiovascular responses were the same size, but respiratory efforts were more often observed. In thirteen of fourteen lambs in which pleural or oesophageal pressure was measured, injection of sodium cyanide ($0{\cdot}27 \pm 0{\cdot}02$ mg/kg) into the left atrium, ascending aorta or both carotids caused an inspiratory effort or brief succession of efforts, which were sometimes sufficient to move a small quantity of fluid into the trachea (Fig. 5). These movements were obtained more regularly as the anaesthesia became lighter, or on application of cold (to the lamb's trunk and hind limbs by a fan and/or ice cubes). When cold had induced quiet regular liquid breathing movements (Dawes, 1968) administration of cyanide caused these to become faster and deeper. The effect of cyanide in exciting respiratory movements persisted after section of the cervical sympathetic and/or vagi, but was abolished by carotid denervation.

After section of the vagi and carotid nerves, asphyxia caused by tying the umbilical cord induced, after an interval of up to a minute, a series of deep gasping efforts similar to those seen in lambs with nerves intact, lasting for 4–5 min in lambs whose rectal temperature was 40–41·5 °C, and longer when it was lower.

DISCUSSION

The results of cyanide injection in lambs of different gestational ages correspond closely with the effects of hypoxaemia described in the preceding paper (Dawes *et al.* 1969). Cardiovascular effects are barely perceptible at 90 days gestational age ($\sim$ 0·6 of term), but after 120 days ($\sim$ 0·8 of term) there are large changes in response to a smaller dose of cyanide, as there are also to hypoxaemia.

In lambs of 120–140 days gestation the effects of injecting 0·28 ml./kg NaCN into the left atrium or ascending aorta closely resemble those of relative hypoxaemia (ascending aorta $P_{O_2} \rightarrow$ 15 mm Hg, Fig. 1). But whereas the effects of hypoxaemia are wholly attributable to stimulation of the aortic chemoreceptors, cyanide injection excites both the aortic and carotid bodies as shown by the effects of denervation. The circumstances differ. Hypoxaemia was induced by giving the mother a low oxygen mixture to breathe and waiting for 6–10 min; there was therefore a steady stimulus applied over a relatively long period of time. Injection of cyanide must have caused an intense but fleeting stimulus to the chemoreceptors, lasting but a few seconds. So the results are consistent with the conclusion that the aortic chemoreceptors are already active even at a P_{O_2} of 28–30 mm Hg, but can be excited to yet greater activity by cyanide, while the carotid chemoreceptors are relatively inactive and require a strong stimulus for excitation in the normal foetal lamb, perhaps stronger than that induced by decreasing the arterial P_{O_2} to $\sim$ 15 mm Hg. The fact that the carotid chemoreceptors can be excited in the foetal lamb by a sufficiently intense stimulus, e.g. by injection of cyanide into both carotids simultaneously, is interesting. Otherwise it might have been argued that the apparent failure of the carotid chemoreceptors to excite a response to hypoxaemia was due to inactivity at or below the normal foetal arterial P_{O_2} (i.e. at, say, $<$ 25 mm Hg). The alternative explanation now becomes more likely, that the range of the carotid chemoreceptors in the foetal lamb is set very low, at $<$ 15 mm Hg. Both views would be consistent with Purves & Biscoe's (1966) failure to record much chemoreceptor activity in the carotid nerves of foetal lambs near term. We are then left with the problem of explaining how it comes about that, shortly after birth, the carotid chemoreceptors are apparently normally functional in rabbits (Dawes & Mott, 1959; Blatteis, 1964) or lambs (Purves, 1966*a*, *b*).

Injection simultaneously into both carotids of doses of cyanide, which are just subthreshold on administration into either carotid alone, caused a considerable cardiovascular effect and often also a brief inspiratory effort or efforts. It is likely that a similar reinforcement might occur between afferent stimuli from the aortic and carotid bodies. So we must use care

in interpreting the results of aortic or carotid denervation. The relatively large reduction in the effects of cyanide injection into the left atrium or ascending aorta could be due to the elimination of a stimulus which was near threshold (just above or just below) on the aortic and/or carotid bodies. There is a further possibility. In lambs near term section of the vagi or aortic nerves causes a fall in arterial pressure and femoral vasodilatation (Dawes *et al.* 1968, 1969), which suggests that there is tonic activity in the aortic chemoreceptors, even at a $P_{O_2} \sim 30$ mm Hg. If this tonic activity were removed by vagal section, the further activity aroused in the carotid bodies by injection of cyanide might be inadequate to excite a response. Hence abolition by vagotomy of a rise in arterial pressure, femoral vasoconstriction or tachycardia (after atropine) might not necessarily imply that the dose of cyanide was acting solely on the *aortic* chemoreceptors as we had supposed. However, in the present experiments it was shown that the cardiovascular response caused by injection of cyanide into the ascending aorta after carotid denervation was abolished by vagotomy. So we are justified in concluding that the response is partly due to excitation of the aortic bodies.

It is also evident that experiments using reflex responses to localize the vascular supply to the aortic chemoreceptors should be preceded by carotid denervation or by some form of cross-circulation to ensure that cyanide, injected into the ascending aorta, does not reach the carotid chemoreceptors directly. The occipitovertebral anastomosis in lambs is large, and constitutes a means by which cyanide, injected into the left atrium, can reach the carotid bodies, even when a long delay loop is inserted into the common carotid arteries (in previous experiments; Dawes *et al.* 1968). It will be more convincing to use impulse traffic in the aortic nerves as an irrefutable index of aortic chemoreceptor activity.

Previous observers failed to elicit respiratory thoracic movements on injection of cyanide in mature foetal lambs (Barcroft & Karvonen, 1948; Reynolds & Mackie, 1961; Dawes *et al.* 1968). Yet this can be seen, in lightly anaesthetized lambs, and is evidently due to carotid chemoreceptor stimulation. Hence intracarotid cyanide injections could be used to assist in the identification of single chemoreceptor afferents in the carotid nerves of the foetus, and hence to determine their sensitivity to blood gas tensions with greater certainty than hitherto.

The fact that asphyxia, caused by tying the umbilical cord, can induce a series of deep gasping movements in lambs after section of the vagi and carotid nerves, which superficially resemble those in lambs with intact nerves, is interesting. It emphasizes the difficulty which Purves & Biscoe (1966) foresaw in the interpretation of their experiments, that asphyxia may initiate respiratory activity in the foetus either by an action on the

systemic arterial chemoreceptors or by activation of centres in the mid-brain and medulla or by a combination of both together.

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