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THE ACTION OF CARBON DIOXIDE AND CYANIDE ON FOETAL RESPIRATORY MOVEMENTS; THE DEVELOPMENT OF CHEMOREFLEX FUNCTION IN SHEEP

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In the sheep foetus, rhythmical movements of a respiratory nature are seen from the 40th day onwards. They can be elicited by various afferent stimuli. From the 50th day onwards they can be initiated by oxygen-want also. The anoxic initiation of the respiration is probably a release of the respiratory centre from the control of 'higher' inhibiting centres, for the same picture can be obtained by sectioning the central nervous system (C.N.S.) at various levels (Barcroft & Barron, 1936, 1937, 1939, 1942; Barcroft, Barron, Cowie & Forsham, 1940; Barcroft, 1946).

Snyder & Rosenfeld (1937) and Rosenfeld & Snyder (1938) compared the effect of CO_2 and anoxia on the respiratory movements of rabbit foetuses and of newborn rabbits. From the 28th day onwards the foetuses often exhibited 'spontaneous' respiratory movements which could be depressed by anoxia and acapnia, but often were slightly stimulated by CO_2 in excess. On the other hand, the respiration of newborn rabbits, even of those prematurely delivered, from the foetal age of 29 days onwards, was stimulated by anoxia and CO_2, and by cyanide from the 28th day. The interpretation given by the authors was that the carotid body is capable of its chemoreceptor function from the 28th or the 29th day, but, before birth, there is inhibition of this function and that, in addition, the foetal respiratory centre is less sensitive to excess CO_2 than that of the adult.

Windle, Monnier & Steele (1938) have reported experiments in which both anoxia and CO_2 have given rise to respiratory movements in cat foetuses. Foetuses younger than 40 days regularly gave a response, whereas at term no stimulation of the respiration could be obtained by either CO_2 or oxygen-want. The authors connected this failure with the foetal anoxia which gradually increases towards full term. In sheep, there have been a few observations showing that CO_2 in excess can stimulate the foetal respiration; and it was noteworthy that the respiratory response was rather different from that obtained by anoxia (Barcroft, 1942).

In the present work, an attempt has been made to observe the response of sheep foetuses to an excess of CO_2, from early midfoetal life until term. In adult animals, CO_2 is known to stimulate both the peripheral chemoreceptors and the respiratory centre (Schmidt & Comroe, 1940). In order to get further information about the nature of the stimulation by CO_2, the effect of cyanide which—at least in adult animals—stimulates the respiration only through the chemoreceptor reflexes (Heymans, Bouckaert & Dautrebande, 1932; Wright, 1935), was also studied.

METHODS

The series consisted of twenty-three experiments performed on twenty-two foetuses and on one lamb. Welsh sheep were used. The dates of the tupping were known, and hence the foetal ages. These ages agreed with those found from the tables of age and crown-rump length given by Barcroft (1946).

The ewes were anaesthetized either with intravenous chloral hydrate, or by a spinal anaesthetic, or by a combination of both. Chloral hydrate was used as a 10% solution. It alone was given to eight ewes (668–678, 703), and the dose required varied between 3·5 and 7·0 g. The spinal anaesthetic alone ('duracaine', 1·5–3·0 c.c.) was given to five ewes (667, 700–702, 704). Some of these ewes exhibited a considerable spontaneous hyperventilation. This was avoided, if the combined anaesthesia was used. Another advantage of the combination was that the dose of chloral hydrate could be reduced to 2·0–4·0 g., and the foetal brain was therefore more likely to exhibit its normal behaviour. In adult animals (decerebrated dogs and cats) chloral hydrate is known to decrease slightly the sensitivity of the respiration to CO_2, whereas the sensitivity to cyanide is slightly increased or remains constant (Dripps & Dumke, 1943). The combined anaesthesia was given to seven ewes (691–699).

Examination of the experimental results shows that the use of different anaesthetics was of very little significance. The foetuses were brought under observation by performing a Caesarean section on the ewe. The operation was carried out in a saline bath at body temperature. The uterus was opened, but the amniotic sac was kept whole until injections into the umbilical vessels were needed. The movements of the foetus were observed through the transparent membrane.

Gas mixtures were administered to the mother through a tracheal cannula. If the ewe had had only a spinal anaesthetic, the cannula was inserted under local anaesthesia. To study excess CO_2, the ewe rebreathed from a big Douglas bag containing 600 l. of a CO_2-air mixture. The CO_2 concentration used in the experiments varied between 5·75 and 8·4%, generally being between 7 and 8%. The respiration of the mother was recorded by using a rubber bellows connected through a side tube to the tracheal cannula. The ewe reacted to the CO_2 with a violent hyperventilation. In two ewes the CO_2 concentration of the arterial blood was measured; it rose as a consequence of the CO_2 administration from 43·6 to 46·2 vol. %, and from 59·3 to 65·8 vol. %, respectively. It was concluded that the expected rise of the CO_2 pressure on the foetal side of the placental barrier occurred, first, because of the flushing of the foetal skin, and second, because the pulse rate and the behaviour of the foetus changed. The O_2-poor mixtures used in four experiments contained 5·0–5·7% oxygen and less than 2·2% CO_2 in nitrogen. They, also, were given from Douglas bags containing 60–80 l. of the mixture. The rebreathing always lasted only for a very short time (1½–4 min.) because the respiration of the mother soon failed.

The cyanide was injected as 0·2 mg. %, 1·33 mg. % or 0·133% KCN solution. The solution was injected into the umbilical veins, except in the foetus of 132 days (692), where a carotid artery was used.

The primary aim of the experiments was to observe the development of the respiratory reflexes. In addition, pulse counts were taken, but only when this could be done without undue interference to the foetus or to the umbilical cord.

RESULTS

Chemosensitivity of foetus

The response of the foetus to CO_2 and to cyanide varied with the foetal age. The results are treated as divided into four age groups each of which showed a more or less consistent behaviour.

Respiratory movements not induced. The youngest age group consists of three foetuses: 43 (667, spinal), 46 (672, chloral) and 49 (668, chloral) days old respectively. The first two did not show any spontaneous movements nor did they react to touch. Their lack of motility does not quite fit the general picture of the functional development of the C.N.S. of the sheep foetus (Barcroft, 1946). CO_2 was administered to the mothers of both, and 13·3 μg. cyanide to the older foetus. Neither induced any foetal movements.

The foetus of 49 days showed spontaneous somatic movements, as well as spontaneous respiratory rhythms of about ten excursions each. The frequency of the respiratory movements was 46 per min. The somatic reflexes were easily elicited; the foetus reacted vigorously to tapping the sac. Nevertheless, administering CO_2 had no effect on the foetal respiratory movements, nor did tying the umbilical cord.

Respiratory movements induced. The second age group comprises five foetuses: 58 (674), 60 (678), 67 (673) and 69 (669, twins) days old, respectively. Until the age of 67 days the foetuses moved spontaneously. Slight rhythmic movements of respiratory nature were seen, the rhythm of which was a little more frequent than before (foetus of 58 days, 48 per min.). The foetuses of 69 days were quieter; only the one later exposed showed a few small spontaneous movements. Stimulation, e.g. tapping the head or pinching the tail of the foetuses, resulted in somatic movements. Only the twins, especially the first one exposed, showed a more subdued reflex behaviour.

The foetus of 58 days behaved very much like that of 49 days. There was no response to CO_2 and none on tying the cord. Injecting 2 μg. of KCN, however, resulted in an immediate deepening of the spontaneous respiratory movements.

The foetuses of 60 and 67 days and the later twin, all responded to CO_2 with rhythmic respiratory movements. Frequencies of 45–78 per min. were observed. Cyanide, 0·24 μg. on the foetus of 60 days, and 4 μg. on that of 67 days, had an effect similar to that of CO_2. A regular respiratory rhythm started at once. The type of the respiratory movements as well as the rhythm were the same when induced by either of these stimuli.

Anoxia, however, produced an entirely different response. An O_2-poor gas mixture was administered to the mother of the foetus of 60 days. The anoxia did not induce any respiratory rhythm in the foetus, except a single gasp.

Clamping the cord of the foetus of 67 days and of the second twin, similarly, produced a few deep gasps, not nearly as frequent as the rhythm produced by CO_2. Anoxia made the later foetus more sensitive to stimulation.

The first twin differed from the rest of the group by not responding to CO_2. Cyanide was not tried. The somatic reflexes seemed to be under a much stronger inhibition than those of the other twin which was exposed later.

Respiratory movements not induced: movements of non-respiratory nature. The foetuses of 91 (691) and 96 (696) days did not exhibit any spontaneous movements. Administering CO_2 or cyanide did not elicit any respiratory movements. Neither of these, however, was quite without an effect. With CO_2 the foetuses reacted with some movements; rhythmic swallowing, chewing, twitches of ears and of shoulder, spasms not involving the thorax.

Cyanide was given to both foetuses in doses of 4 and 27 μg. Both doses gave a few movements similar to those induced by CO_2. The cyanide did not seriously impair the condition of the foetal c.n.s., for the foetus remained sensitive to the movements of the saline solution.

No movements induced. The rest of the foetuses can all be put under the same heading. This group consists of eleven foetuses whose ages were: 107 (676), 112 (679), 132 (692), 139 (694), 141 (701, 703, 704), 142 (699, 702, twins) and 144 (697) days. The foetuses were quiet, but somatic reflexes could often be elicited, e.g. by pinching the fetlock.

CO_2 was tried on ten of these, cyanide on six. CO_2 never gave any respiratory rhythm, and only occasionally some slight movements were seen.

Cyanide, in the dose of 4 μg., generally had no effect. In the foetuses 692, 694 and 699, 27 μg. induced stretching and a few single movements of respiratory nature, but without a rhythm. In the foetuses 679, 697 and 701, even this dose was without effect. Very much higher doses were needed to give a more substantial reaction. In the foetus of 112 days (679), 13·3 mg. of KCN resulted in gasps and in a respiratory rhythm for a short time. In a foetus of 142 days (699), 2·66 mg. gave small jerks, but in the foetus of 144 days (697) the same dose did not give any somatic movements. Such a dose as 2·66 mg.—it has to be kept in mind—is also of an entirely different order from that effective in the younger foetuses. The foetus of 67 days weighs about 100 g. and at term it may be forty times heavier. 2·66 mg. however, is more than 600 times the dose which proved effective at 67 days.

Chemosensitivity at birth

In order to secure information about the possible role of the chemosensitivity at birth, eight of the foetuses mentioned above were experimented upon, on or after the 139th day. (Term is at 147 days.) In addition to the experiments on CO_2 and cyanide, O_2-poor mixtures were given to three foetuses (702, twins; (704), whose mothers were under spinal anaesthetic. The O_2-poor mixture was administered in each experiment first when the foetus was in the amniotic sac. The anoxia made the foetuses very active, they kicked violently and executed swallowing movements. No distinct respiratory movements were seen, certainly no respiratory rhythms. When, however, the nose of the foetus was kept above the surface of the bath, repeating the same procedure produced in all three foetuses the onset of respiration. It started as gasps, not primarily as a rhythm. Only after tying the cord was a rhythm initiated. Bleeding the lambs produced exactly the same type of behaviour as the O_2-poor mixture; violent kicking, shivering, wriggling and gasping.

When one of the twins had started gasping, still having an intact placental circulation, CO_2 was administered to its mother. It produced no apparent change in the behaviour of the foetus, or lamb. The effect of CO_2 was tested also on the respiration of a lamb later, at the age of 3 days. The response was quite similar to that of an adult.

The important role of external stimuli in comparison to the asphyxial ones was very clearly seen in the following experiment:

Sheep 700, 142 days, spinal anaesthetic (1·5 c.c. duracaine).

Experiment conducted with sheep in saline bath.

The mother is quiet. No hyperventilation.

Caesarean section. The foetus is delivered very quickly after starting the operation. The uterus is opened and the head of the foetus is first taken out, but the amniotic sac is left intact. Immediately the nostrils of the foetus are seen to move rhythmically in a respiratory manner. The foetal body also is very soon seen, and then it shows a rather powerful rhythmical respiration. The condition of the foetus is apparently good, the colour of the umbilical vessels markedly bright. The foetus is quite alive, moves vigorously and has a good tonus. Cord is tied. After opening the amniotic sac the foetus tries to make a sound, not entirely without success, and to move about, but very soon it becomes flaccid and ceases to show any rhythmical breathing. After a considerable period it starts to execute several deep gasps, at irregular intervals. At this time the foetal heart beats rather strongly. A respiratory movement of the nature of a small gasp can always be elicited by tapping the nostril lightly with the finger. When artificial respiration was attempted, much mucous fluid comes from the mouth of the foetus. At the end, the foetus is quite cyanotic, the heart beat weakens, and the foetus obviously dies.

Summarizing the protocol: on opening the uterus and exposing the sac, the foetus, which was obviously in a good condition, was seen to execute rhythmic respiratory movements. Consequently, it inspired amniotic fluid, and after delivery, in spite of artificial respiration, could not get rid of this fluid. An asphyxial onset of the respiration certainly was excluded.

DISCUSSION

How do the results of the present work fit the general picture of the development of the function of the C.N.S. in foetal sheep? 'Spontaneous' rhythmic respiratory movements have been observed from the 40th day onwards. Between the 50th and the 70th day the foetal C.N.S. is gradually becoming inhibited, as the midbrain and, later on, the diencephalic centres begin their function (Barcroft & Barron, 1942; Barron, 1941). In the experiments just described, stimulating effects of CO_2 and of cyanide on the respiratory movements were observed between the 58th and 69th days.

Three possibilities thus come into question. Is this stimulation of respiratory movements by CO_2 and by cyanide: (*a*) a true stimulation, or (*b*) a release of inhibition (as the effect of anoxia is understood), or (*c*) a combination of both?

The last possibility seems to be the most probable one. The nature of the respiratory movements elicited by CO_2 or cyanide was quite different from those resulting from anoxia. The respiratory effect of cyanide was momentary; as the dose used was very small indeed it could not possibly produce a condition of 'general anoxia'. These facts obviously suggest, that we are dealing here with something which has its place under the heading 'stimulation'. On the other hand, the possibility of a second component—the release of inhibition—cannot be entirely ruled out.

No direct attempt was made to determine the localization of the cells or tissues responsible for the stimulation obtained. Any suitable experimental procedure at that age—as far as the authors can see—would mean a rather severe exposure of the foetus, too severe to be stood by a foetus some 65 days old. On the principle of analogy, however, two arguments supporting the reflex nature of the stimulation can be put forward. First, the momentary stimulation of the respiratory movements by cyanide in adult mammals is known to be entirely due to the peripheral chemoreceptors, mainly to those of the carotid body (Comroe, 1939; Verdonk, 1941). The respiratory centre, on the contrary, is depressed by cyanide. In other words, this would mean that the foetus has at the age of 60–70 days a functioning carotid body chemoreflex. And secondly, if it were a reflex it should undergo the same development as all the foetal somatic reflex behaviour; it should become inhibited about the middle of pregnancy and be released at birth. This is precisely what happens.

Does the assumption of a functioning carotid body fit the picture of the anatomically existing structure at this foetal age? In adult sheep the carotid body functions similarly to that of other mammalian species (Van Damme, 1933). Unfortunately, there are no histological data about the carotid body of foetal sheep. Boyd's (1937) work on human foetuses shows, that the microscopical development of the carotid body occurs quite early, mainly between the 12·5 and 36 mm. crown-rump length stages. Watzka's (1937) findings on several other mammalian species give a similar picture. The crown-rump length of the sheep foetus of 58 days was 115 mm.; accordingly, perhaps it may be concluded that the foetus of that age probably has a carotid body.

The carotid body sends its messages to the brain through the glossopharyngeal nerve. This reaches a part of the brain (medulla) which starts its function very early. Hence, the anatomical basis for the onset of the carotid body function probably is there.

In addition to CO_2 and cyanide, the carotid body of adult animals is also sensitive to changes in O_2 pressure. In foetal sheep, anoxia never produced an effect like that of CO_2 or of cyanide. Whether this is due to a difference between foetus and adult at the level of the chemosensitive elements or at that of the correlating or effector mechanisms cannot be judged. Our experiments do not rule out the possibility of an anoxic reflexogenic drive, the effects of which were, however, largely modified by the effect of anoxia on the corresponding centres.

In the youngest foetus which gave a positive response to cyanide, CO_2 was not effective. It might be, that the 'spontaneous' rhythms seen on opening the uterus have been CO_2 rhythms. On the other hand, Rosenfeld & Snyder (1938) in their experiments also found a response to cyanide in some premature rabbits before CO_2 gave any effect.

The third age group, in which CO_2 and cyanide induced general motility but no respiratory rhythms, offers a picture of special interest; there was the rather striking occurrence of rhythmic chewing and swallowing movements, whereas no respiratory rhythms were released. Labial and lingual rhythm, chewing and sucking, all have been understood as a relatively late development of the originally respiratory rhythm (Barcroft, 1941). At this phase, the respiratory 'centre' seems to be under a much stronger inhibition than the closely related centres governing the suctorial movements.

Snyder & Rosenfeld (1937) interpreted their findings on rabbit foetuses and on newborn rabbits as indicating: first, that there is an inhibition of the carotid body reflex mechanism during foetal life; secondly, that the foetal respiratory centre is less sensitive to carbon dioxide than it is after birth. Our observations on sheep between the 70th day of foetal life and birth can be expressed as follows. *There is during the second half of the foetal life an inhibition of the nervous mechanism responsible for the respiratory movements.*

As far as it can be seen, the chemosensitivity at the foetal age of 60–70 days does not serve any immediate purpose. On the other hand, it can teleologically be understood as a manifestation of a phase in the ontogenesis of a function needed in future. It is also worth remembering that a reactivity like this is an old property, present already in aquatic vertebrates; the larvae of toadfish react quite similarly to CO_2 and to cyanide (Tracy, 1926).

The next phase, the development of an inhibition, easily finds its 'usefulness' for the animal. If there were no inhibition of the respiratory reflexes, the result would be that observed in the sheep 700; the foetus would be drowned before it had been born. For a foetus near to term it is as essential not to breathe as it is for a newborn lamb to start breathing! The inhibition is associated with the onset of the function of the regions of the brain anterior to the pons (Barcroft & Barron, 1942). On the mechanism of this inhibition the present experiments do not give much light. The inhibition is something which disappears at birth, so much is certain; but what this 'something' is remains a matter of speculation. It has, perhaps, a counterpart in the behaviour of diving mammals, in which contact of the respiratory openings with water stops breathing (Krogh, 1941). In the experiments on the foetuses older than 140 days taking the nose out of the bath was sufficient to release the gasp mechanism, but to obtain a rhythm it was necessary to tie the cord. On the other hand, the foetus 700 started a respiratory rhythm when in the amniotic sac. This foetus seemed to be strikingly well oxygenated for its age; whether this had anything to do with the too early disappearance of the inhibition, cannot be judged. The inhibition may be due to the combined effect of several factors.

SUMMARY

1. Sheep foetuses between the foetal age of 43 days and term have been subjected to increased CO_2 pressures and intravenous cyanide injections. The effects of these on the foetal respiratory movements have been compared to those of anoxia.

2. The effects of CO_2 and of cyanide can be divided into four groups according to the age of the foetus.

(i) 43–49 days. No effect.

(ii) 58 days. Spontaneous respiratory movements deepened by cyanide; CO_2 without effect.

60–69 days. Both CO_2 and cyanide induced vigorous respiratory movements like the 'spontaneous' respiratory movements.

(iii) 91, 96 days. No induction of respiratory movements; some rhythmic swallowing, chewing, twitching of the ears, etc.

(iv) 107–term. No effect.

3. In 60–69 day foetuses anoxia induced 'gasping' respiratory movements of quite a different character to those induced by CO_2 and by cyanide, and after a much longer latent period.

4. The interpretation of the findings is discussed. The induction of respiratory movements by CO_2 and by cyanide is probably a chemoreflex from the carotid body. The failure to induce such movements in the latter half of pregnancy accords with the fact that somatic reflexes become inhibited at that time, only to be released at birth.

5. Some experiments on the role of chemosensitivity during birth are described. They indicate that cutaneous sensory stimulation rather than asphyxia is responsible for the commencement of breathing.

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REFERENCES

Barcroft, J. (1941). *Nature, Lond.*, **147**, 762.
Barcroft, J. (1942). *Lancet*, **2**, 117.
Barcroft, J. (1946). *Researches on Pre-Natal Life.* Oxford: Blackwell.
Barcroft, J. & Barron, D. H. (1936). *J. Physiol.* **88**, 56.
Barcroft, J. & Barron, D. H. (1937). *J. Physiol.* **91**, 329.
Barcroft, J. & Barron, D. H. (1939). *Ergebn. Physiol.* **42**, 107.
Barcroft, J. & Barron, D. H. (1942). *J. comp. Neurol.* **77**, 431.
Barcroft, J., Barron, D. H., Cowie, A. T. & Forsham, P. H. (1940). *J. Physiol.* **97**, 338.
Barron, D. H. (1941). *Biol. Rev.* **16**, 1.
Boyd, J. D. (1937). *Contr. Embryol. Carneg. Instn*, **26**, 1.
Comroe, J. H., Jr. (1939). *Amer. J. Physiol.* **127**, 176.
Dripps, R. D. & Dumke, P. R. (1943). *J. Pharmacol.* **77**, 290.
Heymans, C., Bouckaert, J.-J. & Dautrebande, L. (1932). *C.R. Soc. Biol., Paris*, **109**, 566.
Krogh, A. (1941). *The Comparative Physiology of Respiratory Mechanisms.* Philadelphia: University of Pennsylvania Press.
Rosenfeld, M. & Snyder, F. F. (1938). *Amer. J. Physiol.* **121**, 242.
Schmidt, C. F. & Comroe, J. H. (1940). *Physiol. Rev.* **20**, 115.
Snyder, F. F. & Rosenfeld, M. (1937). *Amer. J. Physiol.* **119**, 153.
Tracy, H. C. (1926). *J. comp. Neurol.* **40**, 253.
Van Damme, J. (1933). *C.R. Soc. Biol., Paris*, **113**, 909.
Verdonk, A. (1941). *Arch. int. Pharmacodyn.* **65**, 111.
Watzka, M. (1937). *Z. ges. Anat.* 1. *Z. Anat. Entw.-Gesch.* **108**, 61.
Windle, W. F., Monnier, M. & Steele, A. G. (1938). *Physiol. Zoöl.* **11**, 425.
Wright, S. (1935). *J. Pharmacol.* **54**, 1.