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PULMONARY VASOMOTOR RESPONSES OF ISOLATED PERFUSED CAT LUNGS TO ANOXIA

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It has been shown previously (Duke, 1951; Nisell, 1950) that ventilation of isolated perfused cat lungs with nitrogen, or gas mixtures containing less than 15 % O_2, causes a rise in pulmonary arterial pressure. The experiments described in this paper were done to see if carbon monoxide and certain known inhibitors of oxidative enzyme systems produced a similar response. For this purpose pulmonary vasomotor responses to ventilation of the lungs with N_2 or CO were compared with those produced by injection of sodium cyanide, sodium azide or sodium monoiodoacetate. Some of the results have already been briefly reported elsewhere (Duke & Killick, 1950, 1951).

METHODS

Isolated cat lungs were perfused at constant volume inflow with the animal's own heparinized blood, as previously described (Duke, 1951). The lungs were ventilated by intermittent positive pressure inflations (Starling 'Ideal' pump), and the tidal air was recorded by Konzett & Rössler's (1940) method under a constant positive pressure which varied between +6 and +12 cm H_2O in different experiments. The amount of air entering the lungs at each inflation could be calculated approximately by subtracting the tidal air overflow volume from the total respiratory pump stroke volume. Pulmonary arterial pressure and lung blood volume were also recorded. Blood gas analysis was done on samples of blood from the pulmonary arterial tubing, and O_2 and CO_2 were estimated in a Van Slyke apparatus (Peters & Van Slyke, 1932). Carboxyhaemoglobin was estimated by means of a Hartridge reversion spectroscope previously calibrated with cat's blood. The concentration of haemoglobin in the blood was determined with a Gowers Haldane haemoglobinometer and the pH of the blood was recorded with a Cambridge meter. The concentration of CO in air samples was analysed by the iodine pentoxide method (Haldane & Graham, 1935). The CO used in the experiments was either the pure gas (as supplied by Imperial Chemical Industries Ltd.) or was made by the action of sulphuric acid on sodium formate.

RESULTS

Nitrogen

Ventilation of the lungs with nitrogen instead of air for periods of 2–10 min produced the increase of pulmonary arterial pressure which has been described previously (Duke, 1951). This pressor response was observed during 105 tests

in twenty-four experiments of the present series at intervals varying from $\frac{1}{4}$ to $5\frac{1}{2}$ hr from the commencement of perfusion of the lungs. In the majority

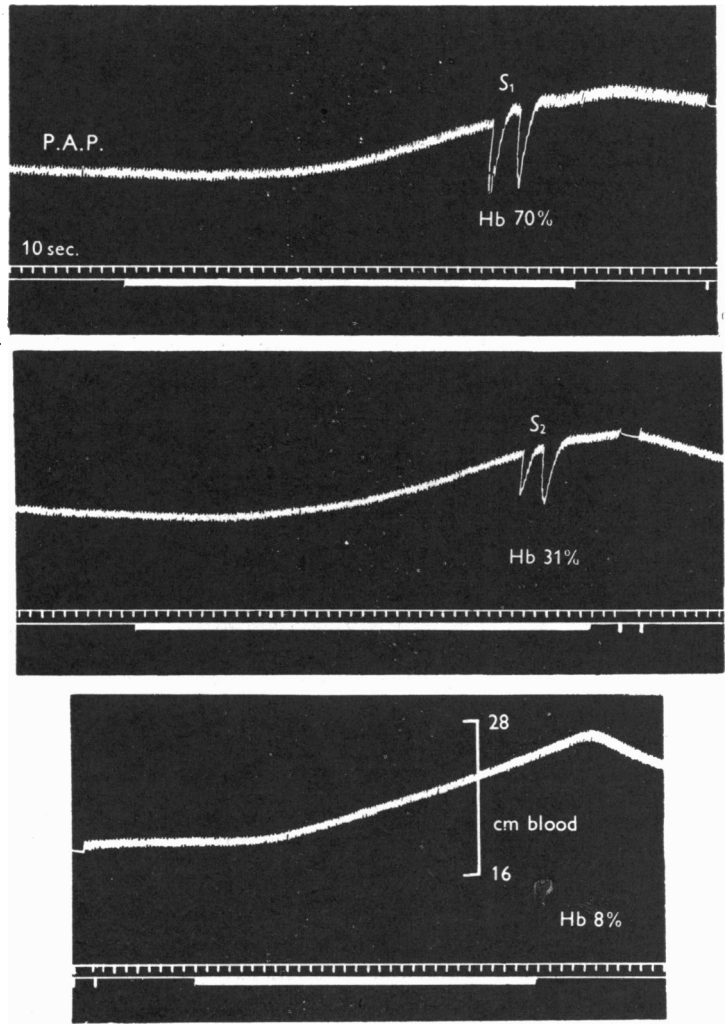

Fig. 1. Cat, 2·8 kg. Blood perfusion begun at 11.22 a.m. Air ventilation, N_2 ventilated during signal. Partial replacement of blood by Dextran. Top, 12.22 p.m.: blood sample at S_1; blood, O_2 9·23 vol. %; CO_2 19·91 vol. %; Hb 70%. Middle, 12.50 p.m.: Hb 31%. Bottom, 1.18 p.m.: Hb 8%. P.A.P. = pulmonary arterial pressure.

of these experiments there was evidence of increasing sensitivity of the lung blood vessels to O_2 lack as the perfusion continued.

Pulmonary pressor responses to nitrogen inhalation also occurred in pre-

parations perfused either with Ringer Locke solution or with Dextran. These solutions were either perfused through the lungs from the beginning of the experiment or they were used as a partial replacement of the blood in the circuit during the course of the experiment. In the latter case the haemoglobin concentration of the perfusate was reduced to values varying from 1 to 20 %, although the total volume of perfusate was not increased. Fig. 1 shows the effect of ventilating with N_2 for 4 min periods in a preparation in which the concentration of haemoglobin in the perfusate was reduced from 70 to 8 % (Haldane scale) by the addition of Dextran to the circuit and withdrawal of blood. With this preparation the blood contained 9·23 vol. % O_2 towards the end of the test with N_2 when the haemoglobin concentration was 70 %. This oxygen content was greater than could have been attained even at full saturation later in the experiment when the haemoglobin concentration was reduced to 8 %. The responses to N_2, however, were very similar under these different conditions, and it appeared that it was the change in O_2 tension in the blood or in the alveolus that was responsible for the appearance of the N_2 effect and not the change in O_2 content of the blood. Moreover, pulmonary pressor responses to N_2 could be obtained when the circulating blood was 60–80 % saturated with CO. In some experiments, however, there was evidence that the vasoconstrictor effect of N_2 was somewhat lessened by diluting the blood with Dextran or by partially saturating it with CO. The reason for this was obscure and was apparently not related to a change in blood viscosity, because there was no significant difference in the viscosity of Dextran and cat's blood when measured under similar conditions.

Carbon monoxide

Ventilation of the lungs with pure CO produced a fall of pulmonary arterial pressure (after a latent period of 30–90 sec) often accompanied by bronchodilatation (see Fig. 2). The depressor effect of CO was observed in nineteen tests in eleven experiments at times varying from 1¼ to 5½ hr from the beginning of the perfusion; the pulmonary arterial pressure decreased by 1–23 % of its initial value after 2–6 min inhalation of CO (see Table 2). In ten out of the thirteen tests made while recording the pulmonary ventilation the bronchi dilated to produce a 1–18 % increase in tidal air. Similar, but less marked, pulmonary depressor responses followed ventilation of the lungs with mixtures containing 1–20 % CO in air, although not every preparation was sufficiently sensitive to respond to 1 % CO. The pulmonary blood vessels also tended to become more sensitive to CO during the course of perfusion.

The addition of 5–10 % CO_2 to the gas mixture did not prevent the appearance of the response to CO, and no conclusive evidence was obtained that inhalation of CO produced any change in blood pH which could account for its effects (see Table 1). The vasomotor effects of CO were not reversed or inhibited by

atropine sulphate (concentration 1 : 250,000) or dihydroergotamine (concentration 1 : 5000) added to the blood either together or separately, and large doses (5–200 μg) of decamethonium iodide were also without apparent effect.

The short latent period between the administration of CO and the fall in pressure, and the rapid return of the pressure to its original level on ventilating the lungs with air, suggested that the depressor response could hardly be

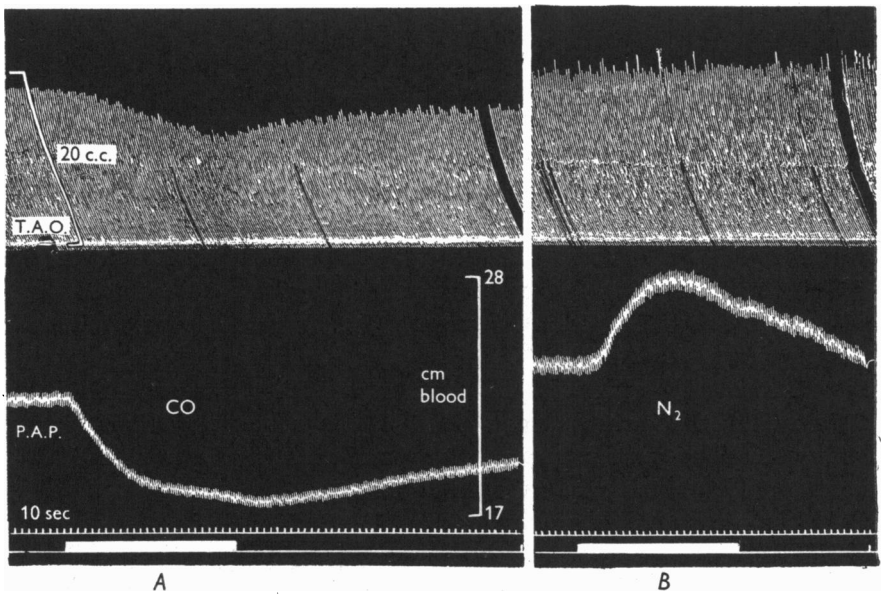

Fig. 2. Cat, 4·0 kg. Perfusion begun 11.35 a.m. Blood flow 180 c.c./min. Ventilation positive pressure 12 cm H_2O, pump 60 c.c./stroke. Air ventilation. *A*, 3.25 p.m.: pure CO ventilated during signal. *B*, 5.00 p.m.: pure N_2 ventilated during signal. Blood COHb saturation; 3.21 p.m. 51%; 3.28 p.m. 64%; T.A.O. = tidal air (overflow volume).

TABLE 1. Pulmonary arterial blood pH changes, as the result of various tests

Expt. no.	Test	Blood pH Before	Blood pH After
18	N_2 inhalation (from air)	8·12	8·00
18	N_2 inhalation (from air)	8·06	8·06
18	N_2 inhalation (from air)	8·08	7·99
18	N_2 inhalation (from air)	8·02	7·97
19	N_2 inhalation (from air)	8·41	8·37
19	N_2 inhalation (from air)	8·41	8·40
20	5% CO_2 in N_2 (from 5% CO_2 in air)	7·46	7·48
22	5% CO_2 in N_2 (from 5% CO_2 in air)	7·51	7·62
18	NaIA	7·97	7·86
19	NaIA	7·32	7·26
20	NaN_3	7·40	7·40
21	NaN_3	7·60	7·62
19	CO inhalation (from air)	8·31	8·27
19	CO inhalation (from air)	8·08	8·06
20	CO inhalation (from air)	7·58	7·60

determined by the concentration of carboxyhaemoglobin in the blood. The percentage saturation of the pulmonary arterial blood with CO was measured

TABLE 2. Inhalation of pure CO (from air)

Expt. no.	Time from beginning of perfusion to test hr	min	Duration of CO inhalation (min)	Percentage decrease P.A.P.	T.A.† change (%)	Blood CO after test (%)
48	3	24	4	20	+18	—
48	3	50	4	11	+11	64
48	5	35	4	18	0	—
3	1	44	6	7	—	75
3	3	56½	6	19	+5	83
10	1	56½	2	8	—	—
11	1	45	2	2	0	86
11	3	6	2	4	+9	86
11	4	1½	2	23	0	83·5
19	2	50	2	8	+1	86
20	3	15	2½	1	—	79
21	3	36	3	2	—	85
34	2	39	2	5	+7	77·5
38*	1	25	1½	23	+1·5	—
47	1	32½	3	9	—	—
47	1	49	3	9	+6	—
47	2	30	3	22	+2	—
48(*a*)	1	13	1½	0·5	+1	—
48(*a*)	2	13	2	13	—	—

* Dextran only as perfusate.
† T.A. = amount of air entering the lungs, see also Table 3.

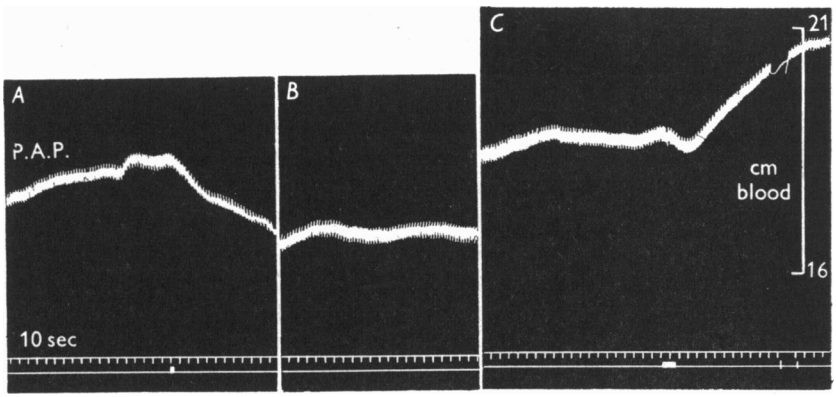

Fig. 3. Cat, 3·7 kg. Perfusion begun 11.30 a.m. Ventilated with 5% CO_2 in air. *A*, 12.44 p.m.: 1% CO and 5% CO_2 in air. *B*, 12.52 p.m.: still ventilated with 1% CO. *C*, 1.28 p.m.: 5% CO_2 in air. Blood COHb saturation: 12.50 p.m. 29%; 1.20 p.m. 81%; 1.33 p.m. 73%.

at various time intervals before, during and after CO inhalation in several experiments of which one is shown in Fig. 3. In this test the maximum fall of pulmonary arterial pressure was reached when the haemoglobin was 29 %

saturated with CO. On ventilating with air the pressure rose steeply to reach a maximum level in 3 min, but the concentration of carboxyhaemoglobin fell from 81% to only 73% in 5 min. Three similar tests in other experiments also showed there was no relationship between the proportion of carboxyhaemoglobin in the blood and the level of the pulmonary arterial pressure. Other indications of a similar nature were that decreasing the blood haemoglobin to 1% or less by withdrawal of blood from the circuit and replacing it with Dextran did not abolish the response; nor was the sensitivity of the response to CO markedly altered when the blood already contained carboxyhaemoglobin at the beginning of the test.

It was important to find out if the difference in the pulmonary vascular response to CO and to N_2 was due to the greater degree of tissue O_2 lack which might have been caused by CO or whether CO was producing some other effect than could be ascribed to its combination with haemoglobin and its effect on the oxygen dissociation curve of the blood. Tests were therefore made to see if the response to CO was affected by the presence of N_2. In the experiment shown in Fig. 4 the fall in pulmonary arterial pressure caused by ventilation of the lungs with 20% CO in air or N_2 was almost identical in the two tests; however, the response was maintained during inhalation of 1% CO in air, but not during inhalation of 1% CO in N_2. In another experiment (see Fig. 5) pulmonary depressor responses were obtained to 5% CO in air and to pure CO, but 5% CO in N_2 produced a pressor effect, though less than that caused by N_2 or 5% O_2 in N_2. Since the pressor effect of N_2 could be superimposed on the depressor effect of CO it appeared improbable that both types of response had a common cause, and possible that CO was exerting some effect that was not due solely to lack of oxygen in the ventilating gas mixture.

Observations were also made of the effects of varying the concentration of O_2 in test gas mixtures which contained known concentrations of CO. For this purpose concentrations (1–5%) of CO were chosen which elicited minimal responses, and a comparison was made of the effect on the pulmonary arterial pressure of inhaling the same concentration of CO in air or in O_2 for similar periods of time (usually 2–4 min). In this way the ratio CO : O_2 could be varied from 1 : 20·7 (1% CO in air) to 1 : 99 (1% CO in O_2). In seven out of eight tests (one of which is shown in Fig. 6) made in seven experiments, CO in O_2 was either without effect on the pulmonary arterial pressure or the response was much less than that produced by the same concentration of CO in air, and the results were equivocal in the remaining test. Analyses of the gas mixtures used for the tests were done on four occasions, and each time the mixture consisting of CO and O_2 contained 0·1–0·3 vol. % more CO than the mixture of CO in air. Table 3 gives a list of the various gas mixtures tested and indicates their effect on the pulmonary arterial pressure. Pulmonary depressor responses were consistently found when the ratio CO/O_2 in the inspired air was greater

than 0·1. When the ratio CO/O_2 was reduced to 0·05 some preparations failed to respond, and further reduction of the ratio to 0·01 abolished the response.

Three experiments have also been performed on isolated rabbit lungs perfused in a similar manner to the cat lungs already described. These

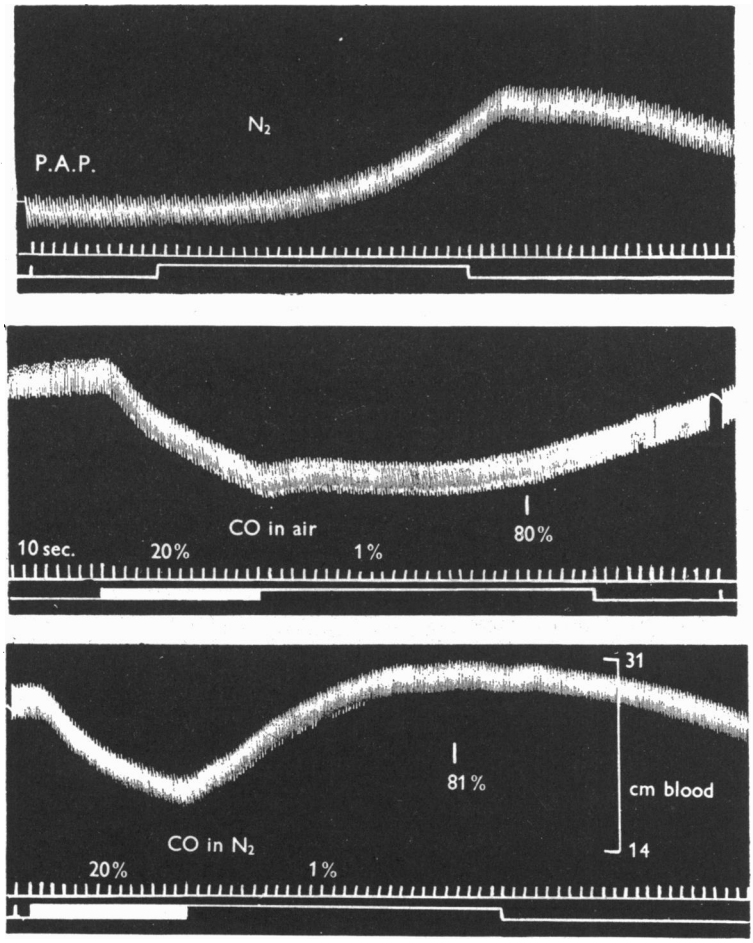

Fig. 4. Cat, 2·4 kg. Perfusion begun 11.50 a.m. Air ventilation. Top: N_2 ventilated during signal. Middle: 20% CO in air followed by 1% CO in air. Bottom: 20% CO in N_2 followed by 1% CO in N_2. % sat. of Hb with CO also shown.

preparations showed pulmonary pressor responses to CO given during the first 1–2 hr of perfusion, although later tests with CO caused a fall in pulmonary arterial pressure. The response to N_2 was pressor throughout the experiments.

The effects of enzyme inhibitors

High concentrations of CO can inhibit oxidative enzymes (Keilin, 1929; Warburg, 1949). With this property of CO in mind other inhibitors of the same enzymes were tested on the isolated lung.

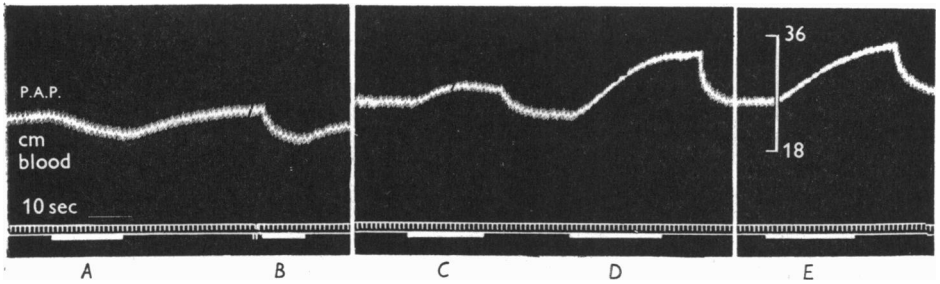

Fig. 5. Cat, 2·5 kg. Perfusion begun 11.40 a.m. Air ventilation. Ventilation with gas mixture during signals. *A*, 2.56 p.m.: 5% CO in air. *B*, 2.56 p.m.: CO. *C* 3.38 p.m.: 5% CO in N_2. *D*, 3.38 p.m.: N_2. *E*, 4·05 p.m.: 5% O_2 in N_2.

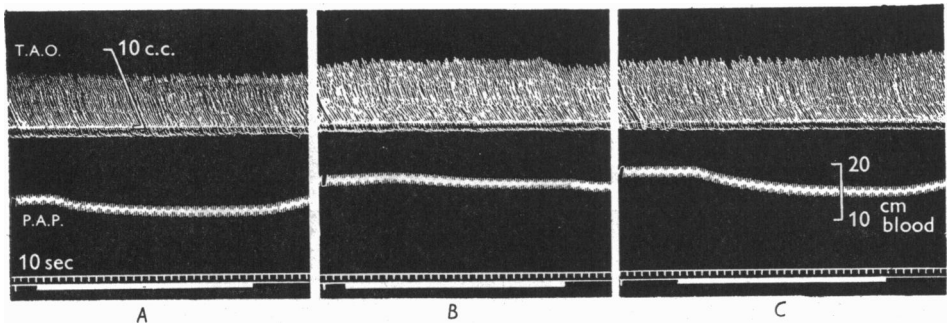

Fig. 6. Cat, 2·3 kg. Dextran perfusion begun 2.40 p.m. Air ventilation. Ventilated with test gases during signals. *A*, 4.15 p.m.: 5% CO in air. *B*, 4.30 p.m.: 5% CO in O_2. *C*, 4.46 p.m.: 5% CO in air.

Injection of sodium azide (dose 4–5 mg) or HCN or sodium cyanide (dose 10 mg) into the pulmonary artery produced a fall of pulmonary arterial pressure (see Table 4). With both drugs the fall of pulmonary arterial pressure occurred after a latent period of 10–20 sec, but cyanide differed from azide in that it sometimes caused bronchodilatation. Pulmonary oedema also usually occurred within 15 min of the injection of cyanide, but sodium azide did not cause oedema so rapidly. After administration of azide, the pulmonary vessels were unresponsive to N_2, CO or HCN, although pulmonary vasomotor responses could still be obtained to ACh and adrenaline for about 1 hr. Fig. 7 shows a typical response to cyanide, and Fig. 8 shows that sodium azide, which itself caused

TABLE 3. The effects of varying proportions of CO and O_2 on the pulmonary arterial pressure

Gas mixture	CO/O_2 ratio	P.A.P. response
100% CO	$100/0=\infty$	*D*
20% CO in O_2	$20/80=0{\cdot}25$	*D*
20% CO in air	$20/20=1{\cdot}0$	*D*
10% CO in air	$10/20=0{\cdot}5$	*D*
10% CO in O_2	$10/90=0{\cdot}1$	*D*
5% CO in air	$5/20=0{\cdot}25$	*D*
5% CO in O_2	$5/95=0{\cdot}05$	?
1% CO in air	$1/20=0{\cdot}05$	?
1% CO in O_2	$1/99=0{\cdot}01$	0

D = pulmonary depressor response.
0 = no effect.
? = doubtful depressor response

TABLE 4. The effects of azide and cyanide on the pulmonary arterial pressure

Expt. no.	Drug and dose (mg)	Percentage decrease P.A.P.	Percentage change in T.A.
2	HCN 10	32	+11
3	HCN 10	23	0
20	NaN_3 4	50	0
21	NaN_3 5	50	0
25	NaN_3 5	7	0
	+2	22	0
24	NaN_3 5	55	0

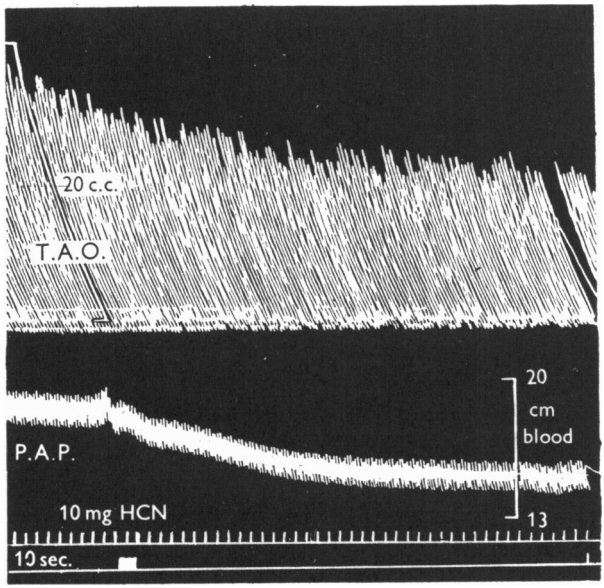

Fig. 7. Cat, 2·4 kg. Perfusion begun 11.50 a.m. Ventilation positive pressure, 10 cm H_2O, pump 60 c.c./stroke. 4.11 p.m. (signal) 10 mg HCN injected into pulmonary artery. (HCN approx. concn. = 0·004 M.)

a slight fall of pulmonary arterial pressure, greatly reduced the response to N_2. The response of the same lungs to ACh and adrenaline is shown later in the same experiment.

Injection of sodium monoiodoacetate (dose 5–10 mg) produced an increase of pulmonary arterial pressure within 10 min in six preparations. The pressure rose gradually to reach a maximum value after 10–15 min (see Fig. 9). Inhalation of N_2 after iodoacetate produced a fall of pulmonary arterial

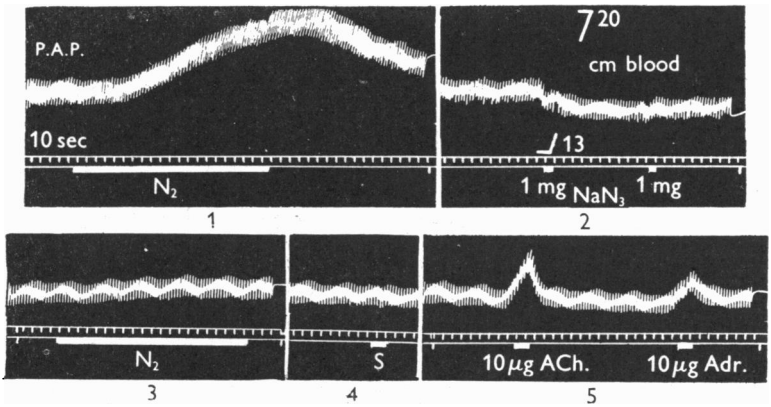

Fig. 8. Cat, 1·9 kg. Perfusion begun 10.20 a.m. Air ventilation. (1) 11.14 a.m.: ventilation with N_2. (2) 11.26 a.m.: 2 mg NaN_3. (3) 12.16 p.m.: ventilation with N_2. (4) 12.15 p.m.: saline control. (5) 12.22 p.m.: 10 μg ACh. 12.25 p.m.: 10 μg adrenaline.

pressure instead of the rise which was obtained earlier in the same experiment (see Table 5), although the response to CO was unchanged. Fig. 10 shows the response to N_2 before and after iodoacetate in the same experiment as in Fig. 8.

DISCUSSION

The experiments here described show that, in conditions in which cardiovascular and respiratory reflexes are excluded, ventilation of isolated lungs with CO produces an effect on the pulmonary blood vessels which differs from that produced by ventilation with N_2. Duke (1951) showed that a pressor response could be produced by ventilating the lungs with H_2, neon, or N_2. It was concluded that this pressor response was due to substitution of an indifferent gas for air or O_2, rather than to any specific effect of the gases. The present results confirm the effect of O_2 lack, and indicate that a change in partial pressure of O_2 in the perfusate or in the alveolar air is responsible for the effect, rather than a change in the O_2 content of the perfusate. Dilution of the blood with Dextran so that its O_2 content was very low caused no change in pulmonary arterial pressure, provided the lungs were ventilated with air or

O_2 so that the partial pressure of the latter gas did not fall below the normal value. Under these conditions ventilation of the lungs with N_2 caused the usual pressor response.

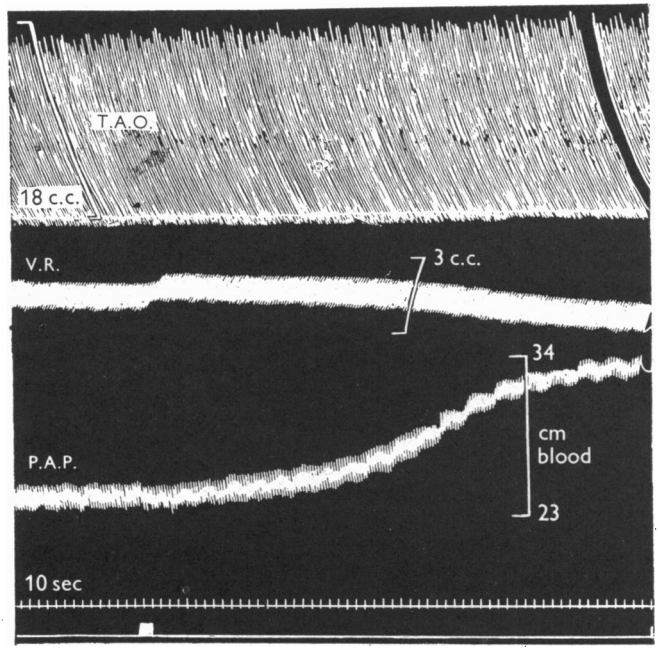

Fig. 9. Cat, 2·5 kg. Perfusion begun 12.30 p.m. Ventilation positive pressure, 10 cm H_2O, pump 45 c.c./stroke. 1.27 p.m. (signal): 5 mg sodium monoiodoacetate injected into pulmonary artery (NaIA concn. 0·00024 M). V.R. = venous reservoir blood volume.

TABLE 5. The effects of sodium monoiodoacetate on the pulmonary arterial pressure

Expt. no.	Dose of NaIA (mg)	Percentage change in P.A.P. to N_2			
		Before NaIA	Time (min)	After NaIA	Time (min)
11	10+10	+41	9	−24	13
13	5	+16	20	−19	10½
	+5			−19	19½
15	5	+ 9	41½	− 3	11½

Similar evidence was provided by experiments in which ventilation of the lungs with CO had resulted in combination of 70–80 % of the haemoglobin in the perfusate with CO. The diminished oxygen content thus produced caused no pressor response if the lungs were ventilated with air, thus maintaining the partial pressure of oxygen.

The observation that ventilation with pure CO caused a fall in pulmonary arterial pressure indicates that some effect was produced by the CO in addition

to that of oxygen lack such as could be produced by ventilation with N_2, H_2 or neon.

The depressor response to CO was not due merely to changes in bronchial calibre, because these were not always present. Nervous stimulation is unlikely

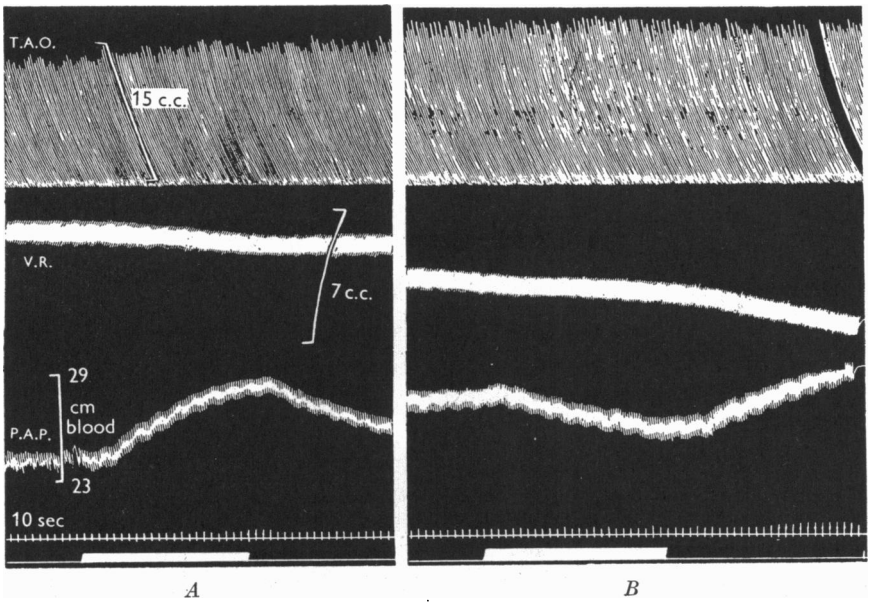

Fig. 10. Cat, 2·5 kg. Perfusion begun 12.30 p.m. Ventilation positive pressure, 10 cm H_2O, pump 45 c.c./stroke. Air ventilation. *A*, 1.07 p.m.: ventilation with N_2 during signal. *B*, 1.46½ p.m.: ventilation with N_2 during signal. Between *A* and *B*, 5 mg sodium monoiodoacetate injected into pulmonary artery.

to be responsible, because the response was not inhibited by atropine, ergotoxine, or decamethonium iodide. It is probable, therefore, that CO directly dilates some part of the pulmonary vascular bed.

The toxic effects of CO in the whole animal have generally been considered to be due to two properties: its strong affinity for haemoglobin compared with oxygen, and its effect in shifting the blood O_2 dissociation curve to the left. The resulting anoxia is thus held to be of the anoxic type (Peters & Van Slyke, 1932). There is much evidence in support of this view (for refs. see Killick, 1940); however, other results have been more equivocal. For instance, Brewer (1937) found that CO had different effects from N_2 on the blood pressure of anaesthetized dogs. Cats are more susceptible to a reduction in O_2 capacity of the blood caused by formation of COHb than formation of methaemoglobin (Lester & Greenberg, 1944). Drabkin, Lewey, Bellet & Ehrich (1943) found that dogs which inhaled CO until their blood was 75 %

saturated with HbCO died, whereas levels of 75 % obtained by transfusion with R.B.C.'s saturated with CO were ineffective in producing signs of anoxia on the e.c.g. Some of these effects may be explained by the maintenance of a normal arterial O_2 tension during poisoning of the whole animal with CO, and consequent lack of chemoreceptor stimulation (Comroe & Schmidt, 1938; Chiodi, Dill, Consolazio & Horvath, 1941). The present experiments, however, support the view that CO has some other effect than can be explained by the anoxia caused by its combination with haemoglobin; the mechanism for this remains to be determined.

Carbon monoxide is known to combine with respiratory haem compounds other than haemoglobin (Millikan, 1936). The tissue oxidative systems, however, have a low affinity for CO; half inhibition of respiration in yeast cells is produced by a CO/O_2 ratio of approximately 10 (Warburg, 1949). Experiments on isolated tissues have yielded results indicating that the cytochrome system of mammalian cells has a similar low affinity for CO. Amongst the respiratory haem compounds, myoglobin is exceptional in having a relatively high affinity for CO; myoglobin is combined to the extent of 50 % with CO and 50 % with O_2 at a CO/O_2 ratio of 1/20 (Millikan, 1936).

In our experiments the response of the pulmonary arterial pressure could be correlated with the CO/O_2 ratio in the gas mixture used to ventilate the lungs (see Table 3). A fall in pressure followed ventilation with mixtures having a CO/O_2 ratio of 1/10 or more. Mixtures containing a lower ratio of CO/O_2 were ineffective, and there was some tendency for the depressor response to become more marked as the CO/O_2 ratio in the gas mixture was increased.

We can only suggest tentatively that CO may have combined with some haem compound other than haemoglobin, and that the inactivation of this compound resulted in a fall of pulmonary arterial pressure. We have no evidence on which to base either a suggestion of the identity of this compound, or of its location.

The observation of a fall in pulmonary arterial pressure resulting from the injection of either sodium cyanide or sodium azide is of interest in this connexion, since both these substances are known inhibitors of haem-containing enzyme systems. It is not possible, as yet, to suggest any correlation between these results and the pressor response to the anoxia caused by ventilating the lungs with N_2.

SUMMARY

1. Isolated cat lungs were perfused through the pulmonary artery with the animal's own blood at constant volume inflow.

2. Ventilation of the lungs with pure CO or 1–20 % CO in air (instead of air) constantly produces a fall in pulmonary arterial pressure usually accompanied by bronchodilatation.

3. The response to CO is not inhibited by atropine, dihydroergotamine or decamethonium iodide, and it is not accompanied by significant changes in blood pH.

4. The vasomotor responses to CO and N_2 can both be produced during perfusion of the lungs with Dextran; and it is probable that it is the change in tension of the gas in the blood or alveolar air which is important.

5. Since the response to N_2 can be superimposed upon that to CO it is suggested that the mechanism of the two effects must be different.

6. Pulmonary vasodilatation is produced by injection of cyanide or azide into the pulmonary artery. Injection of sodium monoiodoacetate produces a gradual rise of pressure with subsequent reversal of the nitrogen effect.

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