J. Physiol. (1969), **201**, *pp.* 87–104
With 5 text-figures
Printed in Great Britain

CARDIOVASCULAR RESPONSES IN APNOEIC ASPHYXIA: ROLE OF ARTERIAL CHEMORECEPTORS AND THE MODIFICATION OF THEIR EFFECTS BY A PULMONARY VAGAL INFLATION REFLEX

BY JENNIFER E. ANGELL JAMES AND M. DE BURGH DALY
From the Department of Physiology, St Bartholomew's Hospital Medical College, London, E.C. 1

(*Received* 15 *May* 1968)

SUMMARY

1. In the spontaneously breathing anaesthetized dog, the systemic circulation was perfused at constant blood flow; there was no pulmonary blood flow and the systemic arterial blood P_{O_2} and P_{CO_2} were controlled independently by an extracorporeal isolated pump-perfused donor lung preparation. The carotid and aortic bodies were separately perfused at constant pressure with blood of the same composition as perfused the systemic circulation.

2. Apnoeic asphyxia, produced by stopping the recipient animal's lung movements and, at the same time, making the blood perfusing the systemic circulation and the arterial chemoreceptors hypoxic and hypercapnic by reducing the ventilation of the isolated perfused donor lungs, caused an increase in systemic vascular resistance.

3. While the systemic arterial blood was still hypoxic and hypercapnic, withdrawal of the carotid and aortic body 'drive' resulted in a striking reduction in systemic vascular resistance. Re-establishing the chemoreceptor 'drive' immediately increased the vascular resistance again.

4. Apnoeic asphyxia carried out while the carotid and aortic bodies were continuously perfused with oxygenated blood of normal P_{CO_2} had little or no effect on systemic vascular resistance.

5. The systemic vasoconstrictor response produced by apnoeic asphyxia was reduced or abolished by re-establishing the recipient animal's lung movements, and this effect occurred in the absence of changes in the composition of the blood perfusing the systemic circulation and arterial chemoreceptors. This abolition of the vasoconstriction was due to a pulmonary reflex.

6. Apnoeic asphyxia slowed the rate of the beating atria due to excita-

tion of the carotid and aortic body chemoreceptors. This response can be over-ridden by an inflation reflex arising from the lungs.

7. It is concluded that the cardiovascular responses observed in apnoeic asphyxia are due, at least in part, to primary reflexes from the carotid and aortic body chemoreceptors engendered by arterial hypoxia and hypercapnia. The appearance of these responses is, however, dependent upon there being no excitation of a pulmonary (inflation) vagal reflex.

INTRODUCTION

It is now well established that the arterial chemoreceptors affect reflexly the cardiovascular system as well as respiration. Stimulation of the carotid bodies causes slowing of the heart (Bernthal, Greene & Revzin, 1951; Daly & Scott, 1958; Downing, Remensnyder & Mitchell, 1962; MacLeod & Scott, 1964; Scott, 1966*a*, *b*) and systemic vasoconstriction (Bernthal, 1938; Daly & Scott, 1962; Daly & Ungar, 1966), whereas excitation of the aortic bodies elicits bradycardia or tachycardia (Comroe & Mortimer, 1964) and vasoconstriction (Daly, Hazzledine & Howe, 1965; Daly & Ungar, 1966).

Although these responses represent the direct or primary reflex effect on the heart and systemic blood vessels, they are not necessarily those which are observed when, for instance, the carotid bodies are excited by hypoxic blood in the spontaneously breathing animal. Under these conditions the predominant responses, at least in the dog, are tachycardia and vasodilatation. The reason for this is that the primary reflex bradycardia and vasoconstriction are masked or over-ridden by secondary mechanisms evoked by concomitant increase in breathing, and by changes in arterial blood pressure and secretions of catecholamines (Daly & Scott, 1958, 1963; Daly & Hazzledine, 1963; Daly & Ungar, 1966). One of the most important secondary mechanisms evoked by hyperventilation is a vagal reflex arising from the lungs when they are inflated, causing tachycardia (Hering, 1871; Anrep, Pascual & Rössler, 1936; Daly & Scott, 1958) and systemic vasodilatation (Salisbury, Galletti, Lewin & Rieben, 1959; Daly, Hazzledine & Ungar, 1967; Daly & Robinson, 1968).

Because of the prominent part played by secondary mechanisms controlling the cardiovascular system, it might appear that the primary cardiac and vascular responses from the arterial chemoreceptors are of academic interest only. However, there are naturally occurring situations in which arterial hypoxia and hypercapnia is accompanied not by hyperventilation but by a reduction or cessation of respiratory movements (apnoeic asphyxia), such as in diving (Andersen, 1966). Under these conditions it might be expected that the primary cardiac and vascular effects

resulting from stimulation of the chemoreceptors by hypoxic hypercapnic blood would be revealed because of the absence of opposing secondary respiratory mechanisms.

In this paper we report the results of experiments which demonstrate that bradycardia and vasoconstriction are elicited by apnoeic asphyxia and are due largely to excitation of arterial chemoreceptors. Our experiments also provide additional information on the interaction between these primary cardiac and vascular chemoreceptor responses and a pulmonary vagal inflation reflex.

Some of our results have been reported briefly elsewhere (Angell James & Daly, 1968).

METHODS

The preparation used in this study was a modification of that described previously by Daly & Ungar (1966), and therefore only the essential details of the technique and modifications will be given here.

Two dogs were used for each experiment and after premedication with morphine hydrochloride (2 mg/kg subcutaneously) were anaesthetized with 2·75 ml./kg of a mixture of α-chloralose (0·055 g/kg, Etablissements Kuhlmann, Paris) and urethane (0·55 g/kg, British Drug Houses, Ltd.) intravenously.

The recipient (test) dogs varied in weight from 11·7 to 16·2 kg. The chest was opened in the midsternal line after positive pressure artificial respiration had been established. The systemic circulation was perfused at constant volume blood flow by means of a Dale–Schuster pump through cannulae inserted into the femoral and vertebral arteries. The ventricles were tied tightly with tape immediately below the atrioventricular groove. The systemic venous blood returning to the right atrium was oxygenated in the isolated perfused lungs of the second donor dog, before being returned to the systemic circulation of the recipient animal. There was therefore no blood flowing through the pulmonary circulation of the recipient dog, but the blood supply to its lung was maintained by the bronchial circulation.

The isolated perfused lungs of the donor animal were ventilated artificially by a Starling 'Ideal' pump at 20 c/min, with a gas mixture of 50% O_2 in N_2. The tidal volume was adjusted to give an end-tidal P_{CO_2} of 35–40 mm Hg, measured with an infra-red CO_2 analyser (Type URAS 4, Hartmann & Braun).

Perfusion of the carotid and aortic body chemoreceptors. The carotid bifurcation regions were isolated from the circulation and perfused with blood through the common carotid arteries by means of a Dale–Schuster pump. Blood from the cannulated external carotid arteries was returned to the main reservoir through a Starling-type resistance by which the pressure could be controlled (Daly & Ungar, 1966). The aortic arch was isolated and perfused by a separate pump as described previously (Daly *et al.* 1965; Daly & Ungar, 1966). The aortic arch pressure was controlled by a second Starling-type resistance.

The two pumps each had an output of approximately 100 ml./min. The pump connexions were such that the two groups of chemoreceptors could be supplied with blood from either (1) the same reservoir that supplied blood to the pump perfusing the systemic circulation or (2) a rotating disk type 'oxygenator' through which a gas mixture of 95% O_2 and 5% CO_2 was pumped at a constant rate.

In all experiments coagulation of the blood was prevented by heparin ('Pularin', Evans Medical, Ltd., 2000 i.u./kg). To correct the metabolic acidosis which develops during the course of perfusion experiments sodium bicarbonate, 75 m-equiv, was added to the blood at the beginning of perfusion and then infused at a rate of 10–15 m-equiv/hr.

Measurement of atrial rate. The rate of the beating atria was recorded from electrodes attached to the right atrium using a pulse frequency meter (J. F. Tonnies, Freiburg Im Breisgau, Western Germany).

Respiration. After the necessary operative procedures had been completed and the four-pump perfusion system established the chest was closed, the air was withdrawn from the thorax and spontaneous breathing restored. The tidal air volume was recorded by means of a balanced spirometer.

Measurement of vascular pressures. Pressures in the carotid sinuses, aortic arch and femoral artery were measured simultaneously. Each pressure was measured with a Statham strain gauge (Model P 23 Gb) and after amplification by means of a carrier amplifier (S. E. Laboratories, Feltham, Middlesex) the pressure was recorded on a direct-writing ultra-violet light recorder (S. E. Laboratories). The mean pressure on each channel was obtained electrically by passing the amplifier output through a simple R-C network with a time constant of either 0·5 or 1 sec.

The manometers were calibrated before and after each experiment using a mercury manometer. Zero reference pressures were obtained post mortem and taken as those recorded when the tips of the needles or catheters were exposed to air.

Calculation of changes in systemic vascular resistance. Since the systemic blood flow remained constant within $\pm 4\%$, the change in vascular resistance may be taken as being proportional to the change in the pressure difference across the systemic circulation, i.e. the mean arterial perfusion pressure minus the mean right atrial pressure. As the right atrial pressure was maintained constant at approximately zero pressure, the change in vascular resistance can be expressed as a percentage change in arterial perfusion pressure.

Exclusion of the lung inflation reflex. Two methods were used. In the first, *changes in activity* of the lung inflation receptors were prevented by maintaining the volume of the lungs constant (Daly & Ungar, 1966). For this purpose a large bore tube (internal diameter 14 mm) was inserted through the fourth right intercostal space near the midsternal line, and the anterior mediastinum was removed so that the tube communicated with both pleural cavities. This tube was kept closed when tidal volume was being recorded during spontaneous breathing. To maintain the lung volume constant, the tubing connecting the tracheal cannula to the spirometer was clamped at the end of a normal expiration, and the tube in the chest wall was opened to the atmosphere. The latter tube acted as a conduit for the air passing in and out of the thorax during rhythmic activity of the respiratory muscles. This procedure was reversible, and normal spontaneous respiration could be re-established by unclamping the tracheal tubing, reducing the pneumothorax and occluding the tube in the chest wall.

The second method of excluding the lung inflation reflex consisted of denervating the lungs by cutting the thoracic vagosympathetic nerve on the left side between the aorta and left pulmonary artery, and on the right side at the level of the azygos vein (Daly & Scott, 1958).

Procedure for producing apnoeic asphyxia. Control records were first taken during spontaneous breathing while the systemic circulation and the carotid and aortic bodies were perfused with oxygenated blood. Apnoeic asphyxia was then produced. Movements of the recipient dog's lungs were stopped by occluding the tracheal tubing in the phase of expiration and opening the tube in the chest wall to the atmosphere. This procedure prevented changes in activity of the pulmonary inflation receptors but did not in itself alter the arterial blood gas composition because only the bronchial circulation of the lungs was perfused. At the same time the blood perfusing the systemic circulation and the carotid and aortic bodies was made hypoxic and hypercapnic by reducing the tidal volume of the isolated perfused lungs to approximately one fifth the control value. These procedures could be reversed at will.

The pressures in the carotid sinuses and aortic arch were maintained constant throughout.

Blood gas analysis. Samples of arterial blood withdrawn anaerobically from a systemic artery were transferred to electrode systems for measuring P_{O_2}, P_{CO_2} and pH as described previously by Daly & Ungar (1966).

Statistical analysis. The results are expressed as means ± 1 standard error of the mean (S.E. of mean). The Student t test was used to evaluate the significance of the difference of two means of grouped values. Where stated the significance of the difference from zero of the mean of the differences between paired observations was also determined.

RESULTS

The reflex activity of the carotid and aortic bodies was determined in each experiment under conditions in which the lungs of the recipient animal were ventilated artificially and the systemic circulation was perfused with oxygenated blood. Tests of separate stimulation of the carotid and aortic bodies were carried out by substituting hypoxic hypercapnic blood for oxygenated blood in the respective perfusion territories as described by Daly & Ungar (1966).

TABLE 1. Effects of stimulation of the carotid and aortic bodies by hypoxic hypercapnic blood on systemic vascular resistance. Recipient dog artificially ventilated. Perfusion in the systemic circulation with oxygenated blood

	No. of expts.	No. of tests	Systemic arterial perfusion pressure: Control (mm Hg)	Systemic arterial perfusion pressure: Increase (mm Hg)	Vascular resistance (% increase)
Carotid bodies	7	11	118·9 ± 4·9 (90–140)	46·6 ± 12·8 (14–160)	41·4 ± 11·5 (12–139)
Aortic bodies	7	11	121·7 ± 4·1 (88–138)	44·8 ± 8·3 (13–100)	38·1 ± 6·9 (10–80)

The open values are the means ± S.E. of mean; those in parentheses the range.

The results of eleven paired tests of stimulation of the carotid and aortic bodies in seven experiments are summarized in Table 1. In every test an increase in systemic arterial perfusion pressure occurred, and since the blood flow was maintained constant this indicates an increase in systemic vascular resistance. Stimulation of the carotid and aortic bodies increased vascular resistance by $41{\cdot}4 \pm 11{\cdot}5\,\%$ and $38{\cdot}1 \pm 6{\cdot}9\,\%$ respectively, and statistical analysis indicates these values are not significantly different from each other ($P > 0{\cdot}7$). Combined stimulation of the carotid and aortic bodies also caused increases in vascular resistance (Fig. 3*C*) and these responses were greater than either of their separate effects. These results confirm those of Daly & Ungar (1966).

In two experiments in which measurements of atrial rate were made, stimulation of the carotid bodies produced an average reduction in rate of 66 and 65 % respectively. Stimulation of the aortic bodies, however, had smaller effects, the average reduction in rate being 8 and 12 % respectively.

Combined stimulation of the two groups of chemoreceptors produced a fall in rate of 75 and 62 % respectively in the two experiments (Fig. 3*C*).

These results show that both the carotid bodies and aortic bodies were active in so far as their vascular and cardiac responses are concerned. Similar results were obtained after completion of the observations on apnoeic asphyxia.

Effects of apnoeic asphyxia on systemic vascular resistance

Table 2 shows the control values for respiratory minute volume, systemic arterial perfusion pressure, and the carotid and aortic arch perfusion pressures after closing the thorax, reducing the pneumothorax and re-establish-

TABLE 2. Initial control values for respiratory minute volume, mean systemic arterial perfusion prossure, mean carotid sinus and aortic arch perfusion pressures, and the composition of the blood perfusing the systemic circulation and isolated perfused carotid and aortic body chemoreceptors. (Seven experiments)

Dog wt. (kg)	13·4 ± 0·63 (11·7–16·2)
Respiratory minute volume (l./min/m²)	5·4 ± 0·55 (3·72–7·65)
Systemic arterial perfusion pressure (mm Hg)	125·7 ± 4·6 (112–140)
Carotid sinus perfusion pressure (mm Hg)	117·2 ± 4·4 (104–132)
Aortic arch perfusion pressure (mm Hg)	111·6 ± 3·5 (98–122)
Blood perfusate	
P_{O_2} (mm Hg)	136·8 ± 14·0 (117–206)
P_{CO_2} (mm Hg)	41·5 ± 2·2 (34–48)
pH	7·38 ± 0·027 (7·31–7·49)

The open values are the means ± S.E. of mean; those in parentheses the ranges.

TABLE 3. Values for blood P_{O_2}, P_{CO_2} and pH before (*A*) and during (*B*) apnoeic asphyxia. Values for equilibrated blood (*C*) used to withdraw the carotid and aortic body 'drive'. (Seven experiments)

	P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)	pH
A. Control (systemic circulation, carotid and aortic body perfusate)	133·4 ± 11·1 (103–172)	42·8 ± 2·2 (37–51)	7·39 ± 0·022 (7·31–7·45)
B. During apnoeic asphyxia (systemic circulation, carotid and aortic body perfusate)	56·6 ± 5·3 (38–74)	58·1 ± 3·5 (49–70)	7·25 ± 0·021 (7·19 – 7·32)
C. Equilibrated blood (carotid and aortic body perfusate)	306·2 ± 82·1 (140–576)	42·0 ± 1·6 (35–47)	7·38 ± 0·006 (7·36–7·40)

The open values are the means ± S.E. of mean; those in parentheses the ranges.

ing spontaneous breathing. Under these conditions blood from the isolated perfused donor lungs perfused the three vascular territories, and its gaseous composition is also shown in Table 2.

In seven experiments, twelve tests of apnoeic asphyxia were carried out as described in Methods. The blood perfusing the systemic circulation and the carotid and aortic bodies gradually became hypoxic and hypercapnic because the ventilation of the isolated perfused lung had been reduced.

The values for P_{O_2}, P_{CO_2} and pH before and after a steady state had been reached during apnoeic asphyxia are shown in Table 3*A*, *B*.

Apnoeic asphyxia caused an immediate small rise in systemic arterial perfusion of up to 20 mm Hg (within 15–20 sec) which in all probability is due to a reflex resulting from a slight diminution in mean lung volume (Daly *et al.* 1967) (Fig. 1*A*). Thereafter there was a more gradual rise in perfusion pressure which reached a new steady level in 4–7 min. The pressure increased by 29–188 mm Hg (mean 77·4 ± 19·1), from a mean control level of 122·0 ± 2·7 mm Hg (range 100–148) to 199·2 ± 19·9 mm Hg (range

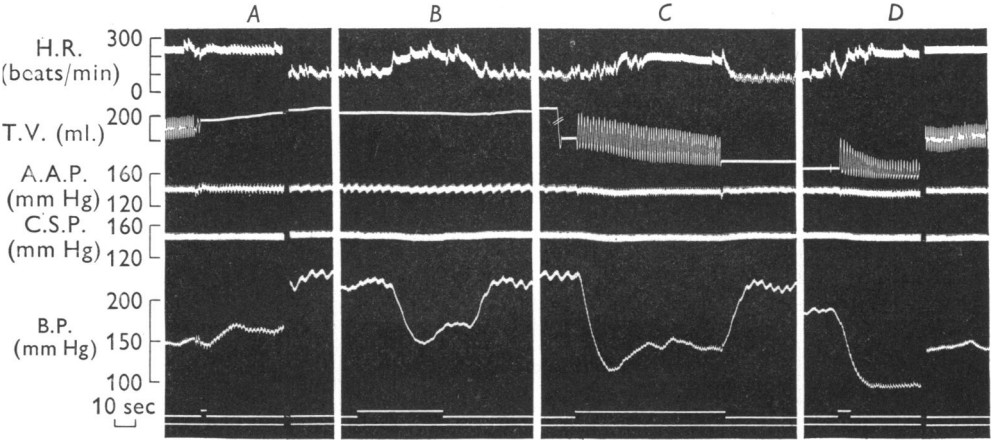

Fig. 1. The effects of withdrawing the arterial chemoreceptor 'drive' and of re-establishing lung movements during apnoeic asphyxia. Dog, male, 13·4 kg. Morphine-chloralose-urethane. Perfusion of the systemic circulation at constant blood flow. Separate perfusions of the isolated carotid sinuses and isolated aortic arch. No pulmonary circulation. Systemic venous blood oxygenated in isolated perfused lungs of a donor animal. Closed chest. Spontaneous respiration. Carotid sinus, aortic arch and arterial blood P_{O_2} 133 mm Hg, P_{CO_2} 45 mm Hg, pH 7·34. *A*, at signal, apnoeic asphyxia carried out by reducing the tidal volume of the isolated perfused donor lungs from 400 to 75 ml., and at the same time creating a bilateral open pneumothorax in the recipient animal, the lungs being held in a semi-inflated position by occluding the trachea. Break in record lasted 3·5 min. *B*, apnoeic asphyxia continued (carotid sinus, aortic arch and arterial blood P_{O_2} 64 mm Hg, P_{CO_2} 70 mm Hg, pH 7·19). During signal, carotid and aortic body chemoreceptor 'drive' withdrawn by substituting oxygenated blood (P_{O_2} 140 mm Hg, P_{CO_2} 44 mm Hg, pH 7·39). *C*, apnoeic asphyxia continued (carotid sinus, aortic arch and arterial blood P_{O_2} 74 mm Hg, P_{CO_2} 70 mm Hg, pH 7·19). During signal, pneumothorax reduced and spontaneous breathing temporarily re-established. *D*, at signal, end of period of apnoeic asphyxia. Spontaneous breathing re-establishing and tidal volume of isolated perfused donor lungs restored to 400 ml. Break in record of 4 min. Time calibration, 10 sec.

In this and in subsequent figures: H.R., heart (atrial) rate; T.V., tidal volume (inspiration upwards); C.S.P., carotid sinus pressure; A.A.P., aortic arch pressure; B.P., systemic arterial perfusion blood pressure; R.M.V., respiratory minute volume.

145–306) ($P < 0{\cdot}005$). This represents an increase in vascular resistance of $63{\cdot}2 \pm 15{\cdot}8$ % (range 25–159). The typical response is shown in Fig. 1*A* and the results of all experiments are summarized in Fig. 2*A*.

In two further tests, apnoeic asphyxia was produced in a different way. Pulmonary ventilation of the isolated perfused donor lung was diminished

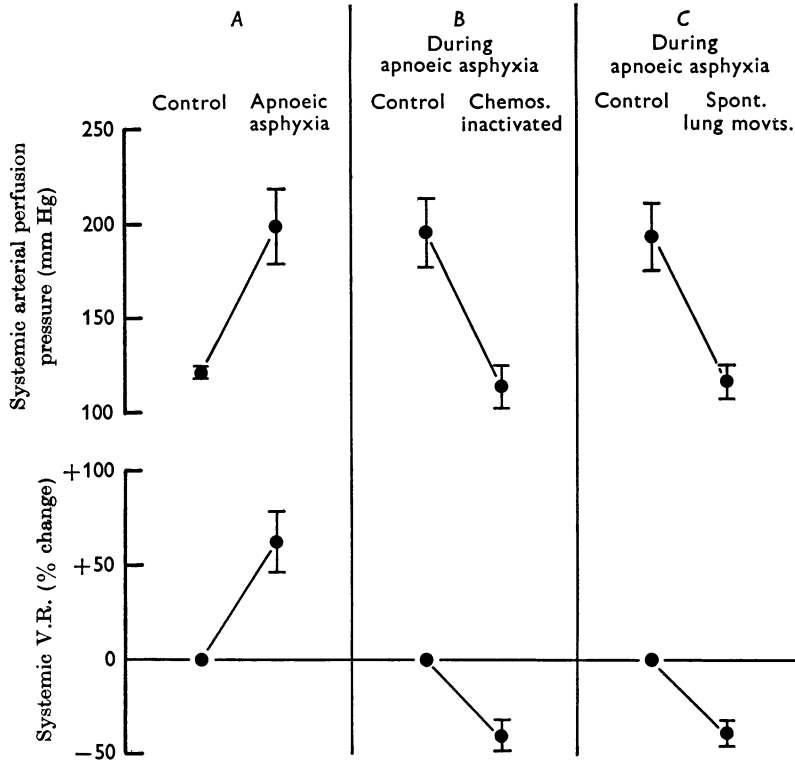

Fig. 2. *A*, the effects of apnoeic asphyxia on systemic arterial perfusion pressure and systemic vascular resistance (twelve observations in seven experiments). *B*, the effects of withdrawing the chemoreceptor 'drive' during apnoeic asphyxia by substituting oxygenated blood perfusion of the carotid and aortic bodies (twelve observations in seven experiments). *C*, the effects of reducing the pneumothorax and re-establishing spontaneous lung movements during apnoeic asphyxia; systemic circulation and carotid and aortic bodies perfused with hypoxic hypercapnic blood (eight observations in five experiments). The mean values (± S.E. of mean) are shown.

to produce systemic hypoxia and hypercapnia but lung movements in the recipient animal were stopped, not by creating a bilateral pneumothorax and occluding the trachea, but by maintaining the chest closed and paralysing the muscles of respiration by injecting into the systemic arterial tubing a neuromuscular blocking agent, decamethonium iodide (0·2 mg/

kg, Light & Co., Ltd.). A rise in systemic arterial perfusion pressure was observed from 115 to 164 mm Hg in one case and from 140 to 195 mm Hg in the other (Fig. 3*A*), representing an increase in vascular resistance of 43 and 39 % respectively.

Effects of withdrawing the chemoreceptor 'drive' during apnoeic asphyxia

The possibility that the increased systemic vascular resistance observed in asphyxia is due, at least in part, to a reflex resulting from stimulation of the carotid and aortic bodies was examined by withdrawing the chemoreceptor 'drive' during the period of apnoeic asphyxia.

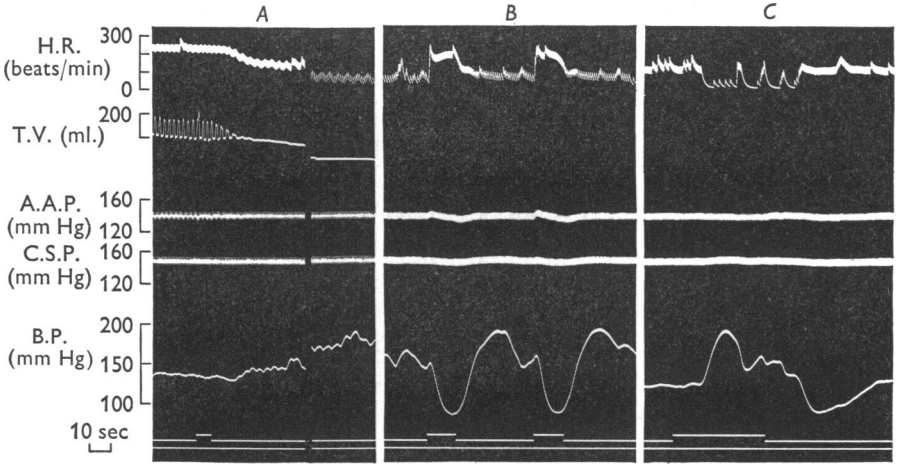

Fig. 3. The effects of inflation of the recipient animal's lungs during apnoeic asphyxia. Same experiment as Fig. 1. Closed chest. Spontaneous respiration. Carotid sinus, aortic arch and arterial blood P_{O_2} 138 mm Hg, P_{CO_2} 42 mm Hg, pH 7·35. *A*, at signal apnoeic asphyxia produced by reducing tidal volume of isolated perfused donor lungs from 400 to 75 ml., and at the same time abolishing the recipient animal's lung movements by injecting decamethonium, 3 mg, into the systemic arterial inflow tube. Break in record lasted 2 min. *B*, apnoeic asphyxia continued (carotid sinus, aortic arch and arterial blood P_{O_2} 70 mm Hg, P_{CO_2} 71 mm Hg, pH 7·19). Two maintained inflations of the lungs with approximately 300 ml. air injected into trachea. Between *B* and *C*, tidal volume of isolated perfused lungs restored to 400 ml. *C*, combined stimulation of the carotid and aortic bodies by hypoxic hypercapnic blood (P_{O_2} 40 mm Hg, P_{CO_2} 51 mm Hg, pH 7·30). Systemic arterial blood P_{O_2} 130 mm Hg, P_{CO_2} 41 mm Hg, pH 7·35. Time calibration, 10 sec.

The typical response is illustrated by Fig. 1*B*. When a steady state had been reached during asphyxia the chemoreceptor 'drive' was withdrawn by changing the carotid and aortic body perfusates simultaneously from hypoxic hypercapnic blood to oxygenated blood obtained from a rotating disk oxygenator supplied with a gas mixture of 95 % O_2 and 5 % CO_2. Values for the P_{O_2}, P_{CO_2} and pH of the oxygenated blood are shown in Table 3*C*. Meanwhile the systemic circulation continued to be perfused

with hypoxic hypercapnic blood. It will be observed (Fig. 1*B*) that a fall in systemic arterial perfusion pressure occurred, which was restored to its original level on re-establishing hypoxic hypercapnic blood perfusion of the chemoreceptors. There was a delay of 10–15 sec in the onset of the responses due to the time taken for the blood to traverse the perfusion circuit.

The results of twelve such tests in seven experiments are summarized in Fig. 2*B*. Withdrawal of the chemoreceptor 'drive' caused a fall in systemic arterial perfusion pressure of 34–163 mm Hg (mean $81{\cdot}0 \pm 13{\cdot}2$), from an initial value of $196{\cdot}0 \pm 19{\cdot}2$ mm Hg (range 138–298) to a final value of $115{\cdot}1 \pm 5{\cdot}6$ mm Hg (range 82–144) ($P < 0{\cdot}001$). This represents a reduction in systemic vascular resistance of $40{\cdot}3 \pm 3{\cdot}9\,\%$ (range 25–60).

Control tests were carried out before producing asphyxia, in which blood from the oxygenator was substituted for the control chemoreceptor perfusate (Table 3*A*). No effect on systemic arterial perfusion pressure was observed.

The role of the arterial chemoreceptors in the production of the increased systemic vascular resistance in apnoeic asphyxia can be further assessed by comparing the values for systemic arterial perfusion pressure during the control state, and during asphyxia with the chemoreceptors inactivated by perfusion with oxygenated blood (Fig. 2*A*, *B*). The two values are $122{\cdot}0 \pm 2{\cdot}7$ and $115 \pm 5{\cdot}6$ mm Hg respectively, and their statistical evaluation, including individual and group comparisons, shows the difference is not significant ($P > 0{\cdot}2$). These results were confirmed in two further experiments in which apnoeic asphyxia was produced while the carotid and aortic bodies were perfused with oxygenated blood from the oxygenator. No change in systemic arterial perfusion pressure occurred in one experiment; in the other, there was a fall of 12 mm Hg.

It is concluded from these experiments that the increase in systemic vascular resistance occurring during apnoeic asphyxia is due largely to stimulation of the carotid and aortic body chemoreceptors by hypoxic hypercapnic blood.

Role of pulmonary vagal inflation reflex

Cessation of lung movements during apnoeic asphyxia determines the observed systemic response through a pulmonary vagal reflex. This was shown in experiments in which the effects of hypoxic hypercapnic blood perfusion of both the systemic circulation and arterial chemoreceptors were compared under two different experimental conditions, viz. in the absence of lung movements, and in the presence of spontaneous rhythmic movements of the lungs.

The results of eight tests in five experiments are shown in Fig. 4*A*.

Perfusion of the recipient animal (systemic circulation and arterial chemoreceptors) with hypoxic hypercapnic blood in the absence of lung movements caused an increase in systemic arterial perfusion pressure of 28–151 mm Hg (mean 70·3 ± 16·8), from an initial mean pressure of 122·6 ± 5·8 to a final pressure of 193·0 ± 19·7 mm Hg ($P < 0{\cdot}005$). By comparison,

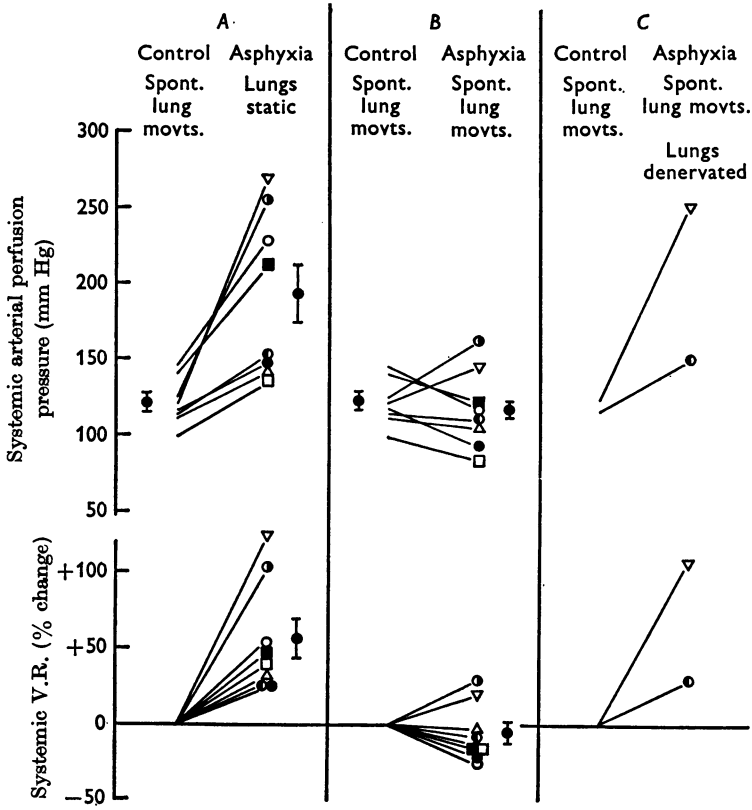

Fig. 4. The effects of perfusion of the systemic circulation, carotid and aortic bodies with hypoxic hypercapnic blood on systemic arterial perfusion pressure and systemic vascular resistance. *Control observations*: *A*, *B* and *C*, closed chest, spontaneous lung movements, oxygenated blood perfusion of the systemic circulation and arterial chemoreceptors. *Experimental observations during asphyxia*: *A*, open pneumothorax, lungs held at constant volume; *B*, closed chest, spontaneous lung movements; *C*, closed chest, spontaneous lung movements, lungs denervated. The mean values (± S.E. of mean) are shown in *A* and *B* for the eight observations in five experiments. In *C*, two observations in two experiments.

perfusion of the animal with hypoxic hypercapnic blood in the presence of spontaneous rhythmic lung movements resulted in a much reduced response in two tests and a fall in pressure in the other six (Fig. 4*B*). The mean change in pressure was a fall of 4·9 ± 8·6 mm Hg (range −30 to

+37), from an initial mean pressure of 122·6 ± 5·8 mm Hg to 117·7 ± 4·6 mm Hg ($P > 0·5$).

The absence of a vascular response in the second group of experiments was due to the over-riding effect of the lung inflation reflex. This was shown in two animals in which the lungs were subsequently denervated. In response to perfusion of the animal with hypoxic hypercapnic blood the arterial perfusion pressure now increased by 30 and 130 mm Hg respectively. These values may be compared with those of −3 and +26 mm Hg before denervation (Fig. 4*B*, *C*), and are reasonably close to the values of 37 and 159 mm Hg respectively for the rise in pressure which occurred under conditions in which asphyxia was produced while the innervated lungs were maintained at a constant volume (Fig. 4*A*, *C*).

The over-riding effect of the lung inflation reflex is also illustrated by Fig. 3*B* taken from an experiment in which the lungs were inflated on two separate occasions with approximately 300 ml. air injected into the trachea during apnoeic asphyxia. This resulted in striking falls in systemic arterial perfusion pressure.

In two experiments *changes in activity* of the pulmonary inflation receptors were prevented by maintaining artificial respiration of the recipient animal's lungs by means of a Starling 'Ideal' pump. Perfusion of the systemic circulation and arterial chemoreceptors with hypoxic blood caused a rise in systemic arterial perfusion of 38 and 89 mm Hg respectively, representing increase in systemic vascular resistance of 32 and 84 %.

Effects of re-establishing lung movements during apnoeic asphyxia. This procedure was carried out in the recipient animal by removing the occlusive clamp on the tracheal tubing, reducing the pneumothorax and occluding the tube in the chest wall (see Methods). Under these conditions the respiratory minute volume increased from zero to 7·86 ± 0·87 l./min, compared with a pre-asphyxial value of 3·38 ± 0·41 l./min.

The typical response is illustrated by Fig. 1*C* and the results of eight such tests in five experiments are summarized in Fig. 2*C*. Restoration of lung movements caused a fall in systemic arterial perfusion pressure of 32–106 mm Hg (mean 76·1 ± 11·6), from an initial value of 193 ± 18·9 mm Hg (range 138–265) to a final value of 117·7 ± 4·6 mm Hg (range 84–162) ($P < 0·001$). This represents an average reduction in systemic vascular resistance of 38·0 ± 3·2 % (range 23–50). No response occurred after denervation of the lungs (Fig. 5).

Effects of apnoeic asphyxia on atrial rate

Several observations were made in two experiments of the changes in rate of the beating atria, and the typical responses are illustrated by Figs. 1 and 3. Apnoeic asphyxia caused a pronounced reduction in atrial rate

from 230 to 115 (Fig. 1*A*) and from 240 to 60 beats/min (Fig. 3*A*) respectively. This slowing of the rate was due largely to stimulation of the carotid and aortic bodies by hypoxic hypercapnic blood, since withdrawal of the chemoreceptor 'drive' by perfusion of the carotid sinuses and aortic arch with oxygenated blood during asphyxia caused an immediate

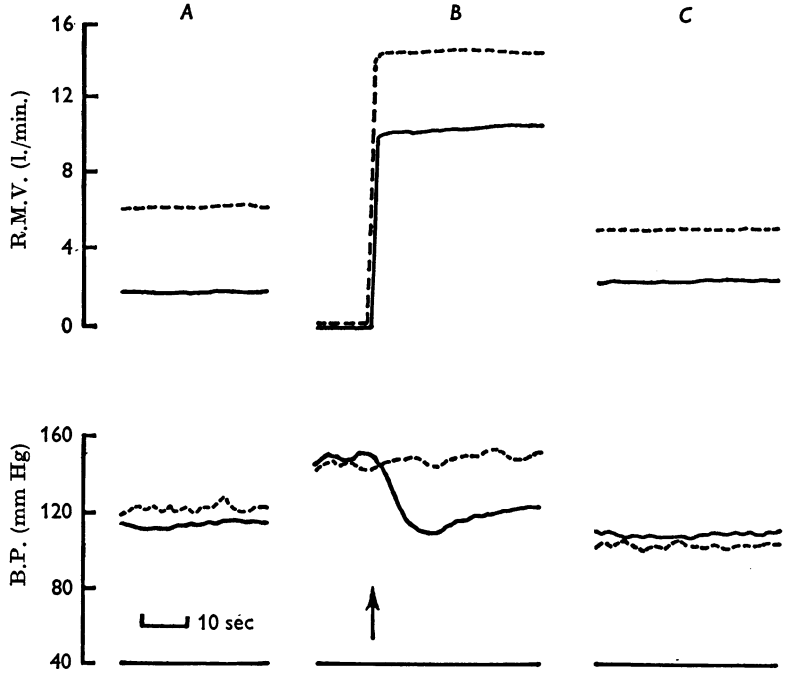

Fig. 5. The effects of re-establishing spontaneous rhythmic movements of the lungs during apnoeic asphyxia before (*continuous lines*) and after (*interrupted lines*) denervation of the lungs. Dog, female, 16·2 kg. Morphine-chloralose-urethane. Perfusion of the systemic circulation at constant blood flow. Separate perfusions of the isolated carotid sinuses and isolated aortic arch. No pulmonary circulation. Systemic venous blood oxygenated in isolated perfused lungs of a donor animal. Closed chest. Spontaneous respiration. *A* and *C*, controls. Systemic circulation and arterial chemoreceptors perfused with oxygenated blood (P_{O_2} 173 mm Hg, P_{CO_2} 44 mm Hg, pH 7·44). *B*, 6·5 min after producing apnoeic asphyxia (blood P_{O_2} 55 mm Hg, P_{CO_2} 52 mm Hg, pH 7·32). At arrow ↑, spontaneous rhythmic movements of lungs re-established. Time calibration, 10 sec.

increase in rate from 115 to 195 beats/min (Fig. 1*B*). This response was reversed on re-introduction of hypoxic hypercapnic blood to the chemoreceptors.

The effects of restoration of lung movements during apnoeic asphyxia are shown in Fig. 1*C*, from which it may be seen that the atrial rate increased from 90 to 190 beats/min. It returned to almost its original value

when lung movements were stopped again. Comparison of the control pre-'asphyxial' value with that during asphyxia but in the presence of spontaneous lung movements shows that there is now much less difference, 230 and 190 beats/min respectively. This suggests that the appearance of the bradycardia during apnoeic asphyxia, as with the vasoconstrictor response, is dependent upon there being no over-riding effect from the pulmonary inflation reflex which is known to cause tachycardia (Hering, 1871; Anrep *et al.* 1936; Daly & Scott, 1958) (see also Fig. 3*B*).

Finally, lung movements were re-established and at the same time ventilation of the isolated perfused donor lungs was increased to its original value (Fig. 1*D*), and this resulted in the atrial rate returning to its control level.

DISCUSSION

Role of the arterial chemoreceptors

Vascular responses. It has been shown that in apnoeic asphyxia in the anaesthetized dog, an increase in systemic vascular resistance occurs. Since the systemic blood flow was maintained constant, this response must be due to predominance of vasoconstriction but no studies have been carried out to establish the vascular territories in which it occurs. The possibility that a decrease in vascular resistance takes place in some areas cannot be ruled out. This vasoconstrictor response occurring in apnoeic asphyxia is due largely to a reflex from stimulation of the carotid and aortic body chemoreceptors by the gradually developing arterial hypoxia and hypercapnia. It has been shown previously that constriction represents the primary vascular effect of stimulation of the carotid and aortic bodies (Bernthal, 1938; Bernthal & Schwind, 1945; Daly & Daly, 1959; Daly & Scott, 1962; Daly *et al.* 1965; Daly & Ungar, 1966). Although in the dog at least both groups of chemoreceptors are equally effective in so far as vasomotor effects are concerned under conditions of oxygenated blood perfusion of the systemic circulation (Daly & Ungar, 1966; present paper), no specific studies have been carried out to determine the relative contribution of the two groups of chemoreceptors in the production of the vascular response observed in apnoeic asphyxia.

From previous observations (Bernthal, Motley, Schwind & Weeks, 1945; Daly *et al.* 1965), it is likely that the efferent pathway mediating the vasoconstrictor responses through stimulation of the arterial chemoreceptors is by way of sympathetic adrenergic nerve fibres. The possibility that the responses are due in part to secretions of catecholamines from the suprarenal medulla cannot be ruled out. However, the fact that withdrawal of the chemoreceptor 'drive' during apnoeic asphyxia caused a maximum response within 40 sec indicates it is largely neurogenic, since this was the

circulation time from the suprarenal glands to the systemic arterioles via the blood reservoirs and isolated perfused lungs.

Cardiac responses. Evidence has been presented that the profound bradycardia occurring during apnoeic asphyxia is due to a reflex from the arterial chemoreceptors. In the two experiments cited it would appear that both the carotid and aortic bodies contribute to the response as their separate stimulations by hypoxic hypercapnic blood also resulted in cardio-inhibition. Nevertheless, a greater contribution emanated from stimulation of the carotid bodies. Comroe & Mortimer (1964) found that stimulation of the aortic bodies by drugs caused reflex bradycardia or tachycardia. It would therefore be profitable to study in a larger series of experiments the cardiac reflexes elicited by excitation of this group of chemoreceptors by means of a natural stimulus.

Bradycardia and vasoconstriction have been observed previously during apnoeic asphyxia, for example, by cessation of artificial respiration in dogs paralysed with a neuromuscular blocking agent (Nahas, 1956; Mithoefer, 1965). Ebert, Greenfield & Austen (1962) showed in dogs with separately perfused systemic and coronary circulations that hypoxia of the systemic circulation alone caused bradycardia or cardiac arrest which was abolished by vagotomy. They concluded that the response was due to stimulation of peripheral receptors. On the basis of the results reported in the present paper, it is likely that stimulation of arterial chemoreceptors plays an important part in the production of the bradycardia under these conditions. Electrical stimulation of the amygdala in the spontaneously breathing monkey causes apnoea and bradycardia, the latter response being abolished by division of the vagus nerves (Reis & McHugh, 1968). These authors presented evidence that the bradycardia was secondary to arterial hypoxia and hypercapnia induced by the apnoea which resulted directly from amygdala stimulation. They suggested that the cardiac response was reflexly engendered through excitation of peripheral arterial chemoreceptors.

Role of a pulmonary vagal reflex

Under conditions in which arterial hypoxia or hypoxia and hypercapnia are present the potency of the primary cardiovascular responses from arterial chemoreceptors depends to a large extent on the concomitant change in pulmonary ventilation. Previous studies have dealt with the importance of the accompanying hyperpnoea in this connexion, and these have demonstrated the over-riding influence (tachycardia and vasodilatation) which secondary respiratory mechanisms, in particular a pulmonary vagal inflation reflex, exert in determining the cardiovascular responses to stimulation of the carotid bodies and to ventilation with gas mixtures of low oxygen content (Daly & Scott, 1958, 1963; Daly & Hazzledine, 1963;

Korner, 1965; Kontos, Mauck, Richardson & Patterson, 1965; Daly & Ungar, 1966; Scott, 1966*a*, *b*; Kontos, Goldin, Richardson & Patterson, 1967; Chalmers, Korner & White, 1967).

The results of the present experiments have confirmed these findings and have shown further that in the absence of lung movements, such as during apnoeic asphyxia, the primary cardiovascular reflex effects from stimulation of the arterial chemoreceptors are prepotent. This is due to the fact that the activity of the pulmonary inflation receptors in lungs static and partially collapsed is minimal (Anrep *et al.* 1936; Daly *et al.* 1967) and in consequence no secondary reflex tachycardia and vasodilatation of pulmonary origin are evoked contemporaneously.

Our findings may have a bearing on certain conditions, such as diving, in which there is cessation of breathing associated with bradycardia and systemic vasoconstriction (see review by Andersen, 1966). One mechanism responsible for these cardiovascular effects is arterial hypoxia and hypercapnia resulting from apnoea (Feigl & Folkow, 1963; Andersen, 1963*a*, *b*). Our results are pertinent in two respects. First, they indicate that excitation of arterial chemoreceptors by hypoxic hypercapnic blood might play an important part in bringing about the cardiovascular effects observed in diving. In this connexion Hollenberg & Uvnäs (1963) found in the anaesthetized duck that asphyxia produced by immersion of the beak caused apnoea and bradycardia, the latter response being abolished by combined denervation of the carotid baroreceptors and chemoreceptors. Secondly, our finding that the appearance of the bradycardia and systemic vasoconstrictor response during apnoeic asphyxia is dependent on there being no increase in the activity of pulmonary inflation receptors suggests that in natural diving, apnoea which occurs reflexly through excitation of nasal receptors (Huxley, 1913), not only serves as a protective reflex to prevent water gaining access to the respiratory tract, but also as a purposeful reflex playing an essential part in the integration of nervous mechanisms leading to the observed cardiovascular adaptations.

We wish to express our thanks to Mr D. R. Bacon for technical assistance, to Dr A. Ungar for help in some of the experiments, and to the Medical Research Council for a grant to one of us (M. de B. D.) defraying part of the expenses of this work. This project was also generously supported by the St Bartholomew's Hospital Endowment Fund.

REFERENCES

ANDERSEN, H. T. (1963*a*). Factors determining the circulatory adjustments to diving. I. Water immersion. *Acta physiol. scand.* **58**, 173–185.

ANDERSEN, H. T. (1963*b*). Factors determining the circulatory adjustments to diving. II. Asphyxia. *Acta physiol. scand.* **58**, 186–200.

ANDERSEN, H. T. (1966). Physiological adaptations in diving vertebrates. *Physiol. Rev.* **46**, 212–243.

ANGELL JAMES, J. E. & DALY, M. DE B. (1968). Role of the peripheral arterial chemoreceptors in the cardiovascular adaptations occurring during simulated dives in the anaesthetized dog. *J. Physiol.* **198**, 52–53*P*.

ANREP, G. V., PASCUAL, W. & RÖSSLER, R. (1936). Respiratory variations of the heart rate. The reflex mechanism of the respiratory arrhythmia. *Proc. R. Soc.* B **119**, 191–217.

BERNTHAL, T. (1938). Chemo-reflex control of vascular reactions through the carotid body. *Am. J. Physiol.* **121**, 1–20.

BERNTHAL, T., GREENE W., JR. & REVZIN, A. M. (1951). Role of carotid chemoreceptors in hypoxic cardiac acceleration. *Proc. Soc. exp. Biol. Med.* **76**, 121–124.

BERNTHAL, T. G., MOTLEY, H. E., SCHWIND, F. J. & WEEKS, W. F. (1945). The efferent pathway of chemoreflex vasomotor reactions arising from the carotid body. *Am. J. Physiol.* **143**, 220–225.

BERNTHAL, T. G. & SCHWIND, F. J. (1945). A comparison in intestine and leg of the reflex vascular response to carotid-aortic chemoreceptor stimulation. *Am. J. Physiol.* **143**, 361–372.

CHALMERS, J. P., KORNER, P. I. & WHITE, S. W. (1967). The relative roles of the aortic and carotid sinus nerves in the rabbit in the control of respiration and circulation during arterial hypoxia and hypercapnia. *J. Physiol.* **188**, 435–450.

COMROE, J. H. & MORTIMER, L. (1964). The respiratory and cardiovascular responses of temporally separated aortic and carotid bodies to cyanide, nicotine, phenyldiguanide and serotonin. *J. Pharmac. exp. Ther.* **146**, 33–41.

DALY, I. DE B. & DALY, M. DE B. (1959). The effects of stimulation of the carotid body chemoreceptors on the pulmonary vascular resistance in the dog: the 'vasosensory controlled perfused living animal' preparation. *J. Physiol.* **148**, 201–219.

DALY, M. DE B. & HAZZLEDINE, J. L. (1963). The effects of artificially induced hyperventilation on the primary cardiac reflex response to stimulation of the carotid bodies in the dog. *J. Physiol.* **168**, 872–889.

DALY, M. DE B., HAZZLEDINE, J. L. & HOWE, A. (1965). Reflex respiratory and peripheral vascular responses to stimulation of the isolated perfused aortic arch chemoreceptors of the dog. *J. Physiol.* **177**, 300–322.

DALY, M. DE B., HAZZLEDINE, J. L. & UNGAR, A. (1967). The reflex effects of alterations in lung volume on systemic vascular resistance in the dog. *J. Physiol.* **188**, 331–351.

DALY, M. DE B. & ROBINSON, B. H. (1968). An analysis of the reflex systemic vasodilator response elicited by lung inflation in the dog. *J. Physiol.* **195**, 387–406.

DALY, M. DE B. & SCOTT, M. J. (1958). The effects of stimulation of the carotid body chemoreceptors on heart rate in the dog. *J. Physiol.* **144**, 148–166.

DALY, M. DE B. & SCOTT, M. J. (1962). An analysis of the primary cardiovascular reflex effects of stimulation of the carotid body chemoreceptors in the dog. *J. Physiol.* **162**, 555–573.

DALY, M. DE B. & SCOTT, M. J. (1963). The cardiovascular responses to stimulation of the carotid body chemoreceptors in the dog. *J. Physiol.* **165**, 179–197.

DALY, M. DE B. & UNGAR, A. (1966). Comparison of the reflex responses elicited by stimulation of the separately perfused carotid and aortic body chemoreceptors in the dog. *J. Physiol.* **182**, 379–403.

DOWNING, S. E., REMENSNYDER, J. P. & MITCHELL, J. H. (1962). Cardiovascular responses to hypoxic stimulation of the carotid bodies. *Circulation Res.* **10**, 676–685.

EBERT, P. A., GREENFIELD, J. & AUSTEN, W. G. (1962). The mechanism of cardiac arrest during hypoxia. *Surg. Forum* **13**, 187–189.

FEIGL, E. & FOLKOW, B. (1963). Cardiovascular responses in 'diving' and during brain stimulation in ducks. *Acta physiol. scand.* **57**, 99–110.

HERING, E. (1871). Über den Einfluss der Atmung auf den Kreislauf. Zweite Mittheilung. Über eine reflectorische Beziehung zwischen Lunge und Herz. *Aber. Akad. Wiss. Wien* **64**, 333–353.

HOLLENBERG, N. K. & UVNÄS, B. (1963). The role of the cardiovascular response in the resistance to asphyxia of avian divers. *Acta physiol. scand.* **58**, 150–161.

HUXLEY, F. M. (1913). On the reflex nature of apnoea in the duck in diving: I. The reflex nature of submersion apnoea. *Q. Jl exp. Physiol.* **6**, 147–157.

KONTOS, H. A., GOLDIN, D., RICHARDSON, D. W. & PATTERSON, J. L., JR. (1967). Contribution of pulmonary vagal reflexes to circulatory response to hypoxia. *Am. J. Physiol.* **212**, 1441–1446.

KONTOS, H. A., MAUCK, H. P., JR., RICHARDSON, D. W. & PATTERSON, J. L., JR. (1965). Mechanism of circulatory responses to systemic hypoxia in the anaesthetized dog. *J. appl. Physiol.* **209**, 397–403.

KORNER, P. I. (1965). The role of the arterial chemoreceptors and baroreceptors in the circulatory response to hypoxia of the rabbit. *J. Physiol.* **180**, 279–303.

MACLEOD, R. D. M. & SCOTT, M. J. (1964). The heart rate responses to carotid body chemoreceptor stimulation in the cat. *J. Physiol.* **175**, 193–202.

MITHOEFER, J. C. (1965). Breath holding. In *Handbook of Physiology*, section 3, Respiration, ed. FENN, W. O. & RAHN, H., vol. II, pp. 1011–1026. Washington D.C.: American Physiological Society.

NAHAS, G. S. (1956). Heart rate during short periods of apnea in curarized dogs. *Am. J. Physiol.* **187**, 302–306.

REIS, D. J. & MCHUGH, P. R. (1968). Hypoxia as a cause of bradycardia during amygdala stimulation in monkey. *Am. J. Physiol.* **214**, 601–610.

SALISBURY, P. F., GALLETTI, P. M., LEWIN, R. J. & RIEBEN, P. A. (1959). Stretch reflexes from the dog's lung to the systemic circulation. *Circulation Res.* **7**, 62–67.

SCOTT, M. J. (1966*a*). The effects of hyperventilation on the reflex cardiac response from the carotid bodies in the cat. *J. Physiol.* **186**, 307–320.

SCOTT, M. J. (1966*b*). Reflex effects of carotid body chemoreceptor stimulation on the heart rate of the rabbit. *Aust. J. exp. Biol. med. Sci.* **44**, 393–404.