J. Physiol. (1976), **259**, *pp.* 575–596
With 5 text-figures
Printed in Great Britain

RESPIRATORY AND CARDIOVASCULAR INTERACTIONS IN DUCKS: THE EFFECT OF LUNG DENERVATION ON THE INITIATION OF AND RECOVERY FROM SOME CARDIOVASCULAR RESPONSES TO SUBMERGENCE

BY OWEN S. BAMFORD* AND DAVID R. JONES†
From the Zoology Department, University of British Columbia, Vancouver, B.C., V6T 1W5, Canada

(*Received 28 April 1975*)

SUMMARY

1. Lung denervation in ducks, by sectioning all vagal branches to one lung following mid-cervical vagotomy on the other side, resulted in immediate bradycardia and fall in breathing frequency. Some 3–5 weeks after lung denervation breathing frequency was within the normal range but the lung inflation reflex, present in unilaterally vagotomized sham-operated ducks, was abolished.

2. During 2 min dives there were no significant differences between sham-operated and denervated ducks in heart rate, arterial blood pressure, blood gas tensions and pH_a. However, during recovery from diving heart rate increased more slowly in denervates and breathing rate was significantly below that attained by shams, although tidal volume rose to a maximum of 225% of the pre-dive value in denervates in contrast to a maximum increase of 139% of pre-dive in sham-operated ducks. Both sham-operated and denervated ducks exhibited a significant fall in diastolic blood pressure 60 sec after emergence.

3. A single passive lung inflation with air during a dive caused a significant increase in heart rate in normal and sham-operated but not denervated ducks. Addition of 60 ± 4 mmHg P_{CO_2} to the inflating gas prevented this cardio-acceleration. However, in unoperated, sham-operated, and denervated animals a significant increase in heart rate occurred with the first voluntary breath terminating a dive, even if the animal was surfaced into air containing 60 ± 4 mmHg P_{CO_2}.

4. Respiratory motor neurone activity was monitored in an intercostal nerve of paralysed ducks either on unidirectional ventilation or during

* Killam Post-Doctoral Fellow.
† Killam Senior Fellow.

asphyxia. Cardio-acceleration and hypertension accompanied each inspiratory burst. The increase in heart rate was $43{\cdot}9 \pm 3{\cdot}2\%$ of the maximum possible change which could have occurred (i.e. maximum rate in that duck minus the pre-burst rate). Increases in heart rate usually preceded those in blood pressure although only the former were abolished by bilateral vagotomy.

5. It is concluded that denervation of the lungs has no obvious effect on the initiation of the cardiovascular responses to diving. Termination of these responses can be achieved either by feed-back from pulmonary receptors or 'central irradiation' of impulses from respiratory to cardiovascular controlling neurones.

INTRODUCTION

The initiation and maintenance of apnoea and concomitant cardiovascular adjustments to diving displayed by many air breathing vertebrates have been the subject of intensive investigation (Scholander, 1940; Anderson, 1966; Angell James & Daly, 1972*a*; Jones & Johansen, 1972). In ducks there is no bradycardia in the absence of apnoea (Butler & Jones, 1968) and Cohn, Krog & Shannon (1968) proposed that, in birds, cessation of thoracic motion was the initiating factor in the development of the cardiovascular responses to submergence. Undoubtedly generation of the diving responses in both birds and mammals is either prevented or reduced by continued rhythmic lung ventilation (Angell James & Daly, 1969; Drummond & Jones, 1972; Butler & Taylor, 1973) and Lin, Matsuura & Whittow (1972) even suggested that, in the California sea lion, diving bradycardia is solely the result of the lack of cardio-acceleration normally caused by rhythmic lung inflation. The influence of the act of breathing on the diving response is exemplified on emergence for, in virtually all divers, reversal of the diving cardiovascular adjustments begins as soon as the first breath is taken, although in some aquatic mammals cardio-acceleration sometimes starts before emergence (Jones, Fisher, McTaggert & West, 1973). Butler & Taylor (1973) report heart rate increasing tenfold with the first inspiratory effort in ducks and many authors have noted the propensity for sinus arrhythmia in the early period of recovery from a dive (Andersen, 1963). The most attractive explanation of this rapid recovery is the so-called respiratory–heart rate response (Anrep, Pascual & Rössler, 1936*a*, *b*) although, as yet, the relative contributions of 'central irradiation' and peripheral input from the lungs in causing this response have not been elucidated.

Undoubtedly, respiratory related cardiovascular reflexes are involved in both the initiation of and recovery from the cardiovascular responses to diving. Therefore, the present experiments were designed to examine

the roles of both peripheral input and 'central irradiation', alone and together, on some cardiovascular adjustments to diving. First, selective pulmonary denervation was used to establish the effects of permanent withdrawal of peripheral input on cardiovascular and respiratory variables before, during, and after diving. The role of peripheral input from the lungs was studied in intact ducks by making single artificial lung inflations during a dive, while the role of central irradiation alone was assessed by monitoring heart rate and blood pressure changes occurring with the first active inspiration terminating a dive in denervated animals. However, since the majority of avian pulmonary receptors appear to be inhibited by high concentrations of CO_2 in the inhaled gas (Burger, 1968; Fedde, Gatz, Slama & Scheid, 1974*a*, *b*) it was also possible to determine the contribution of these chemosensitive pulmonary receptors to the peripherally generated heart rate response by inflation with high CO_2 gas mixtures, thus obviating the need for surgical denervation. Finally, the effects of 'central irradiation' alone were also studied in intact ducks by correlating respiratory neurone discharge, recorded from the intercostal nerve of a paralysed duck, with heart rate and blood pressure changes both before and during periods of apnoeic asphyxia.

METHODS

The experiments were performed on forty-three Khaki Campbell ducks (*Anas platyrhynchos* var.) between 5 and 15 weeks old. The average weight of these animals when adult (10+ weeks) was $1{\cdot}6 \pm 0{\cdot}1$ kg. The adjective 'initial', when referring to any of the measured variables, describes them before submergence. The adjective 'normal' refers to an animal which did not undergo any nerve section whereas 'sham' refers to an animal in which at least one lung remained innervated but which had nevertheless undergone some surgical procedures. All experiments were carried out at the temperature at which the ducks were maintained (20–22° C).

(*a*) *Recording techniques and data analysis*

In the majority of experiments the animals were restrained, ventral side down, on an operating table and submergence was effected by manually lowering the duck's head into a beaker of room-temperature water. All operative procedures for implantation of cannulae were of a superficial nature and were performed under local anaesthesia (2% lignocaine). Furthermore, the area of any wounds was periodically infiltrated with local anaesthetic throughout the course of an experiment.

To monitor arterial and venous blood pressures a sciatic artery and ulnar vein were exposed under local anaesthesia and cannulated with polyethylene cannulae attached to Hewlett–Packard 267 BC pressure transducers. The venous cannula was advanced from its position of insertion in the wing until its opening was close to the heart. The cannulae, of various sizes depending on the size of the vessel, were filled with heparinized avian saline (40 i.u./ml.). The frequency response of the

manometric system with the smallest cannulae used was 40 Hz with damping of 0·2 of critical.

Breathing was monitored with a pneumotachograph attached to a tracheal cannula and the flow signal integrated to give tidal volume. The tracheal cannula was inserted high in the neck after exposing the trachea under local anaesthesia. In experiments in which ducks were paralysed by injection of curare (1 mg/kg; D-tubocurarine chloride) artificial ventilation was effected using either a Harvard respirator or a unidirectional flow of warm humidified air + 4% CO_2 (2–3 l./min). In the unidirectionally ventilated preparation air entered via the tracheal cannula and exited through an opening in the cervical air sac. The level of relaxation achieved by the initial injection of curare was maintained throughout the rest of the experiment by continuous intramuscular infusion of curare (0·36 mg/kg.hr).

Heart rate was obtained from the e.c.g., the latter being recorded with bipolar copper wire electrodes (Butler & Jones, 1968) and after amplification the signal was fed into an instantaneous heart-rate-meter to give pulse frequency. Electroneurograms were recorded from an intercostal nerve using bipolar platinum hook electrodes.

Arterial blood samples and gas samples were analysed using a Radiometer BMS 3 blood gas analyser with appropriate electrodes. Before analysis the oxygen and carbon dioxide electrodes were calibrated using precision gas mixtures and the pH electrode with Radiometer precision buffer solutions.

All signals were amplified by conventional means and blood pressures, breathing frequency or integrated tracheal air flow, the e.c.g. or instantaneous heart rate, the e.n.g. or pulse frequency per burst were displayed on both a Tektronix 502A oscilloscope and a Hewlett–Packard 4-channel thermal pen writer, writing on rectilinear co-ordinates. At the same time all variables were recorded on an 8-channel FM tape system for later analysis by computer. The stored data were analysed using a specially prepared computer programme for a Digital PDP Lab 8e computer. This programme yielded the mean values of the analogue data, e.g. mean blood pressure, heart rate, nerve discharge over pre-set but variable time periods. When necessary, signal averaging, cross- and auto-correlation, time interval histograms and post-stimulus time histograms were generated from the analogue data using conventional software. In the text and figures numerical values, when referring to determinations of variables in a group of animals, are given as means ± S.E. of the mean. Statistical analysis of the results was done by a two sample t test and 5% was considered the fiducial limit of significance.

(b) Denervation of the lungs

Five ducks were dissected to determine the pattern of vagal innervation of the lungs. It was apparent that there was considerable variability in innervation with between five and seven vagal branches to the lung in individual ducks. Since the lungs are overlaid by the air sacs, which had to be punctured to allow denervation, we decided to denervate the lungs on only one side, denervation of the other side being effected by mid-cervical vagotomy.

At least 1 week after unilateral mid-cervical vagotomy a duck was restrained, ventral side up, on an operating table and given 1 g/kg urethane (I.M.). When a level of surgical anaesthesia had been attained the skin over the sternum was parted and the furculum and sternum bisected in the mid line. The sternum was reflected, on the side with the intact vagus, to expose the air sacs which were then punctured to reveal the ventral surface of the lung. All branches leaving the vagus and coursing across the lung were then sectioned near their point of origin from the vagus. Section of all pulmonary vagal branches caused an immediate

fall in heart rate and breathing frequency. Typically, heart rate fell to 33–50% and breathing frequency to 20% of the values pertaining before nerve section. The sternum and furculum were sewn together using surgical gut and the wound was sprayed with antibiotic powder. The muscles, anterior air sac and skin were closed in layers. The animals were quickly removed from the operating table and attended until they could maintain equilibrium. Initially the respiratory frequency was low and the animals tended to breathe through their mouths with gasping inspirations; however, by one week post-operation and during the complete recovery period (3–5 weeks) breathing was apparently normal. The ducks were given penicillin injections (70,000 i.u./kg I.M.) every day for 10 days post-operation. Sham operations were performed on a number of ducks in which the branches of the pulmonary vagus were exposed and identified but not cut. The animals were then closed and treated in the same manner as lung denervates.

(c) *Experimental procedures*

1. *The lung inflation reflex.* The efficacy of lung denervation was tested by monitoring the duration of apnoea provoked by pulmonary inflation over a range of pressures. The inflation reflex was investigated in normal, right vagotomized sham-operated, and denervated ducks. The trachea was either intubated with a soft vinyl tube of appropriate diameter inserted through the glottis or cannulated high in the neck. The exposed end of the cannula was attached to a polyethylene T-piece, one arm being open to the atmosphere and the other connected to a pressure reservoir (Eaton, Fedde & Burger, 1971). While monitoring intratracheal pressure, the respiratory system was inflated by connecting the tracheal cannula to the pressure reservoir after occluding the connexion to the atmosphere. For convenience of data analysis inflation of the respiratory system was usually performed at end inspiration and the period to the next inspiration measured. To inflate the respiratory system to the desired pressure took 250–500 msec. The inflations were usually made with air although on some occasions a mixture of air + 8% CO_2 was used. The pressure in the system was released after the bird had made two or three respiratory movements.

2. *Effect of lung denervation on respiratory and cardiovascular responses to submergence.* These experiments were performed on twenty-one ducks, of which ten were denervated following right vagotomy, nine were sham-operated after right vagotomy, and two were subjected to no operative procedures (normal). Following any operative procedures the animals were given from 3 to 5 weeks for recovery. Thirteen animals, six of which were denervates, were subjected to dives of 2 min duration in which heart rate, arterial and venous blood pressures, arterial blood gas tensions and pH, breathing frequency and tidal volume were monitored. The effects of single cycles of lung inflation and deflation after 75–90 sec submergence on some of these variables were investigated in another group of eight ducks, four of which were denervated. Before the start of a dive a T-piece was attached to the pneumotachograph and air was passed through its cross-arms at a rate of between 1 and 3 l./min. When respiration was stable, as judged from the record of tidal volume, the animal's head was submerged, and the tracheal tube clamped with haemostats. The flow rate through the T-piece was then adjusted so that when lung inflation was performed it would approximate the inhalation rate and volume normally observed on the first breath at the end of a dive. After 75–90 sec submergence the tracheal clamp was removed and the open end of the T-piece occluded thereby forcing air into the respiratory system. At the desired peak inflation the occlusion was released and the lungs deflated passively. A high flow rate (6–10 l./min) was then established through the cross-arms in preparation for

emergence. On some occasions two inflations were performed during a single dive. In other tests lung inflations were made with air + 8 % CO_2, the latter being mixed in an anaesthetic gas unit equipped with precision flow meters. Before diving the animal breathed air but after submergence the air flow was replaced by the air–CO_2 mixture and the respiratory system inflated as described above. The flow of gas mixture was maintained until after the animal surfaced so that the first few breaths were of air + CO_2. The partial pressure of CO_2 in the gas mixture was determined with the BMS 3 blood gas analyser. At the conclusion of the experiments each duck was tested for reflex apnoea on lung inflation.

3. *Correlation between central respiratory periodicity, heart rate and blood pressures.* Central respiratory periodicity was monitored by recording afferent activity from an intercostal nerve. Twenty-two ducks were anaesthetized using Halothane inhalation while secured in a prone position with the wings spread to expose the back. Feathers were removed from one side of the back and an incision made to expose the dorsal parts of ribs 5–7 and their overlying musculature. The costal nerve associated with rib 6 was usually chosen for recording. The intercostal muscle caudal to the rib was carefully scraped from its attachment to the rib, exposing the air sac overlying the lung. The nerve was usually visible across the air sac surface parallel with the rib, though it was sometimes attached to the central face of the rib.

The intercostal nerve was cut as far peripherally as possible, de-sheathed and placed on a pair of platinum hook electrodes. Petroleum jelly was applied to the nerve to avoid desiccation. When an active nerve had been placed on the electrodes, activity was recorded for a short period during which the duck breathed normally. From this, the activity was characterized as expiratory or inspiratory. Curare (1 mg/kg, I.M.) was then given, and paralysis maintained until the end of the experiment by supplementary doses when necessary while the area of the wound was infiltrated with local anaesthetic periodically. Tidal or unidirectional artificial ventilation was applied as soon as normal breathing failed but any correlation between heart rate, arterial and venous blood pressure and intercostal nerve activity was investigated only under conditions of unidirectional ventilation. In such studies the heart rate was controlled by varying the composition of the ventilating gas stream. Arterial blood samples were taken for blood gas analysis before paralysis and after each change in the ventilating gas mixture.

To investigate the relationship between nerve activity and heart rate and blood pressure under transient conditions, asphyxia was induced by switching off artificial ventilation. Blood samples were taken for blood gas analysis both before and after 90 sec of asphyxia. Finally, the correlation between respiratory neurone activity, as monitored in the intercostal nerve, and cardiovascular variables was analysed in unidirectionally ventilated animals following bilateral cervical vagotomy.

RESULTS

(a) *The effect of lung denervation on the lung inflation reflex*

Breathing frequency in the ducks under investigation decreased with age from 33 ± 2 breaths/min in younger ones to $17{\cdot}5 \pm 1$ in adults, so, in these tests, the period of apnoea provoked by lung inflation was expressed as a percentage of the mean period of the ten breathing movements before lung inflation which was performed at end inspiration. An inflation pressure of around 5 cmH_2O in normal animals caused extension of the

interbreath interval of $322 \pm 17\%$, varying in individuals from 232 to 423 %. The average length of the apnoeic period increased by $62{\cdot}8 \pm 0{\cdot}9\%$ per cmH_2O applied pressure over the pressure range tested (5–12 cmH_2O). Apnoeic periods similar to those in normal ducks were provoked by inflating the respiratory system of right vagotomized sham-operated individuals to 5 cmH_2O but at the highest inflation pressures (10–12 cmH_2O) the apnoeic periods were invariably shorter than in normal individuals. As has been reported previously (Eaton *et al.* 1971) inflation with air + 8 % CO_2 virtually eliminated the apnoeic response to inflation even at applied pressures of 15 cmH_2O. Following pulmonary denervation the inflation reflex was abolished even when applied pressures were 2–3 times those which caused pronounced apnoea before denervation. Arterial blood pressure and heart rate were also monitored in three sham-operated and three denervated ducks during these periods of lung inflation. There were no pronounced changes in any of these variables except that, in normal animals, bradycardia developed after about 10 sec of apnoea.

(*b*) *The effect of pulmonary denervation on the initiation of and recovery from some cardiovascular responses to submergence*

In a preliminary series of experiments only heart rate and breathing rate were monitored before, during and after dives of 2 min duration in six normal and six sham-operated ducks. Three dives were performed on each animal with some 45–60 min allowed for recovery between dives. Three of the sham-operated ducks had only undergone right vagotomy and their responses were examined separately. There were no significant differences in either the cardiovascular or respiratory response to 2 min submergence between normal, right vagotomized or sham-operated animals. Consequently there seemed no reason why the responses of these animals could not be examined together. Therefore, in the main series of experiments, arterial blood pressure, blood gas tensions and tidal volume were monitored in addition to heart and breathing frequencies in two groups of animals, one with the lungs totally denervated and the other with innervation to at least one lung being intact (shams).

There were no significant differences in any of the variables between the two groups at rest, before submergence (Table 1). The minute volumes of the shams was 524 ml./kg.min which was similar to that of the denervates (492 ml./kg.min). During dives there were no significant differences between the two groups of ducks with respect to heart rate, systolic or diastolic blood pressures. As has been described previously (Butler & Jones, 1971), by the end of the 2 min dive both systolic and diastolic blood pressures had fallen below the pre-dive levels but the fall was not significant in this instance. On emergence, systolic blood

TABLE 1. Cardiovascular and respiratory responses in sham-operated and denervated ducks before, during, and after submergence of 2 min duration. Mean values (± S.E. of mean) of dives on seven sham-operated ducks and six ducks which had undergone left pulmonary denervation after right mid-cervical vagotomy. n = number of determinations contributing to the mean

(*a*) Sham (mean wt. = 1·51 ± 0·07 kg)

Time (sec)	Heart rate (beats/min) (n = 14)	Systolic pressure (mmHg) (n = 6)	Diastolic pressure (mmHg) (n = 6)	Breathing rate (breaths/min) (n = 14)	Tidal volume (ml.) (n = 6)	P_{a,O_2} (mmHg) (n = 6)	P_{a,CO_2} (mmHg) (n = 6)	pH (n = 6)
Pre-dive	207·1 ± 10·4	135·4 ± 5·7	109·0 ± 4·6	17·4 ± 1·2	45·5 ± 5·1	99·0 ± 3·5	31·0 ± 1·5	7·5 ± 0·01
Dive								
5	170·6 ± 5·9	142·3 ± 5·8	113·4 ± 3·7	—	—	—	—	—
10	155·1 ± 6·5	142·1 ± 6·5	115·6 ± 4·5	—	—	—	—	—
15	147·4 ± 5·4	142·3 ± 6·3	115·6 ± 4·6	—	—	—	—	—
30	112·3 ± 6·7	136·8 ± 6·6	114·3 ± 6·7	—	—	—	—	—
60	75·8 ± 7·8	128·4 ± 4·9	97·0 ± 10·9	—	—	—	—	—
90	—	—	—	—	—	51·4 ± 3·3	38·6 ± 3·1	7·4 ± 0·02
120	58·3 ± 6·4	129·2 ± 5·6	99·4 ± 7·7	—	—	—	—	—
Recovery								
5	284·3 ± 22·8	141·5 ± 8·3	114·3 ± 8·7	46·2 ± 3·7	63·4 ± 4·0	—	—	—
10	255·7 ± 27·1	145·7 ± 6·6	110·8 ± 7·1	42·0 ± 3·9	59·5 ± 2·8	—	—	—
15	254·3 ± 27·0	145·9 ± 7·7	112·2 ± 9·4	36·8 ± 3·1	52·9 ± 3·9	—	—	—
30	282·9 ± 31·5	137·6 ± 5·2	102·0 ± 7·8	35·1 ± 4·2	51·2 ± 2·3	—	—	—
60	266·5 ± 18·0	122·0 ± 1·9	82·3 ± 4·6	28·4 ± 2·2	49·8 ± 1·4	—	—	—
120	260·0 ± 12·4	119·6 ± 3·0	75·9 ± 2·0	26·6 ± 1·4	52·7 ± 4·4	—	—	—

TABLE 1 (*cont.*)

(*b*) Denervate (mean wt. = 1·5 ± 0·02 kg)

Time (sec)	Heart rate (beats/min) ($n = 12$)	Systolic pressure (mmHg) ($n = 6$)	Diastolic pressure (mmHg) ($n = 6$)	Breathing rate (breaths/min) ($n = 12$)	Tidal volume (ml.) ($n = 6$)	P_{a,O_2} (mmHg) ($n = 6$)	P_{a,CO_2} (mmHg) ($n = 6$)	pH ($n = 6$)
Pre-dive	213·2 ± 5·4	137·2 ± 4·7	109·0 ± 6·6	15·2 ± 1·2	48·6 ± 3·2	102·7 ± 1·5	27·5 ± 1·4	7·4 ± 0·02
Dive								
5	182·3 ± 8·0	144·7 ± 5·6	114·8 ± 4·5	—	—	—	—	—
10	156·0 ± 7·4	146·8 ± 5·7	114·7 ± 5·0	—	—	—	—	—
15	155·8 ± 9·9	145·3 ± 5·4	112·7 ± 5·0	—	—	—	—	—
30	98·5 ± 12·7	146·2 ± 3·4	111·3 ± 2·9	—	—	—	—	—
60	52·0 ± 7·4	134·4 ± 4·2	98·7 ± 5·0	—	—	—	—	—
90	—	—	—	—	—	56·0 ± 2·6	36·7 ± 2·0	7·4 ± 0·01
120	45·0 ± 7·3	134·0 ± 5·7	98·0 ± 7·2	—	—	—	—	—
Recovery								
5	159·0 ± 29·4	123·4 ± 5·1	88·5 ± 6·1	24·4 ± 4·6	109·3 ± 10·2	—	—	—
10	174·7 ± 26·5	127·0 ± 4·4	89·1 ± 6·3	21·9 ± 4·0	100·0 ± 10·5	—	—	—
15	177·5 ± 23·4	128·2 ± 3·4	93·1 ± 4·2	22·3 ± 3·9	89·4 ± 7·0	—	—	—
30	187·5 ± 28·1	128·5 ± 6·3	82·6 ± 9·4	20·1 ± 3·6	80·8 ± 7·0	—	—	—
60	216·0 ± 27·8	127·5 ± 5·6	80·9 ± 7·1	19·4 ± 3·4	89·1 ± 7·2	—	—	—
120	249·6 ± 24·1	129·9 ± 5·6	97·9 ± 4·6	20·6 ± 3·0	83·5 ± 3·6	—	—	—

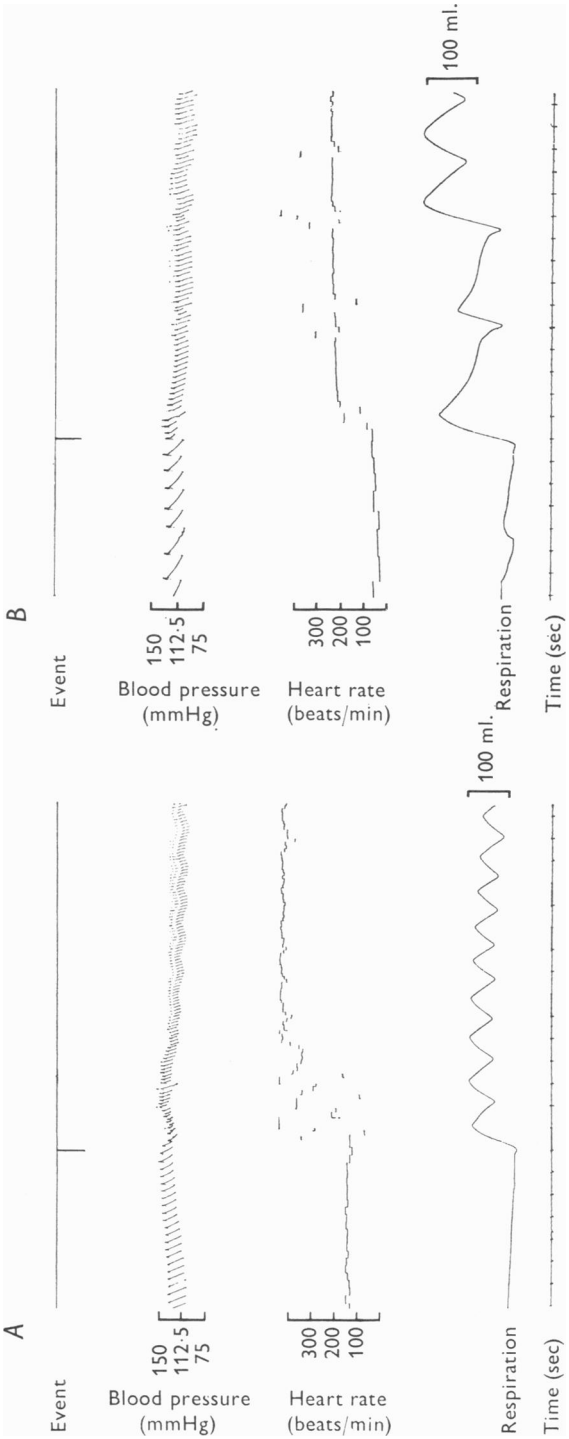

Fig. 1. The effect of the resumption of breathing, terminating a 2 min dive, on heart rate and blood pressure in *A* a normal and *B* a pulmonary denervated duck. Traces from top to bottom in both *A* and *B*: 1, event, downward deflexion signals emergence; 2, arterial blood pressure (mmHg); 3, instantaneous heart rate (beats/min); 4, tidal volume (ml.) up on trace = inspiration; 5, time (sec).

pressure fell in denervates but rose to slightly exceed the pre-dive levels in shams, although the difference between groups was not significant. Sixty seconds after emergence diastolic arterial blood pressures were nearly identical in both groups, and significantly below the pre-dive values (Table 1). Heart rate rose markedly during the first breath after emergence in both sham and denervated ducks (Fig. 1*A*, *B*) and in the ormer was at pre-dive levels 2·5 sec after emergence whereas in denervates heart rate fluctuated considerably (Fig. 1*B*; Table 1), the pre-dive level being attained 60 sec after emergence. That oxygen conserving cardiovascular adjustments were equally effective in both sham-operated and denervated animals was confirmed by measurements of P_{a,O_2}, P_{a,CO_2} and pH_a before and after 90 sec submergence. Before diving there were no significant differences in any of these variables between groups and this was also the case after 90 sec submergence, although P_{a,O_2} and pH_a had fallen significantly from their respective initial levels (Table 1).

In both groups heart rate rose spectacularly when the first breath was taken at the end of the dive (Fig. 1*A*, *B*). A point of interest which has not been reported previously is that, after an initial small exhalation on emergence, the first inhalation was considerably larger than the following ones and the expiratory phase of the first breathing movement was often, particularly in normal animals displaying tachypnoea, little more than half the volume of the initial inhalation (Fig. 1*A*). In effect then, at the end of the dive, the animal restores or elevates its reserve volume (Fig. 1*A*, *B*). Fig. 1*A*, *B* also illustrates the marked difference in breathing rate and tidal volume between sham and denervated ducks on recovery. In shams breathing frequency rose to 265 % of the pre-dive rate, 5 sec after emergence, whereas in the denervates rate had only increased to 160 % of the pre-dive rate at this time (Table 1). Tidal volume increased to 225 % of pre-dive volume, 5 sec after emergence in denervates, but in shams was only 139 % of the initial value at this time. In the latter, minute volume attained 5·7 times the pre-dive level at 5 sec recovery and, although falling throughout recovery, was still some 2·6 times the pre-dive value at 120 sec recovery (Table 1). Minute volume of denervates showed a similar decline with time although, despite the very large increase in tidal volume, maximum minute volume (1778 ml./kg. min) only reached three-fifths the maximum value observed in shams.

(*c*) *On the role of 'central irradiation' and peripheral input in the respiratory–heart rate response; the effect of lung denervation on the cardiovascular responses to single lung inflations during and terminating a dive*

In both sham-operated and denervated animals the first breath terminating a normal dive was associated with an increase in heart rate of

some 100–150 beats/min by peak inspiration (Figs. 2 and 3). The data plotted in Figs. 2 and 3 represent an analysis of results from two sham-operated, two normal, and four denervated animals in respect of absolute changes in heart rate and percentage changes in systolic and diastolic arterial pressures during single active lung inflation terminating, or passive lung inflation during, a dive. Single lung inflations, regardless of the length of each cycle of inflation and deflation, were divided into

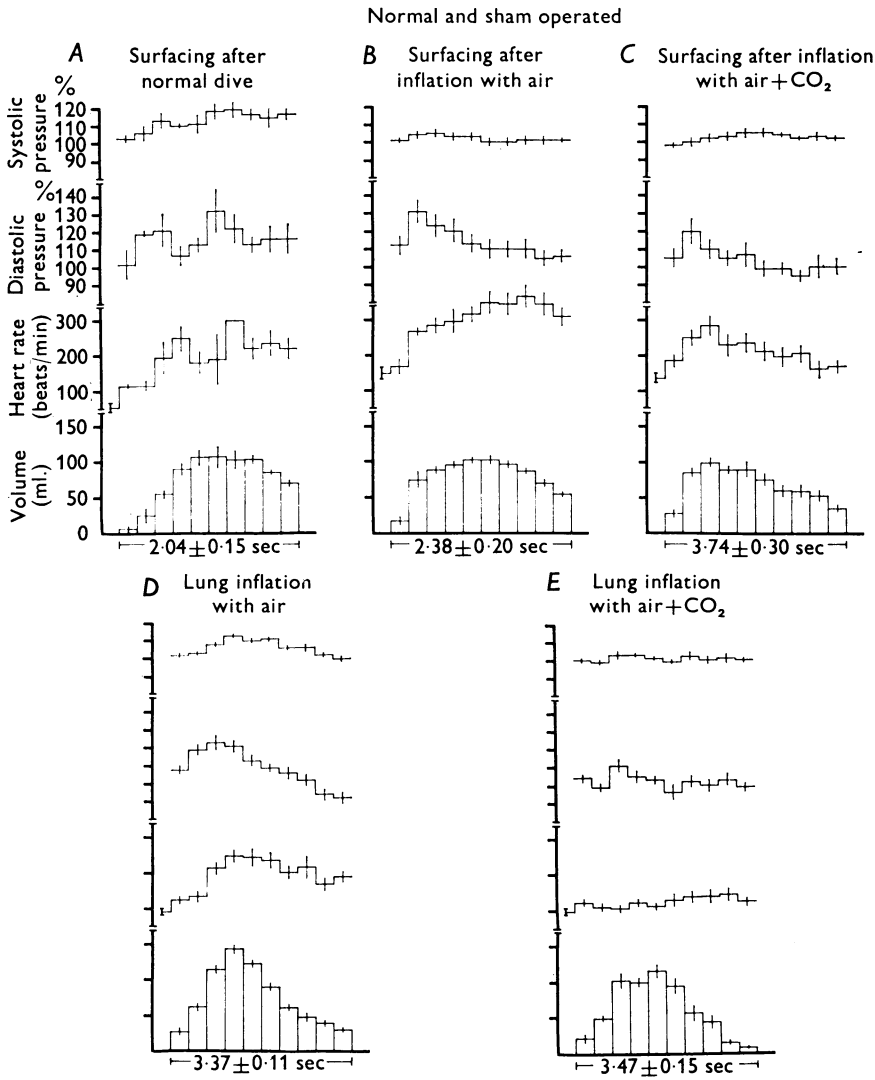

Fig. 2. For legend see facing page.

ten time bins and the individual values for volume inhaled or exhaled, heart rate and blood pressures which occurred in each time bin were noted. All data occurring in corresponding time bins from all animals in a group were averaged. The mean length of all cycles of inflation and deflation ($\pm$ S.E. of mean) is given on the abscissa in Figs. 2 and 3. The increase in heart rate with the breath terminating a normal dive was accompanied by an elevation in both systolic and diastolic pressures of some 10 and 30 % respectively (Figs. 2 and 3). The first breath, terminating dives in which passive lung inflations of air or air + CO_2 (mean P_{CO_2} = 60 ± 4 mmHg) had been made, resulted in a similar heart rate increase as was observed at the end of a normal dive (Figs. 2 and 3). However, passive lung inflation with air during a dive caused a marked increase in heart rate in sham-operated and normal ducks but no significant changes in denervates (Figs. 2, 3 and 4). The heart rate response to passive inflation was virtually abolished in sham-operated and normal ducks by addition of 60 ± 4 mmHg P_{CO_2} to the inflating gas (Fig. 2). Passive lung inflations with air or air + CO_2 caused only slight changes in systolic blood pressures in either sham-operated and normal or denervated animals over a time period of up to 20 sec from the start of inflation. However, air inflation of sham-operated and normal ducks caused a decline in

---

Fig. 2. Changes in heart rate and systolic and diastolic arterial blood pressure provoked in normal and sham-operated ducks by: *A*, the first breath terminating an uninterrupted dive; *B*, the first breath terminating a dive which was interrupted by one or two passive lung inflations with air; *C*, the first breath terminating a dive which was interrupted by one or two passive lung inflations with air + 60 ± 4 mmHg CO_2; *D*, a single passive lung inflation with air; and *E*, a single passive lung inflation with air + 60 ± 4 mmHg CO_2. For data analysis, the cardiac intervals were converted into rates (beats/min), the value to the extreme left in the graphs representing the frequency obtaining in a 10 sec period before lung inflation. The start of lung inflation was taken as time zero and heart rate at the time of the first QRS complex to appear after the start of inflation was obtained from the interval between it and the previous QRS. This process was repeated for every heart beat throughout the cycle of lung inflation and deflation. Each tidal volume was then divided into ten equal time bins and the inflation volume, mean systolic and diastolic blood pressure, and heart rate occurring in corresponding time bins for all inflations was averaged to give the values presented $\pm$ S.E. of mean. When only one value fell within a particular time bin it has not been represented in the Figure. Blood pressures are expressed as the percentage occurring in each time bin with the pre-inflation value = 100 %. The mean length of each group of inflations is given on the abscissa. All data was obtained from two normal and two sham-operated ducks and involved analysis and averaging of (in *A*) 8 cycles of lung inflation, (*B*) 28 cycles, (*C*) 26 cycles, (*D*) 44 cycles, (*E*) 43 cycles.

diastolic pressure which had fallen significantly below the pre-inflation level 5 sec after the start of inflation (Fig. 4*A*). This decline in diastolic blood pressure was associated with a heart rate which was significantly above the pre-inflation rate. Even in denervates heart rate was significantly

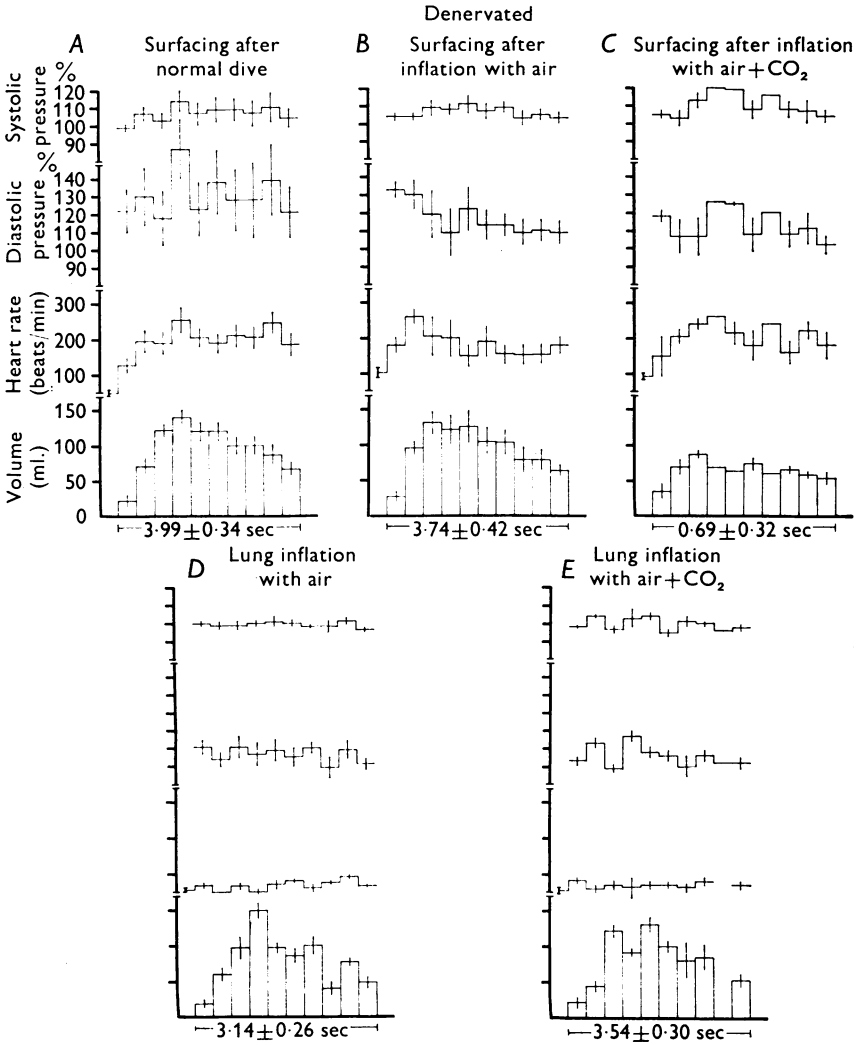

Fig. 3. Changes in heart rate and systolic and diastolic arterial blood pressure provoked in pulmonary denervated ducks by similar interventions to those described in the caption for Fig. 2. Data analysis was as performed for Fig. 2 and concerns data from four ducks involving analysis and averaging of (in *A*) 10 cycles of inflation, (*B*) 14 cycles, (*C*) 7 cycles, (*D*) 21 cycles, and (*E*) 16 cycles.

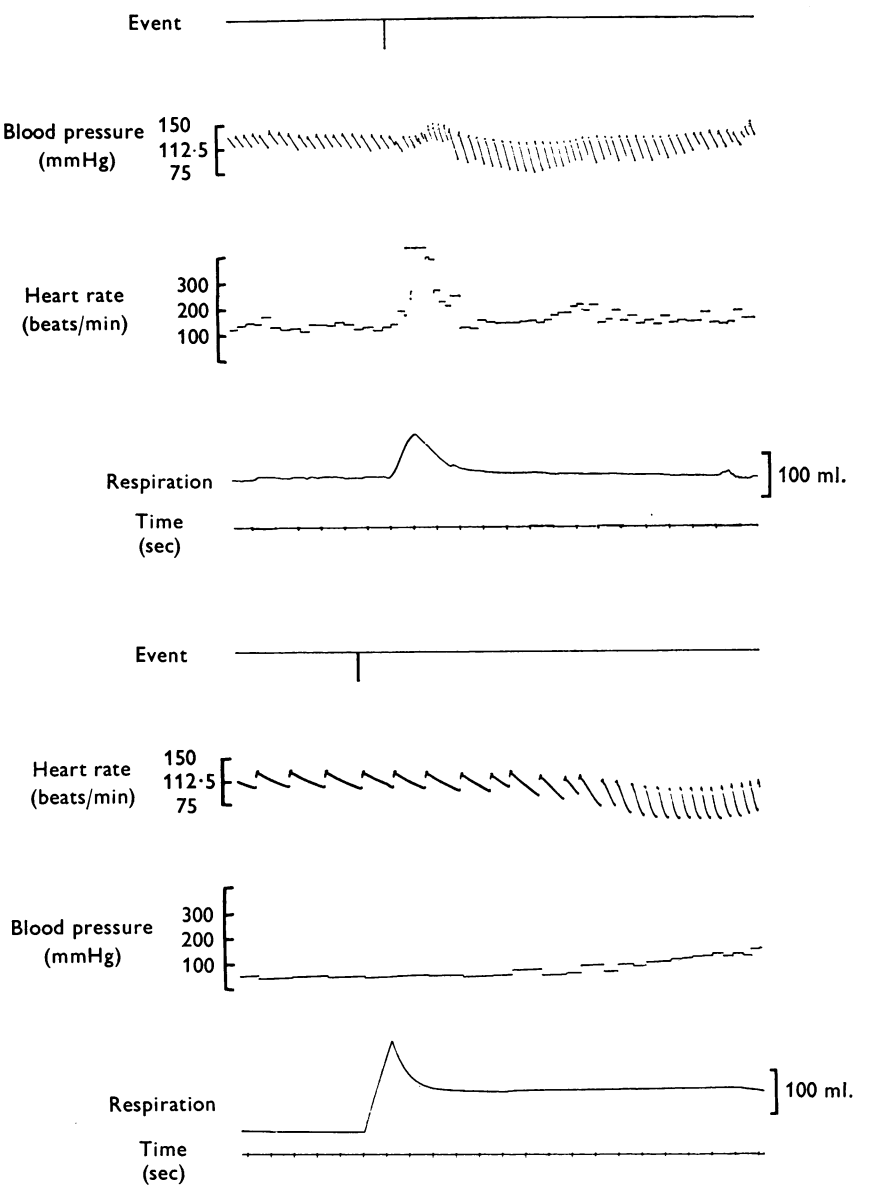

Fig. 4. The effect of single passive lung inflations, after 90 sec submergence, on heart rate and blood pressure in (*A*) a sham-operated, and (*B*) a pulmonary denervated duck. Traces from top to bottom in both *A* and *B* as for Fig. 1 except that the event channel signifies the onset of lung inflation.

elevated and diastolic blood pressure significantly reduced 10 sec after the start of inflation (Fig. 4*B*). Following air + CO_2 inflation a significant increase in heart rate occurred in normal and sham-operated, and denervated ducks, 12·5 sec after inflation and diastolic pressure declined significantly at this time in the former but, although it also fell in denervates, at no time were the changes significantly different from the pre-inflation value.

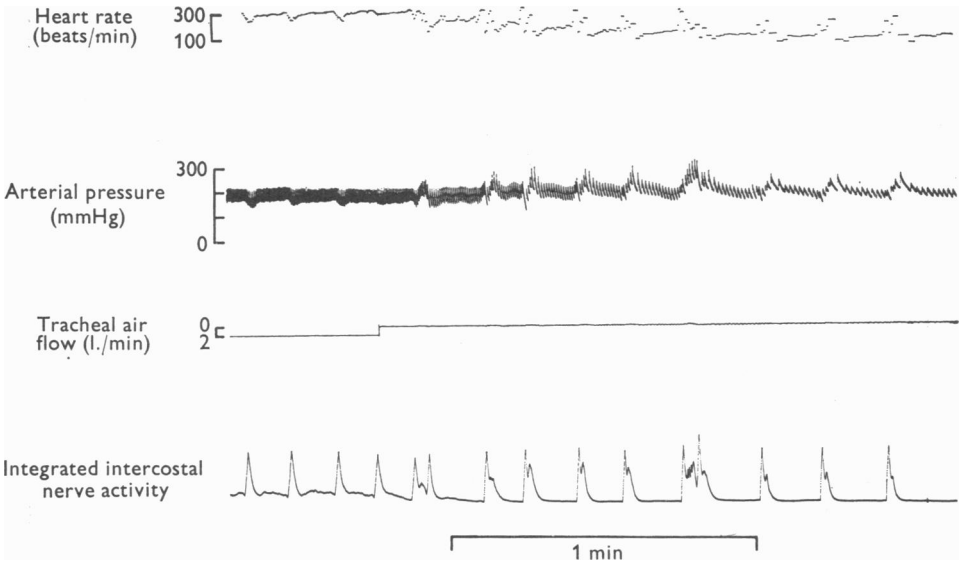

Fig. 5. The relationship between respiratory motor neurone activity, recorded from an intercostal nerve, heart rate and arterial blood pressure during unidirectional ventilation and asphyxia in a paralysed duck. Traces from top to bottom: 1, instantaneous heart rate; 2, arterial blood pressure (mmHg); 3, tracheal air flow (l./min), the period of asphyxia starting when the air flow is discontinued; 4, integrated intercostal nerve activity which was associated with inspiration before paralysis.

(d) On the role of 'central irradiation' alone in the respiratory–heart rate response; the relationship between respiratory motor discharges, heart rate and blood pressures

In all animals bursts of activity in the intercostal nerve persisted after paralysis, showing the respiratory neurones to be active in the absence of rhythmic feed-back. These bursts appeared to be identical in pattern to those recorded in the same nerve before paralysis. Fibres whose discharges were associated with inspiration were usually isolated from the intercostal nerve of the normally breathing animal since these discharges were more clearly defined. Consequently, in this section, associations

between intercostal nerve discharge and cardiovascular variables are given with respect to inspiratory motor activity. Apnoeic asphyxia and changes in the O_2 and carbon dioxide tensions of the unidirectionally ventilating gas stream were used to cause changes in the mean heart rate pertaining between bursts of inspiratory motor activity. This allowed extension of the effects of 'central irradiation' to heart rates in the range of those occurring at the end of a dive.

Over 1500 discrete nerve discharges were monitored in this series of experiments, and, on average, increases in mean arterial pressure occurred with 80 % of the bursts, and increases in heart rate occurred with 88 % of the bursts. On the other hand changes in venous pressure only accompanied about 30 % of the bursts.

It proved difficult to perform adequate analysis of the relationship between intercostal nerve inspiratory activity and heart rate by means of cross-correlation functions. Obviously, these two events were very closely related in time in that peak heart rate and peak nerve discharge frequency often occurred simultaneously (Fig. 5). However, there was considerable temporal variation between these events from one nerve discharge to another even in the same animal. On yet other occasions the increase in heart rate associated with an inspiratory burst was followed by a fall in rate of virtually the same magnitude in the early part of the interburst period, before heart rate rose again to a level that was maintained throughout the rest of the period (Fig. 5). The absolute increase in heart rate which accompanied each burst was $43{\cdot}9 \pm 3{\cdot}2$ % of the maximum possible change which could have occurred (i.e. maximum rate observed in that duck minus pre-burst rate). Since the maximum rate observed in these ducks was around 400 beats/min, then increases in rate of 150 beats/min were observed with each burst when initial heart rate was in the range of 80–120 beats/min.

Cross-correlograms computed between nerve discharge rate and mean arterial pressure (MAP) were quite consistent. Mean arterial pressure rose with each inspiratory motor burst, although there was considerable variation in the temporal relationship of the two events between animals. For instance, in some animals peak pressure virtually coincided with peak discharge, whereas in others the peak pressure was delayed by up to 3 sec. This kind of variation however was not apparent in a series of bursts recorded from a single animal. Generally, the changes in blood pressure were preceded by the acceleration in heart rate but both the magnitude and temporal relationship of the former to the peak nerve discharge were unaffected by bilateral vagotomy, while the heart rate changes were eliminated by this procedure. Changes in venous pressure which occurred with each nerve burst were somewhat inconsistent but

it appeared that the venous pressure increase was greatest when the heart rate was low, though changes in venous pressure were still observed after bilateral cervical vagotomy.

DISCUSSION

Although there can be no doubt that, on occasion, both baroreceptor and immersion reflexes may play a part in generation of diving bradycardia in ducks (Andersen, 1963; Andersen & Blix, 1974; Jones, 1973; Bamford & Jones, 1974), at least 85 % of this response is due to stimulation of carotid body chemoreceptors by the progressively hypoxic and hypercapnic blood during a dive (Jones & Purves, 1970). In mammals, withdrawal of pulmonary input during apnoea causes an enhancement of the arterial chemoreceptor primary reflex autonomic pathways (Daly & Scott, 1958, 1963; Daly & Hazzledine, 1963; Daly & Ungar, 1966; Angell James & Daly, 1969, 1972*a*) but that this is not the case in ducks is shown by the fact that there is virtually no difference in the time course of diving bradycardia in intact or denervated animals. Furthermore, there is no evidence that elimination of central interaction between respiratory and vasomotor/cardiac neurones is at all important for initiation of the cardiovascular responses to apnoea since they are similar in submerged intact and apnoeic curarized ducks (Butler & Taylor, 1973; Bamford & Jones, 1974), the present experiments showing that the respiratory neurones are rhythmically active in the latter. Consequently, any direct role played by reduction in feed-back from pulmonary receptors and/or 'central irradiation' in initiating the diving reflex remains to be demonstrated.

On the other hand, central respiratory neurone activity or lung inflation, either alone or in concert, causes pronounced effects on cardiovascular variables during diving. In normal or sham-operated animals a single passive lung inflation during a dive promotes a large increase in heart rate with a time course which precludes relief of anoxaemia at the arterial chemoreceptors. This acceleration is eliminated by pulmonary denervation or addition of 8 % CO_2 to the inflating gas in normal animals. The latter suggests that CO_2 sensitive pulmonary receptors are vitally important to this response for the concentration of CO_2 used was sufficient to inhibit the discharge of many of these receptors (Burger, Osborne & Banzett, 1974; Osborne & Burger, 1974; Fedde *et al.* 1974*a*, *b*). However, longer term (up to 20 sec) changes in heart rate and/or arterial blood pressure followed all inflations, although the time taken for significant heart rate elevation and fall in diastolic pressure after inflation with air in normal and sham-operated ducks (5·0 sec) was about half that observed with air + CO_2 inflation in normal and sham-operated ducks or air inflation

in denervates (10 sec). It may be that the fall in diastolic blood pressure is indicative of a pulmonary vasodilator reflex (Daly & Robinson, 1968) in air-inflated intact ducks. The slower time course for changes in these variables in denervated or air + CO_2 inflated intact ducks suggests a chemoreceptor–baroreceptor interaction in that the former is responsible for the heart rate elevation which is accompanied by vasodilation in most cases.

In relaxed ducks on unidirectional ventilation respiratory motor activity was usually accompanied by an increase in heart rate and blood pressure. In most instances the increase in heart rate preceded the rise in blood pressure but the amount by which heart rate changed was not related to the amount of change in MAP. The latter suggests a lack of a causal relationship between these two events which was substantiated in vagotomized ducks where MAP changes with respiratory activity occurred while heart rate remained constant. In intact ducks therefore central irradiation must cause an inhibition of vagal restraint on the heart while sympathetic effector activity must be directed at the heart (enhancing the inotropic and chronotropic effects of the decline in vagal activity), the periphery, or both of these. Since the latencies between respiratory burst and rise in MAP were short a cardiac sympathetic effect is indicated, although, even in vagotomized ducks, an increase in inotropic state might also be expected to cause a chronotropic effect on the heart (McCrady, Vallbona & Hoff, 1966).

It is interesting that the degree of cardio-acceleration caused by active lung inflation in ducks was virtually identical to that evoked by passive lung inflation or 'central irradiation' acting alone. This situation is also seen in the mammal (Anrep *et al.* 1936*b*). In fact the similarities between the present data from ducks and that existing in the recent literature for mammals precludes the necessity for detailed discussion (Anrep *et al.* 1936*a*, *b*; Clynes, 1960*a*, *b*, 1961; McCrady *et al.* 1966; Daly & Scott, 1958; Daly, Hazzledine & Ungar, 1967; Daly & Robinson, 1968; Daly, 1972; Angell James & Daly, 1969; 1972*a*, *b*; 1973; Hainsworth, 1974; Valentinuzzi & Geddes, 1974). Undoubtedly CO_2-sensitive pulmonary receptors (Fedde & Peterson, 1970; Leitner, 1972; Fedde *et al.* 1974*a*, *b*; Osborne & Burger, 1974) are responsible both for generation of the lung inflation reflex in birds and for supplying vagal afferent information which provokes the peripherally generated respiratory-heart rate response. A question not resolved by the present experiments however is whether these afferents exert their effect directly on cardiac-vasomotor neurones (Hering, 1871; Anrep *et al.* 1936*a*) or via respiratory neurones which interact with the cardiac neurones. Undoubtedly during diving lung inflation evoked cardio-acceleration when it might be assumed that the

respiratory neurones were inactive. However, there is no doubt that peripheral pulmonary input can 'drive' the respiratory neurones (Kun & Miller, 1974) so that inflation could have provoked a concomitant respiratory neurone activation insufficient to generate a breathing cycle. Certainly passive inflation with 8% CO_2 in nitrogen during a dive nearly always caused the animal to breathe voluntarily within 1–2 sec from the start of inflation which indicates respiratory neurone sensitivity to other inputs was increased by inflation, the resultant respiratory activity being sufficient to override water-initiated apnoea (Butler & Jones, 1968; Bamford & Jones, 1974). The fact that the degree of cardio-acceleration under our experimental conditions was similar whether caused by passive lung inflation or 'central irradiation' at least suggests that similar central nervous interconnexions are involved. In other words, a coupling network may exist between the respiratory and cardiac neurones (Valentinuzzi & Geddes, 1974), so that the so-called direct effects on the cardiac neurones by peripheral input take place via this network.

We are grateful to the Killam Foundation for award of fellowships and to the B.C. Heart Foundation for a grant-in-aid which allowed purchase of the computational facilities used in this study and provided for operating expenses.

REFERENCES

ANDERSEN, H. T. (1963). *The Elicitation of Physiological Responses to Diving.* Norwegian Research Council for Science and Humanities, Universitets-forlaget.

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

ANDERSEN, H. T. & BLIX, A. S. (1974). Pharmacological exposure of components in the autonomic control of the diving reflex. *Acta physiol. scand.* **90**, 381–386.

ANGELL JAMES, J. E. & DALY, M. DEB. (1969). Cardiovascular responses in apnoeic asphyxia; role of arterial chemoreceptors and the modification of their effects by a pulmonary vagal inflation reflex. *J. Physiol.* **201**, 87–014.

ANGELL JAMES, J. E. & DALY, M. DEB. (1972*a*). Some mechanisms involved in the cardiovascular adaptations to diving. *Symp. Soc. exp. Biol.* **26**, 313–341.

ANGELL JAMES, J. E. & DALY, M. DEB. (1972*b*). Reflex respiratory and cardiovascular effects of stimulation of the receptors in the nose of the dog. *J. Physiol.* **220**, 673–696.

ANGELL JAMES, J. E. & DALY, M. DEB. (1973). The interaction of reflexes elicited by stimulation of carotid body chemoreceptors in the nasal mucosa affecting respiration and pulse interval in the dog. *J. Physiol.* **229**, 133–149.

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

ANREP, G. V., PASCUAL, W. & RÖSSLER, R. (1936*b*). Respiration variations of the heart rate. II. The central mechanism of the respiratory arrhythmia and the interrelations between the central and reflex mechanism. *Proc. R. Soc.* B. **119**, 218–230.

BAMFORD, O. S. & JONES, D. R. (1974). On the initiation of apnoea and some cardiovascular responses to submergence in ducks. *Resp. Physiol.* **22**, 199–216.

BURGER, R. E. (1968). Pulmonary chemosensitivity in the domestic fowl. *Fedn Proc.* **27**, 328.

BURGER, R. E., OSBORNE, J. L. & BANZETT, R. B. (1974). Intrapulmonary chemoreceptors in *Gallus domesticus*: adequate stimulus and functional localization. *Resp. Physiol.* **22**, 87–97.

BUTLER, P. J. & JONES, D. R. (1968). Onset of and recovery from diving bradycardia in ducks. *J. Physiol.* **196**, 255–272.

BUTLER, P. J. & JONES, D. R. (1971). The effect of variations in heart rate and regional distribution of blood flow on the normal pressor response to diving in ducks. *J. Physiol.* **214**, 457–479.

BUTLER, P. J. & TAYLOR, E. W. (1973). The effect of hypercapnic hypoxia, accompanied by different levels of lung ventilation, on heart rate in the duck. *Resp. Physiol.* **19**, 176–187.

CLYNES, M. E. (1960*a*). Respiratory sinus arrhythmia: laws derived from computer simulation. *J. appl. Physiol.* **15**, 863–874.

CLYNES, M. E. (1960*b*). Respiratory heart rate reflex (RHR) in man: mathematical law. In *Medical Physics*, vol. 3, ed. GLASSER, O., pp. 184–193. Chicago: Year Book Publishers.

CLYNES, M. E. (1961). Unidirectional rate sensitivity: A biocybernetic law of reflex and humoral system as physiologic channels of control and communication. *Ann. N.Y. Acad. Sci.* **92**, 946–969.

COHN, J. E., KROG, J. & SHANNON, R. (1968). Cardiopulmonary responses to head immersion in domestic geese. *J. appl. Physiol.* **25**, 36–41.

DALY, M. DEB. (1972). Interaction of cardiovascular reflexes. *Lect. scient. Basis Med.* pp. 307–332.

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

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

DALY, M. DEB. & 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.

DRUMMOND, P. C. & JONES, D. R. (1972). Initiation of diving bradycardia in muskrats. *J. Physiol.* **222**, 165–166*P*.

EATON, J. A., FEDDE, M. R. & BURGER, R. E. (1971). Sensitivity to inflation of the respiratory system in the chicken. *Resp. Physiol.* **11**, 167–177.

FEDDE, M. R. & PETERSEN, D. F. (1970). Intrapulmonary receptor response to changes in airway-gas composition in *Gallus domesticus*. *J. Physiol.* **209**, 609–625.

FEDDE, M. R., GATZ, R. N., SLAMA, H. & SCHEID, P. (1974*a*). Intrapulmonary CO_2 receptors in the duck. I. Stimulus specificity. *Resp. Physiol.* **22**, 99–114.

FEDDE, M. R., GATZ, R. N., SLAMA, H. & SCHEID, P. (1974*b*). Intrapulmonary CO_2 receptors in the duck. II. Comparison with mechanoreceptors. *Resp. Physiol.* **22**, 115–121.

HAINSWORTH, R. (1974). Circulatory responses from lung inflation in anaesthetized dogs. *Am. J. Physiol.* **226**, 247–255.

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

JONES, D. R. (1973). Systemic arterial baroreceptors in ducks and the consequences of their denervation on some cardiovascular responses to diving. *J. Physiol.* **234**, 499–518.

JONES, D. R., FISHER, H. D., MCTAGGERT, S. & WEST, N. H. (1973). Heart rate during breath-holding and diving in the unrestrained harbor seal (*Phoca vitulina richardi*). *Can. J. Zool.* **51**, 671–680.

JONES, D. R. & JOHANSEN, K. (1972). The blood vascular system of birds. In *Avian Biology*, vol. 2, ed. FARNER, D. S. & KING, J. E., pp. 157–285. New York: Academic Press.

JONES, D. R. & PURVES, M. J. (1970). The carotid body in the duck and the consequences of its denervation upon the cardiac responses to immersion. *J. Physiol.* **211**, 279–294.

KUNZ, A. L. & MILLER, D. A. Pacing of avian respiration with CO_2 oscillation. *Resp. Physiol.* **22**, 167–178.

LEITNER, L.-M. (1972). Pulmonary mechanoreceptor fibres in the vagus of the domestic fowl. *Resp. Physiol.* **16**, 232–244.

LIN, Y. C., MATSUURA, D. J. & WHITTOW, G. C. (1972). Respiratory variation of heart rate in the California sea lion. *Am. J. Physiol.* **222**, 260–264.

MCCRADY, J. D., VALLBONA, C. & HOFF, H. E. (1966). Neural origin of the respiratory–heart rate response. *Am. J. Physiol.* **211**, 323.

OSBORNE, J. L. & BURGER, R. E. (1974). Intrapulmonary chemoreceptors in *Gallus domesticus*. *Resp. Physiol.* **22**, 77–85.

SCHOLANDER, P. F. (1940). Experimental investigations on the respiratory function in diving mammals and birds. *Hvalråd. Skr.* **22**, 1–131.

VALENTINUZZI, E. E. & GEDDES, L. A. (1974). The central component of the respiratory heart-rate response. *Cardiovasc. Res.* **12**, 87–103.