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ONSET OF AND RECOVERY FROM DIVING BRADYCARDIA IN DUCKS

By P. J. BUTLER† and D. R. JONES*
From the School of Biological Sciences, University of East Anglia, Norwich

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SUMMARY

1. No evidence was found of a 'postural reflex' in ducks. Neither the position of the head nor the water temperature affected the cardiac response to diving.

2. In ducks with access to air through a tracheal cannula, submersion did not invariably cause apnoea until the water level reached the glottis. Heart rate was closely related to respiratory frequency, and bradycardia did not occur during submersion unless there was a reduction in respiratory frequency or a cessation of ventilation altogether.

3. When apnoea and bradycardia did occur during submersion, the first inspiration upon surfacing was 2–3 times larger than normal and was accompanied by an instantaneous rise in heart rate.

4. Atropinization or cold block of the vagus abolished diving bradycardia. Only one vagal trunk was involved in cardiac chronotropic control at any one time. This vagal trunk also appeared to be more important in control of respiratory frequency.

5. β-adrenergic receptor blockade did not affect either diving bradycardia or post-dive tachycardia.

6. The results show that the cardiac chronotropic response both during and after submergence is controlled solely by changes in parasympathetic vagal activity.

INTRODUCTION

During submersion air breathing vertebrates are forced into an apnoeic condition and all habitual divers show a reduction in heart rate (Andersen, 1966). The actual mechanisms involved in the maintenance of apnoea and the initiation of bradycardia during submersion are not completely understood. Ducks become apnoeic and exhibit bradycardia upon assuming certain positions in air, and Huxley (1913*b*) claimed that this postural

† Present address: Department of Zoology and Comparative Physiology, Birmingham University.
* Present address: Department of Zoology, The University, Bristol.

reflex was important during submersion. Koppányi & Kleitman (1927) later reported that during natural submersion ducks never assume the positions that produce apnoea and bradycardia in air. Andersen (1963*a*) found that wetting the beak induced apnoea even if the duck had access to air through a trachael cannula. Bradycardia during submersion is generally thought to be the result of a rise in vagal tone (Andersen, 1966). This increase in parasympathetic activity may be reflexly induced by wetting water-sensitive areas in the beak and nostrils (Andersen, 1963*a*), by sudden apnoea causing progressive hypoxia and hypercapnia (Andersen, 1963*b*) or by a combination of these factors.

As with the onset of diving adjustments the termination of diving adjustments on surfacing has been attributed to a variety of factors, such as a decrease in nervous influence from peripheral receptors (Andersen, 1963*a*) or a conscious realization of surfacing (Irving, Scholander & Grinnell, 1941). However, it is only following the first inspiration that complete recovery and post-dive tachycardia occurs (Andersen, 1963*a*). A reduction in inhibitory vagal tone due to either interaction from proprioceptors in the lungs (Anrep, Pascual & Rössler, 1936), or baroreceptors in the walls of arteries or veins may be important in this respect (Andersen, 1963*a*; Jones, 1966).

The purpose of the present investigation is to determine whether the wetting of water-sensitive areas on the beak and nostrils is important in the onset of bradycardia and the maintenance of apnoea during submersion of the duck. The respective roles of the sympathetic and parasympathetic nervous systems in both onset and recovery from diving bradycardia are also studied.

METHODS

Twenty mallard ducks (*Anas platyrhynchos*) and sixteen domestic ducks (*A. domesticus*) of both sexes were used. They weighed between 0·9 and 1·7 kg. During experiments the birds were restrained horizontally, ventral side down. Heart rate was determined from the electrocardiogram which was obtained from two wires sewn into the left thigh and right shoulder (lead II). The signal was amplified by a Tektronix 122 low-level preamplifier and displayed on an A.E.I. pen oscillograph. Respiratory frequency was determined from a pneumograph. An air-filled ballooon was fastened over the dorsal surface of the animal and pressure changes in the balloon, coinciding with respiratory movements, were detected by a Sanborn 270B pressure transducer. Respiratory tidal volume was measured by a pneumotachograph connected to a tracheal cannula. The pressure difference across a fine nylon mesh screen (350 mesh, aperture size 45 μ, area of screen 1·3 cm^2) was directly proportional to air flow up to a rate of 10 l./min, and was detected by a Sanborn 270B differential pressure transducer. This system had a frequency response of about 20 c/s. The signal was displayed on an A.E.I. pen recorder and gas volume was calculated from the area under the flow curve. The pneumotachograph was used to measure the volume of both inspired and expired gas. Because of this, there was the danger that condensation on the nylon screen would alter the calibration characteristics of the system. The screen was not heated, and in trial runs on the

resting animal the output of the system began to change after about 75 sec. Therefore in experiments air flow measurements were not taken for longer than 1 min at a time. The pneumotachograph was calibrated before each experiment.

All the operations were of a superficial nature and were performed after a local injection of 2 % w/v xylocaine (lignocaine hydrochloride with adrenaline 1:80,000, Astra-Hewlett Ltd, Watford). This produced local anaesthesia for long periods of time and the animals showed no signs of distress during the operations nor during the experiments except sometimes after atropinization. The trachea and vagi are just below the skin and easily exposed. Division and intubation of the trachea were both performed after spraying the necessary area with xylocaine. All animals that had undergone operations were killed at the end of each experiment with an overdose of Nembutal (Pentobarbitone Sodium, Abbot Laboratories Ltd, Queenborough).

Various authors (Andersen, 1963*a*, *b*; Johansen & Aakhus, 1963) have found that the cardiovascular response to submersion can be elicited in the duck by simply covering the head with water. This fact was made use of, and the words 'dive' and 'submersion' as applied to the present investigation mean immersion of the head only into water. Similarly, the words 'surface' and 'emersion' signify the removal of the head from water. When determining effect of posture on the diving response, a Perspex box was fitted over the duck's head and a rubber membrane formed a water-tight seal around the neck, without impeding ventilation. Air was passed through the box at a rate of 2·5 l./min before the dive. The head was held in the required position, air flow stopped and the box was filled with water in order to simulate a dive. At the end of the diving period the water was run out of the box and air flow resumed.

'Simplex' dental cement (Dental Fillings, London) was used to secure a polythene tube over the nostrils. As the volume of the tube was about 10–15 ml. it was aerated with fresh air thus enabling the submerged animal to have access to air. Allowing access to air during a dive was also achieved by intubating the trachea with a polythene cannula, which was held above the water surface. The beak was strapped to keep it closed and the cannula held in position at the corner of the mouth. The size of the cannula varied according to the size of the trachea, but the volume of the largest tube was not more than 3 ml. Eyes alone were wetted by placing a Perspex box over the eyes with the head in a vertically down position. A rubber membrane formed a water-tight seal below the eyes, so that filling the box with water wetted only the eyes. The larynx and epiglottis could be wetted while still allowing the ducks access to air by inserting two polythene tubes into the trachea. The trachea was exposed and divided, one cannula was inserted towards the lungs and the other towards the mouth. The duck was able to breathe through the former and water could be poured down the latter to wet the internal respiratory passages.

The vagi were exposed in the neck. Reversible cold block was achieved by hooking one of the nerve trunks over a length of brass wire 1 mm in diameter which was attached to the cooling stage of a freezing microtome ('Pelcool', C. W. Brown Ltd., Hertford). The temperature at the tip of the wire was $-5°$ C. Drugs were administered through the left wing vein. Acetylcholine was given at a dose of 0·5 mg/kg and transitory bradycardia caused by this was blocked by 2·5 mg/kg atropine. β-Adrenergic block was achieved by using Inderal (Imperial Chemical Industries). Its effectiveness was determined by its ability to block the tachycardia which followed the injection of 5 mg/kg isoprenaline sulphate, the most potent activator of the β-adrenergic receptors. A dose of 0·5 mg/kg Inderal was found to be effective in this respect.

In the present account, 'left' and 'right' refer respectively to the left and right sides of the animal. The *t* test was used in the statistical analysis of the data and 'significant' in the present report means at the 95 % level ($P < 0·05$).

RESULTS

Control of apnoea and its relationship to bradycardia

Preliminary experiments. A number of experiments were performed on mallards to determine whether postural reflexes or water temperature were likely to influence the results of the present experiments. Varying the position of the duck's head had no noticeable effect on heart rate (Fig. 1*a*). Consequently, the two most convenient positions, with the head 45° down

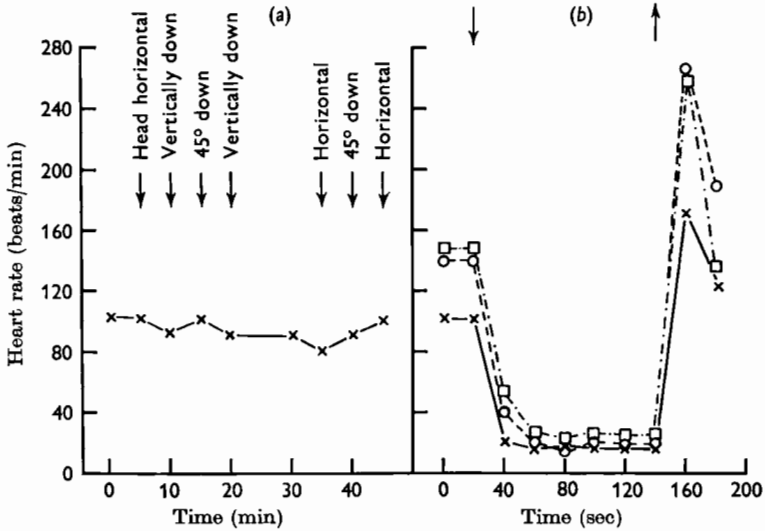

Fig. 1. (*a*) *A. platyrhynchos* 1·46 kg. Unanaesthetized. The effect of different positions of the head in air on heart rate. (*b*) The effect of different positions of the head on diving response in *A. platyrhynchos*. Downward pointing arrow indicates submersion, upward pointing arrow indicates emersion. □ Head vertically down (mean values from 4 experiments). ○ Head 45° down (mean values from 5 experiments). × Head in natural position (mean values from 3 experiments).

or vertically down were chosen for the experiments described in the later sections of this paper. The positions of the head also had no effect on the diving response itself (Fig. 1*b*). Onset and degree of bradycardia were almost identical whether the head was in the natural position (i.e. position that the animal usually holds its head when recumbent) or vertically down. These results support those of Andersen (1963*a*). The fall in heart rate during submersion is unaffected by water temperature. Six observations on three mallards gave a mean fall in heart rate of 79 % when the head was submerged into water at room temperature (18–20° C) as compared with a fall in rate of 76 % when the water temperature was near that of the duck's body temperature (37–40° C).

Water immersion. Several types of experiments were performed to determine if stimulation of peripheral receptors in the beak and nostrils during water immersion is important in the onset of the diving response. In the first series, a wide polythene tube was placed over the nostrils and cemented into position. This gave the submerged animal access to air thus allowing it to breathe. The ducks were submerged slowly and the effect of water at different levels was recorded. Table 1*a* gives the average results of eight experiments performed on two mallards. In seven of these tests ventilation continued with water above the level of the nostrils. In six cases water was raised above the eyes and on four occasions ventilation ceased and heart rate fell. In each instance, there was no change in heart rate unless accompanied by a change in respiratory frequency.

In those cases where apnoea and bradycardia occurred, then a large increase in heart rate and respiratory frequency to values above normal followed surfacing. When ventilation continued during submersion there was little or no change in cardiac frequency when the head was raised into air.

In the experiments already described the external nares were protected from water by the polythene tube. In order to find if wetting the nostrils would induce apnoea and bradycardia, a tracheal cannula was inserted into some mallards and the previous experiments repeated. Ducks had access to air via the cannula during submersion. Table 1*b* gives the average results of seventeen experiments on five animals. In sixteen tests breathing continued with water above the nostrils, and no bradycardia was apparent. When the water level was raised above the eyes, ventilation stopped in thirteen cases, but started a few seconds later in two cases. It therefore appears that water over the eyes may be important in the onset of apnoea and bradycardia. To test this, the eyes alone were covered with water and the average results of fifteen experiments on eight mallards are shown in Table 1*c*. In six of these tests, ventilation stopped initially upon covering the eyes with water, but on three occasions the animal started to breathe again. It therefore appears that wetting the head in the region of the eyes is not very important in the onset of apnoea and bradycardia.

The glottis is at the same level as the eyes when the head is vertically down, and it is possible that water in contact with the internal respiratory passages was the cause of apnoea. Two tubes inserted into the trachea enabled the ducks to breathe through the lung-facing cannula, whilst water was poured down the oral-facing tube. Ducks were submerged until water covered the nostrils but not the eyes. Only in those cases in which the animals continued to breathe was water then poured down the oral cannula. Eight experiments were performed upon three mallards (Table 1*d*). In each case apnoea and bradycardia occurred during the period that

TABLE 1. Variations in heart rate and respiratory frequency in ducks, in response to water immersion under various conditions. In assessment of mean values the number of experiments included is shown in parentheses. In a few animals responses varied and these have been included twice; see text for explanation. H.R. = heart rate, R.F. = respiratory frequency

				Submersion (level of water indicated)						Emersion (level of water indicated)					
				Tip of beak		Above nostrils		Above eyes		Below eyes		Below nostrils		Tip of beak	
Procedure	No. of Experiments	Before submersion H.R.	Before submersion R.F.	H.R.	R.F.	H.R.	R.F.	H.R.	R.F.	H.R.	R.F.	H.R.	R.F.	H.R.	R.F.
a. Tube over nostrils	8	92·5	11·75 (8)	107·0	12·6 (8)	108·0	12·0 (7)	80·0	10·5 (2)	—	—	—	—	199·0	23·0 (5)
		—	—	—	—	50·0	0·0 (1)	34·25	0·0 (4)	—	—	—	—	—	—
b. Tube in trachea	17	160·0	16·0 (17)	—	—	160·0	15·3 (16)	142·0	11·0 (5)	262·5	27·4 (7)	202·5	19·75 (3)	—	—
		—	—	—	—	70·0	0·0 (1)	48·5	0·0 (13)	50·0	0·0 (3)	—	—	—	—
c. Box over eyes	15	150·5	14·8 (15)	—	—	—	—	141·5	11·8 (12)	234·0	24·25 (3)	—	—	—	—
		—	—	—	—	—	—	56·5	0·0 (6)	—	—	—	—	—	—
								Water down tube		Water stopped					
d. Two tubes in trachea	8	176·25	17·0 (8)	—	—	175·0	16·6 (8)	—	—	300·0	27·75 (4)	392·5	33·75 (4)	—	—
		—	—	—	—	—	—	55·0	0·0 (8)	43·3	0·0 (4)	—	—	—	—

water flowed down the tube. On four occasions ventilation began and heart rate increased as soon as water stopped flowing down the cannula, but in the other four instances recovery was only seen when the nostrils were clear of the water. Thus, water in the internal respiratory passages will induce apnoea and bradycardia even when water over the nostrils has no such effect.

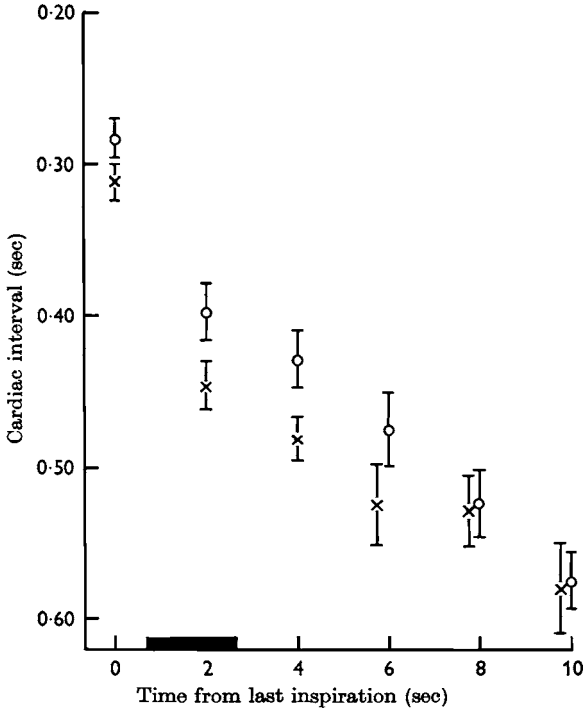

Fig. 2. Development of bradycardia in response to natural respiratory pauses in air (×), and submergence (○). Time 0 sec represents the end of one inspiration and the marker indicates the period during which submersion took place. (Mean values from 16 experiments in each case, vertical lines indicate S.E. of mean).

Relationship between apnoea and bradycardia. The normal post-dive tachycardia occurred simultaneously with the performance of the first large inspiration (Fig. 6*b*). On no occasion when animals were surfaced quietly was any large decrease in cardiac interval observed until the first inspiration. Similarly, as results in the previous section show, onset of bradycardia was not dependent upon water immersion itself, but it did seem to be related to apnoea. Some ducks showed short periods of apnoea with their heads in air. The change in cardiac interval during these periods was compared with changes caused by immersion of the whole head into water. The average results of determinations made on sixteen animals are shown in Fig. 2.

Respiratory pauses usually occurred in the expiratory position and cardiac interval was measured from the end of one inspiration to the start of the next. Cardiac interval was also measured from the end of the last inspiration before submergence. Submergence always occurred between 1 and 3 sec after completion of the last inspiration. No obvious discontinuities were introduced into the gradual onset of bradycardia by sub-

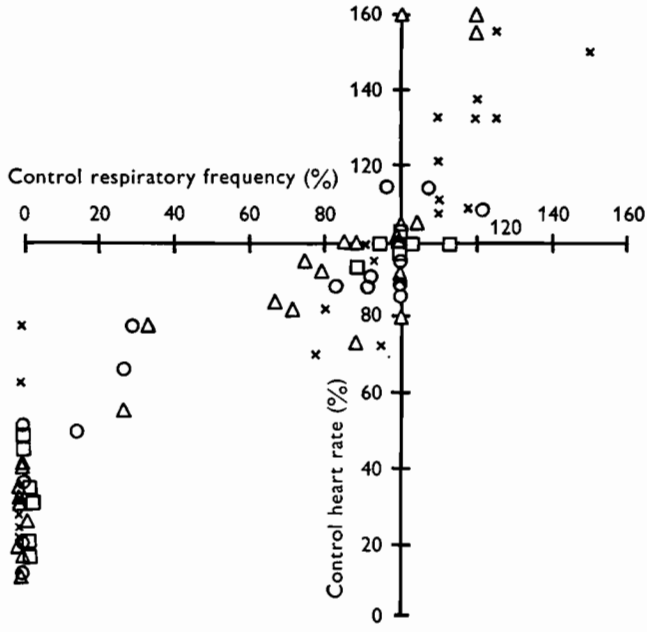

Fig. 3. Relation between respiratory frequency and heart rate in mallard ducks. Both parameters expressed as % pre-submersion value. Different parts of the head and beak were submerged, but the level of submersion is not important. All animals had access to air. ×, Animals with tube over nostrils. △, Animals with tube in trachea. ○, Animals with box over eyes. □, Animals with two tubes in trachea. For details see text.

mergence. However, the number of times that animals showed respiratory pauses of the order of 10 sec was few. In order to confirm that water immersion had no effect on development of bradycardia, unless accompanied by changes in respiratory frequency, the relationship between heart rate and respiratory frequency during various levels of submersion was examined. Changes in heart rate and respiratory frequency were expressed as a percentage of the resting level and the results are shown in Fig. 3. On some occasions immersion of the tip of the beak induced an increase in heart rate and this was accompanied by an increase in respiratory frequency. In the majority of cases diving bradycardia was only

present when respiratory frequency was reduced or ventilation ceased (Fig. 3). Reduction in respiratory frequency was related to varying degrees of bradycardia and only in a few instances was there slight bradycardia with no change in respiratory frequency.

The patterns of air flow recorded by means of the pneumotachograph before and after a dive are shown in Fig. 4. During inspiration peak flow

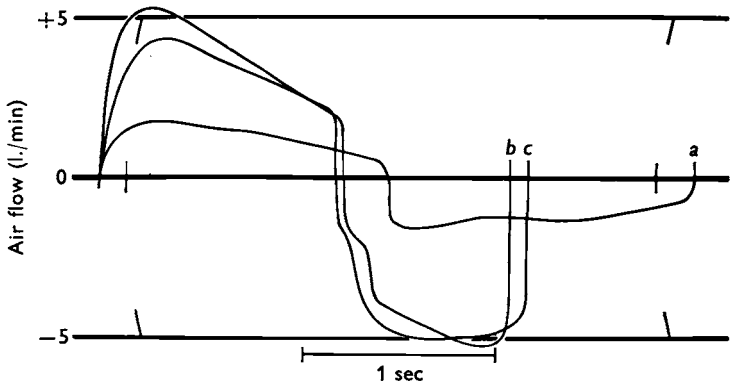

Fig. 4. Pattern of air flow during inspiration (upwards on trace) and expiration (downwards) of a duck. Tracings from original figures. (*a*) Resting, (*b*) immediately after a dive, (*c*) 30 sec after emergence. Curved lines show writing radius of the pen and coincident points in time.

rate was immediately established and thereafter flow rate decreased but seldom reached zero before expiration. This pattern was observed under both resting and post-dive conditions (Fig. 4*a–c*). However, the air flow pattern recorded during expiration was variable. In the resting animal flow rate decreased steadily from a peak value established at the start of expiration (Fig. 4*a*). These pneumotachograms of the resting animal are similar to those recorded by Amoroso, Scott & Williams (1964) in both the chicken and duck. Expiration was usually longer than inspiration in the present experiments taking an average of 2·23 sec (twenty-seven determinations on six ducks) compared with 1·47 sec for a complete inspiration. Expiratory flow pattern changed immediately 'post-dive' to one of increasing flow throughout (Fig. 4*b*). During this period, inspiration took 1·08 sec (twenty-four determinations on six ducks) and was 0·14 sec longer than expiration. Respiratory frequency was therefore increased. Within 10–30 sec from the end of a dive, expiratory air flow remained at a more or less constant rate (Fig. 4*c*) and this phase took 1·09 sec (twenty-one determination on six ducks), whereas inspiration took 1·18 sec. Soon after this period the flow pattern characteristic of the resting animal was established although the complete return of temporal relationships was

delayed. In some animals fluctuations in air flow corresponding to ventricular contractions were superimposed upon the pneumotachograms. This effect has also been described in some mammals (Amoroso *et al.* 1964).

In the resting animal the mean value for minute volume was 700 ml./min (range 450–880 ml./min for ten experiments on four ducks). During sub-

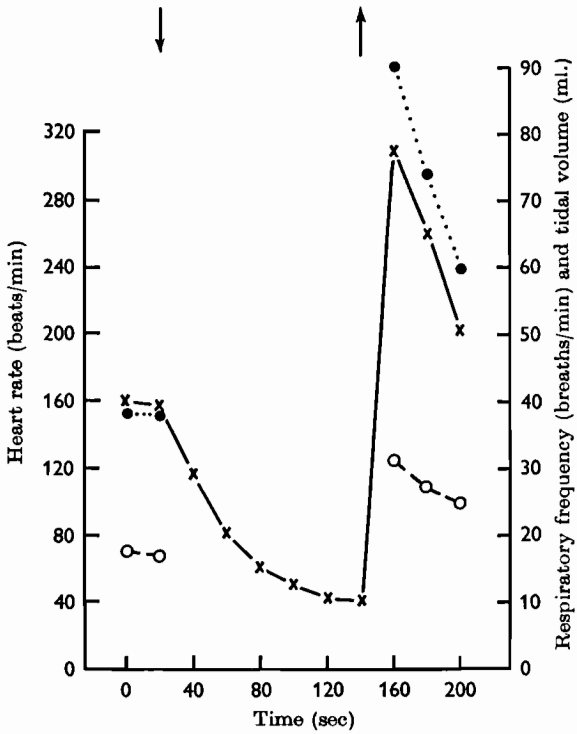

Fig. 5. Relations between heart rate (×), respiratory frequency (○) and tidal volume (●) before and after submersion. Arrows indicate submersion and emersion as in Fig. 1. Each point is average of 10 experiments performed on four mallard ducks. Tidal volume immediately on emersion is that of first inspiration.

mersion the tracheal cannula was clamped to prevent the animal having access to air and just before emersion the clamp was removed. Upon surfacing the first breath was much larger than in the resting animal and tidal volume of this inspiration was 230% the 'pre-drive' value (Fig. 5).

Role of the autonomic nervous system

In these experiments no duck had access to air during submersion and the whole head was immersed in water when simulating a dive. As already mentioned, accompanying the first, large inspiratory effort at the end of a dive is an instantaneous increase in heart rate. To test the importance of the

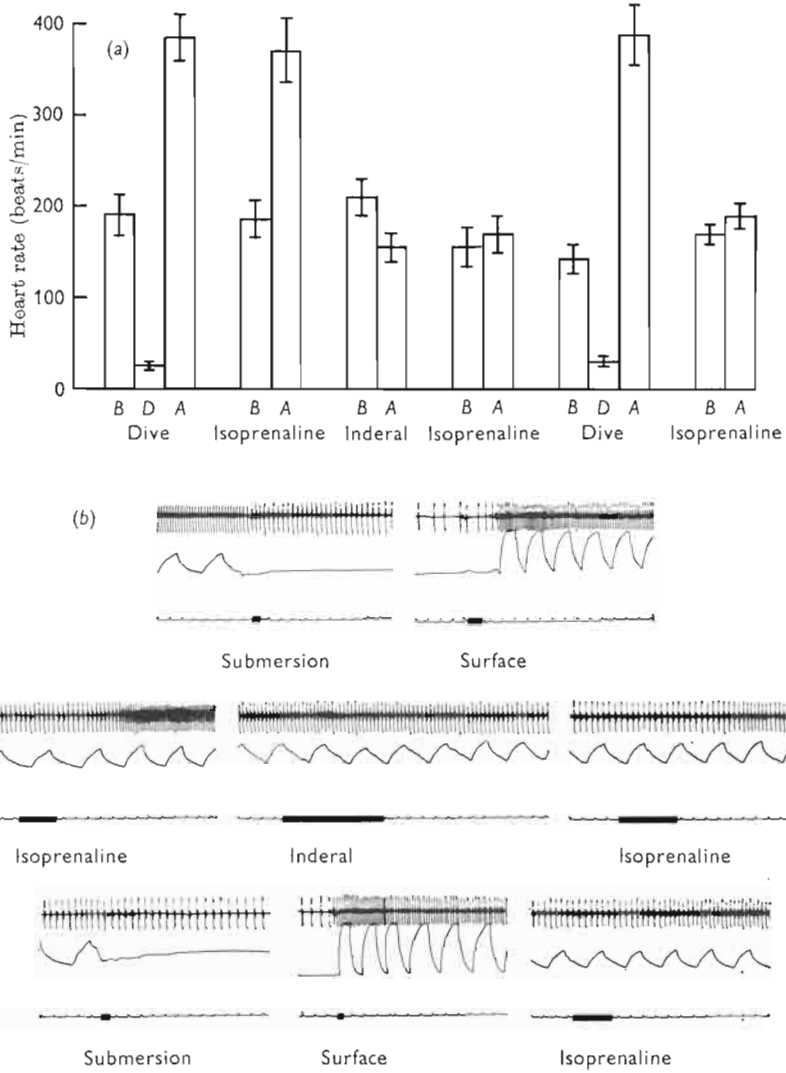

Fig. 6. Effect of β-receptor blockage on heart rate before, during and after submersion in mallard ducks. (*a*) Mean results from five ducks (vertical lines indicate S.E. of mean). *B.* Heart rate recorded before submersion or injection of drug. *D.* Heart rate at lowest level during submersion. *A.* Heart rate measured at first inspiration after surfacing or at a time of maximum response after injection of drug. Each dive was of 2 min duration. (*b*) *A. platyrhynchos* 1·6 kg. Unanaesthetized. Traces showing effect of β-receptor blockage on heart rate before, during and after submersion. In each series, upper trace, electrocardiogram; middle trace, pneumogram (up on trace-inspiration); lower trace, time marker in sec.

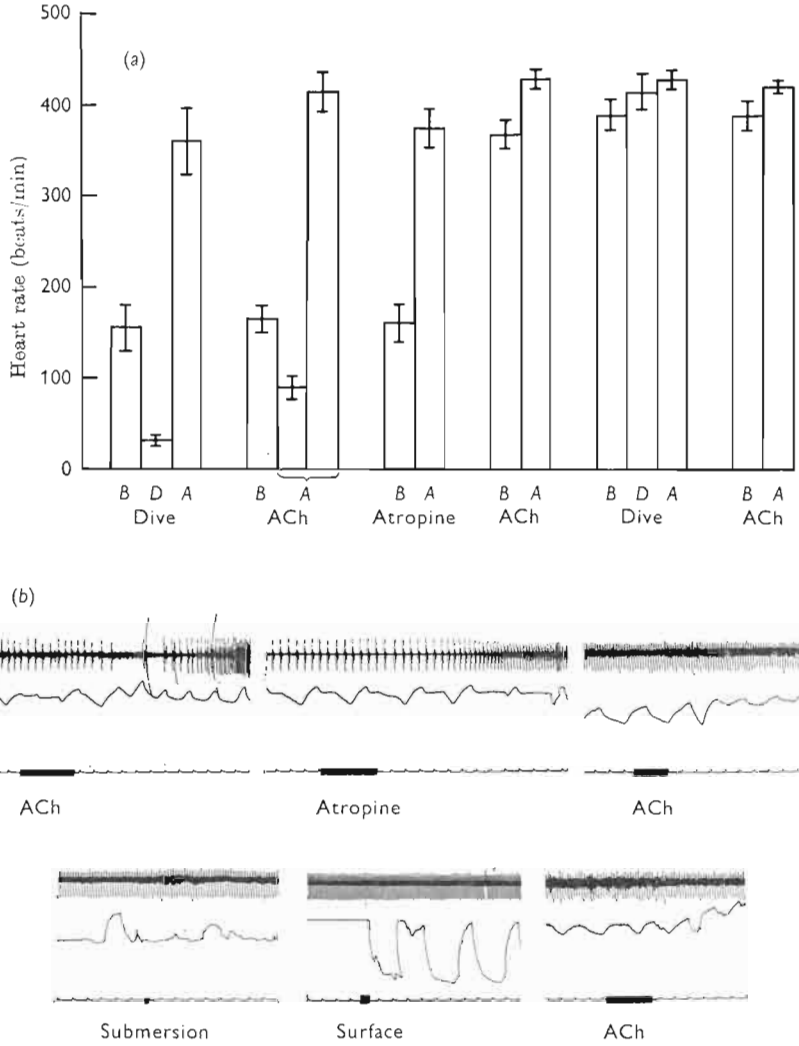

Fig. 7. Effect of blocking parasympathetic nervous system on diving response in mallard ducks. (*a*) Mean results from five ducks (vertical lines indicate S.E. of mean). *B*. Heart rate recorded before submersion or injection of drug. *D*. Heart rate recorded at lowest level during first dive, and just before surfacing in second dive. *A*. Heart rates recorded at first inspiration after surfacing or a time of maximum response after injection of drug. Before atropinization, injection of acetylcholine induced two responses which were both measured. Each dive was of 1 min duration. (*b*) *A. platyrhynchos* 1·59 kg. Unanaesthetized. Traces showing effect of atropine on heart rate before, during and after submersion. In each series, upper trace, electrocardiogram; middle trace, pneumogram (up on trace-inspiration); lower trace, time marker in sec. Struggling during submergence displaced the pneumogram, necessitating readjustment on emergence.

sympathetic nervous system in this response, adrenergic β-receptor activity was blocked by injecting Inderal intravenously into some ducks. The effectiveness of Inderal was ascertained before and after each dive by its ability to block the tachycardia caused by injection of isoprenaline (Fig. 6*b*). Figure 6*a* shows the mean results of five such experiments each performed on a different mallard duck. The length of each dive was 2 min. A dive was performed on a duck before β-receptor blockage. Isoprenaline was injected and caused a significant increase in heart rate (Fig. 6*a*). After injection of Inderal there was no significant tachycardia following isoprenaline injection. The ducks were then made to dive again. The bradycardia was not significantly different from that seen before β-receptor blockage, nor indeed was the 'post-dive' tachycardia (Fig. 6*a*). Further injection of isoprenaline caused no significant tachycardia, showing the Inderal to be active. Thus the sympathetic nervous system is not essential for the diving bradycardia or the 'post-dive' increase in heart rate. Following the injection of Inderal there was a fall in heart rate but this was not significantly different from the normal level (Fig. 6*a*).

Another five experiments on mallards using atropine confirmed that bradycardia during the dive is caused solely by parasympathetic activity (Fig. 7*a*, *b*). Atropine abolished the temporary slowing of heart rate caused by acetylcholine injection, and also the diving bradycardia (Fig. 7). Three animals struggled during the dive and there was actually a slight increase in heart rate during submersion. This struggling was taken to indicate that atropinized ducks were less able to tolerate submersion than intact animals. Therefore the length of each dive was reduced to 1 min. Bradycardia caused by acetylcholine injection lasts for only about 5 sec. After this there is a large increase in heart rate (Fig. 7*b*).

Johansen & Reite (1964) noted that section of the right vagus of the duck caused no lasting changes in heart rate, whereas section of the remaining left vagus caused lasting tachycardia. In the present investigation the vagus trunks were exposed in the neck region of domestic ducks and reversibly cold blocked one at a time, before and during a dive. Each vagus trunk consists of afferent and efferent fibres to and from various organs. Only fibres affecting heart rate were of importance in the present experiment, so heart rate was used as an indicator of the state of activity of each vagus trunk. When referring to the 'active' and 'inactive' vagus, these terms only apply as far as cardiac chronotropic control is concerned. A local anaesthetic was used when exposing the vagi. The fact that heart rate of the ducks was not altered after exposure of the vagi indicated that the anaesthetic had not affected the nerve fibres concerned with cardiac control. Before submersion, selective blockage of one vagus trunk had no effect on heart rate, whereas selectively blocking the other caused an

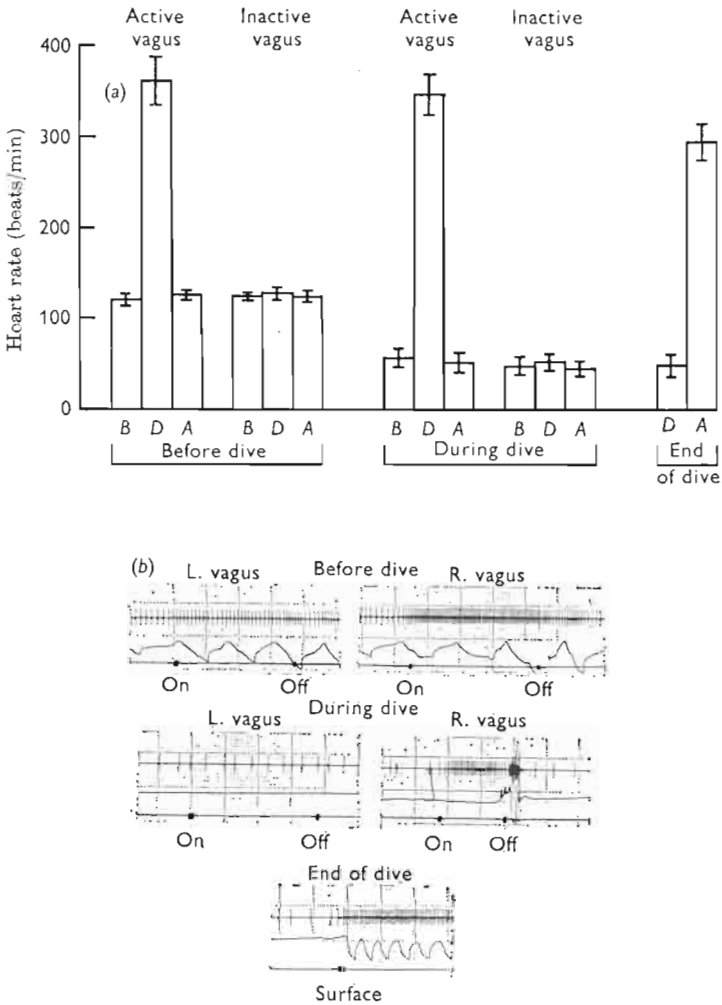

Fig. 8. Effect of reversible cold block of vagi on heart rate before and during submersion in domestic ducks.

(*a*) Mean results from 7 experiments on four ducks (vertical lines indicate S.E. of mean). Right vagus active in two animals and left vagus active in the other two. Before and during dive, *B*. Heart rate before cold block, *D*. Heart rate during cold block, *A*. Heart rate after cold block. At end of dive, *D*. Heart rate during dive, *A*. Heart rate at first inspiration after surfacing.

(*b*) *A. domesticus* 1·62 kg. Unanaesthetized. Traces showing effect of vagal cold block on heart rate before and during submersion. In each series, upper trace, electrocardiogram; middle trace, pneumogram (up on trace-expiration); lower trace, time in sec. 'On' and 'off' respectively refer to the placing of the vagus trunk on and off the cold metal hook.

immediate tachycardia which lasted as long as the cold block was applied. These results were substantiated by unilateral vagotomy. If the active vagus was sectioned there was an increase in heart rate, whereas if the inactive trunk was sectioned no change in heart rate occurred. In each case one vagus trunk was left intact. If after section of the active trunk the inactive nerve was cut, there was no further increase in heart rate. Therefore the so called inactive vagus nerve had in fact exerted no control on heart rate. The fact that cutting the active vagus caused a similar increase in heart rate as cold block of the same trunk indicated that the latter operation interrupted activity in most of the fibres concerned with control of heart rate. After cold block, heart rate always returned to the 'pre-block' value, therefore no permanent damage was done to the cardiac fibres.

Figure 8*a* shows the mean results of seven cold block experiments carried out on four ducks. In two of these animals the right vagus was active (Fig. 8*b*), and in the other two the left vagus was the one that influenced heart rate. Both before and during a dive, blockage of the active vagal trunk caused an increase in heart rate and the level reached was not significantly different in each case. Blockage of the inactive nerve trunk had no significant effect on heart rate in either instance. Upon surfacing, the 'post-dive' tachycardia was not significantly different from the increase in heart rate seen during blockage of the active vagus (Fig. 8*a*, *b*). From these experiments it is obvious that it is not just one particular vagus trunk that carries active nerve fibres to the heart. From a total of sixteen domestic ducks the right vagus was active in twelve, whereas the left caused changes in heart rate in the remaining four. In one particular animal the left vagus was initially active with the right one inactive. One hour later however the right vagus trunk had become active and the left one inactive.

The effects of cold block on respiration were more variable. Blocking the vagus active in cardiac control caused respiratory frequency to increase in five ducks, whereas a sixth consistently showed a reduction in frequency. Blocking the other vagus also caused an increase in respiratory frequency but this was usually not so pronounced as when the active vagus was blocked. Following bilateral cervical vagotomy of five ducks, breathing movements were erratic and respiratory frequency reduced. It was noticed in one duck that following section of the inactive vagus, section of the active vagus trunk caused an acceleration in respiratory frequency which lasted some 20 sec. This was about the period for which cold block of the active vagus was applied. Control experiments were performed in which the vagi were lifted on to uncooled brass wire. This procedure caused no obvious change in heart rate or respiratory frequency.

DISCUSSION

In ducks with access to air, apnoea during diving appears to be reflexly induced by water in contact with the glottis or some other area within the internal respiratory passages. In many instances, ducks continued to breathe whilst water covered the external nares and only raising the water to the level of the eyes caused apnoea. However, covering the eyes with water was not important in this respect since only on a few occasions did wetting the eyes alone induce apnoea. Wetting the internal respiratory passages of ducks invariably caused apnoea. These results in fact substantiate the deductions of Huxley (1913*a*) that only complete submersion of the head, so that the glottis is under water, causes apnoea.

No evidence has been obtained that water immersion by itself plays an important part in development of diving bradycardia, unless an apnoea also occurs. Indeed, the present work illustrates the close relationship between respiratory frequency and heart rate. Bradycardia was only observed during water immersion when breathing slowed or ceased altogether and this slowing in heart rate was similar to that observed during natural respiratory pauses in air. A relationship of this type was previously noted by Koppányi & Dooley (1928), who claimed that insufficient ventilation and accumulation of CO_2 in the blood were responsible for bradycardia both during submersion and 'postural' apnoea. Suggestion of the existence of definite 'immersion reflexes' in ducks in that cardiac slowing occurs when the beak tip touches the water may be explained in terms of this relationship. That lung ventilation alters the character of diving bradycardia is well documented (Andersen, 1963*b*; Feigl & Folkow, 1963; Reite, Krog & Johansen, 1963) but complete recovery with a large tachycardia only occurs, according to Andersen (1966), if tidal volumes much larger than normal are administered. In fact, the average value of tidal volume that occurs on surfacing is 2–3 times normal and in this light the above result is not surprising. However, the diving situation is obviously more complex than just a simple relationship between apnoea and heart rate. Even within these parameters variations exist. Tracheal occlusion results in a reduction in heart rate which is not so severe as occurs during submersion (Andersen, 1963*b*; Feigl & Folkow, 1963). A tentative explanation of this difference could be that during a normal dive exhalation of air, and therefore collapse of lungs or air sacs, is necessary for full development of diving bradycardia and this act will be prevented by tracheal occlusion. In fact, Eliassen (1960) found that birds which did not expel air during a dive developed diving bradycardia slowly. Furthermore, during tracheal occlusion birds frequently attempt to breathe (Andersen, 1963*b*) and this act may have a stimulating effect on heart rate.

Although there is a close relationship between apnoea and bradycardia, the sensory mechanisms responsible for the initiation and maintenance of the cardiovascular response during submersion are still not clear. Repeated submersion may 'condition' the animal so that a certain action makes it aware that submersion is imminent. This 'awareness' of submersion may in fact be an important influence when the animal is in its natural state. Experimental dives on unconditioned animals may only stimulate peripheral reflex mechanisms, whereas in normal situations or with trained animals higher nervous centres may anticipate the dive, and enhance the onset of the response.

The 'post-dive' tachycardia in terms of the chronotropic response is unaffected by adrenergic β-receptor blockage. Combined with the fact that atropinization completely abolishes diving bradycardia, this indicates that the entire cardiac chronotropic response during and upon recovery from diving is under the influence of the parasympathetic nervous system. This is also supported by the fact that selectively cold blocking the active vagus trunk before and during a dive produced a tachycardia not significantly different from that seen at the end of the dive. That a reduction in parasympathetic inhibitory activity is necessary for complete recovery from diving bradycardia and possible mechanisms whereby this may be achieved in the frog has already been discussed (Jones, 1966). In the present experiments the nerves concerned at any one time were confined to one vagal trunk, either right or left, and it was possible for this activity to switch from one trunk to the other illustrating a certain degree of lability in the avian central nervous system.

The present results throw some light on the pattern of normal respiration in ducks. In the resting animal, both during inspiration and expiration, air flow is asymmetrical, peak flow being established immediately after the start of inspiration or expiration. This type of air flow is unusual compared to that recorded for man (Mead & Agostoni, 1964) and a series of mammals studied by Amoroso *et al.* (1964). Our results do not elucidate the role of the vagus in the control of normal respiration. Undoubtedly bilateral vagotomy, in the short term, caused a reduction in rate and a tendency towards arrhythmia. Unilateral cold block, however, gave results which are in conflict with present literature (Sturkie, 1965). Apart from one experiment where unilateral vagal blockage consistently caused a decrease in rate, in all other experiments unilateral vagal block caused acceleration. The vagus active in cardiac control was more effective in this respect than the non-active trunk. Weak stimulation of the central end of the cut vagus generally causes increased respiratory frequency (Sturkie, 1965). That the method of freezing may have caused weak stimulation at some point more central to the cold block is possible but numerous other

explanations could be advanced. In view of the confusion already in the literature regarding the role of the vagus in respiratory control, it seems pertinent to leave any analysis until the results of further experiments on this subject are obtained.

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