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FACTORS AFFECTING THE RECOVERY FROM DIVING BRADYCARDIA IN THE FROG

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INTRODUCTION

A relationship between respiratory movements and heart rate has been shown to exist in a number of vertebrates. It has been claimed that in elasmobranchs maximal ventilation of the gills coincides with maximum blood flow through the vessels of the respiratory lamellae (Satchell, 1960). In air-breathing vertebrates this type of direct linkage would have little functional significance and apart from periods of sinus arrhythmia, when heart rate increases during inspiration and slows during expiration, the relationship between heart rate and respiratory movements is seldom apparent. However, in the mammal, respiratory movements have a tonic effect on the heart. Hyperventilation, in response to decreased oxygen, is usually accompanied by an increase in heart rate which is abolished or reduced by lung denervation (Daly & Scott, 1958). This response appears to be a reflex, initiated by proprioceptor discharges from the lung, resulting in reduced activity in the cardiac vagus (Anrep, Pascual & Rössler, 1936).

Terrestrial vertebrates during submersion, and aquatic vertebrates on removal from water, display a pronounced decrease in heart rate (Andersen, 1961, 1963; Garey, 1962; Irving, Scholander & Grinnell, 1941*b*; Johansen, 1959). Fish, birds, and mammals generally show a rapid adjustment of the heart rate, whereas in frogs $\frac{1}{2}$ hr. or so may elapse before the complete expression of bradycardia (Jones & Shelton, 1964). In the frog oxygen lack is the major cause of the fall in heart rate, whereas in the bird full development of diving bradycardia is due to a summation of oxygen lack and excess carbon dioxide (Andersen, 1963). However, in all vertebrates, recovery on surfacing is much more rapid than can be explained by the animal renewing its oxygen supply or eliminating excess carbon dioxide. In fact, in frogs, complete recovery from diving bradycardia occurs even when the animals surface into nitrogen (Jones & Shelton, 1964). This suggests that a neuronal connexion between respiratory movements and heart rate may be important for a rapid recovery although, in this type of experiment, it is possible that elimination of carbon dioxide is aiding recovery. In the duck artificial ventilation of the lungs, during submersion, with a gas mixture which potentiates the effect of hypoxia and hypercapnia has little effect on heart rate whereas large increases in rate usually occur if one or both are relieved (Andersen, 1963). Irving, Scholander & Grinnell (1941*a*) claimed a 'conscious realization' of the end of a dive by the porpoise. The anticipation of breathing is sufficient to cut off, either partially or completely, a number of the physiological

adjustments to diving before the start of respiratory movements. In the duck specific 'immersion reflexes', triggered by wetting of receptors in the region of the beak, depress heart rate during submersion so that on surfacing the heart rate increases due to cessation of the inhibitory influence; but complete recovery does not occur until respiratory movements begin (Andersen, 1963; Feigl & Folkow, 1963).

Three explanations have been given of the rapid recovery from diving bradycardia These are: (1) a linkage between heart rate and respiratory movements; (2) renewal of the oxygen supply or elimination of excess carbon dioxide, both of which are dependent on respiratory movements; (3) a 'conscious' or reflex element which is independent of respiratory movements. The purpose of the present investigation is to study these in the amphibian by determining the effect of hypoxia and hypercapnia on the recovery response and the role of the autonomic nervous system when the heart rate is changing rapidly.

METHODS

Experiments were performed on seven toads (*Bufo bufo*), forty-eight *Rana pipiens* and seventy-nine *R. temporaria*. Although there are quantitative differences between the reactions of these animals to submergence (Jones, in preparation), these were unimportant from the point of view of the current investigation. The experiments were carried out in two tanks of 12 and 3 l. capacity and the animal was restrained by clamping it either to a Perspex board or to a wax block. In addition a number of free-choice experiments were performed in which the animal was allowed to move freely in the box and to surface at will. The electrocardiogram (e.c.g.) was detected by thin copper wire sewn into the chest, and, after amplification in a Tektronix type 122 pre-amplifier, was displayed on an A.E.I. pen recorder. Movements of the buccal cavity were recorded photo-electrically and flank movements by means of an R.C.A. mechano-electrical transducer. Submersion and emersion were achieved by changing the water level in the tank. A Perspex box was inverted over the water surface in such a way that on emersion the animal was obliged to breathe from the box. The gas concentrations in the box were varied by introducing nitrogen or carbon dioxide. The oxygen tension was determined polarographically by a Beckman 777 analyser and the carbon dioxide concentration by estimation with a Roughton-Scholander capillary syringe.

In those experiments involving cannulation of the lungs the animal was fixed to a wax block and a cannula was introduced through a small slit in the body wall into the tip of the lung. The lungs were inflated with 1–3 c.c. of gas delivered from an hypodermic syringe. The dead space of the cannulation tube was less than 0·05 c.c. so that virtually complete anoxia could be maintained on inflation from a syringe filled with nitrogen. The tenth cranial nerve was approached in the angle of the jaw as described previously (Jones & Shelton, 1964). The sympathetic nervous system was exposed by making a 0·5 cm. incision in the roof of the pharynx, and after removal of the overlying muscle the first sympathetic ganglion could easily be seen. From this position it was possible to expose the first four sympathetic ganglia. Control experiments were always carried out before nerve section to eliminate possible effects of the operation to expose the nerves. In some experiments yohimbine hydrochloride, an adrenaline antagonist, was injected into the dorsal lymph sac (0·1–0·125

ml. of 1 in 10^{-3} per 10 g. wt.) to inhibit the action of the sympathetic nervous system on the heart.

Recordings from the pulmonary and cardiac vagus were made with a pair of fine silver-wire electrodes, and the signal, amplified by conventional means, was displayed on a Tektronix 502A oscilloscope. The pulmonary and cardiac branches of the vagus were usually exposed by ligaturing the superior vena cava on one side, and after the vessel had been cut the nerves could be seen under a magnification of 50 to 100 times. In about one-third of the experiments it was possible to isolate the nerves without ligaturing the superior vena cava. The sympathetic nervous system was exposed dorsally, and recording was attempted from the connexion between the first sympathetic ganglion and the vagus ganglion. All operations were performed under MS 222 Sandox anaesthesia (300 mg/l) and the experiments were carried out on the animals after recovery from the anaesthetic.

RESULTS

(1) *Effect of anoxia and hypercapnia on recovery from bradycardia*

After submersion for 30–45 min. animals were allowed to surface into atmospheres of reduced oxygen concentration. Bradycardia was immediately released, the heart rate returning within 1 or 2 min. to virtually the pre-immersion value at all concentrations between 21 and 0·2% oxygen (Fig. 1).

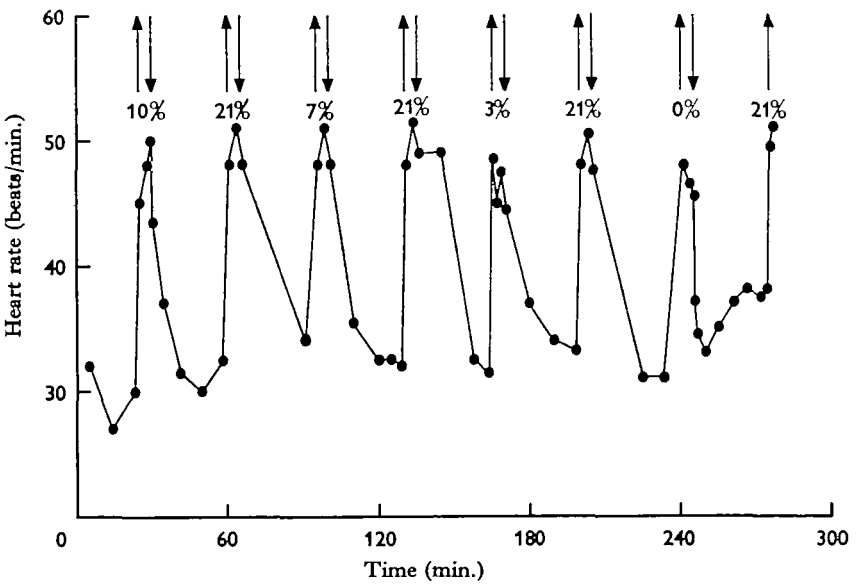

Fig. 1. The effect on heart rate of allowing a frog to surface into air of reduced oxygen content. Upward-pointing arrows indicate emersion, downward ones submersion. Percentage oxygen content of the air is shown beneath the arrows.

The heart rate was usually maintained at this level throughout the period at the surface except when oxygen content of air which the animal was breathing was less than 3%. At these low levels, after an initial increase, heart rate declined but it was unusual for bradycardia as pronounced as that seen during diving to occur. Re-

submersion after exposure to low levels of oxygen frequently caused a greater fall in heart rate than usually occurred at first submersion, but this was not invariably the case and seemed to depend on the duration of the period of exposure. As a control between successive emersions into reduced oxygen the animal was allowed to surface into air. All the animals survived these experiments. A number of experiments were performed in which animals could choose between surfacing into air devoid of oxygen or remaining in well-aerated water. As before, bradycardia which developed during submersion was released on surfacing. Occasionally animals remained at the surface for protracted periods of time until they became moribund. It seems probable that oxygen receptors in the frog and toad have similar deficiencies to those of man, though the ability to exchange gas through the skin may be a complicating factor in the amphibian.

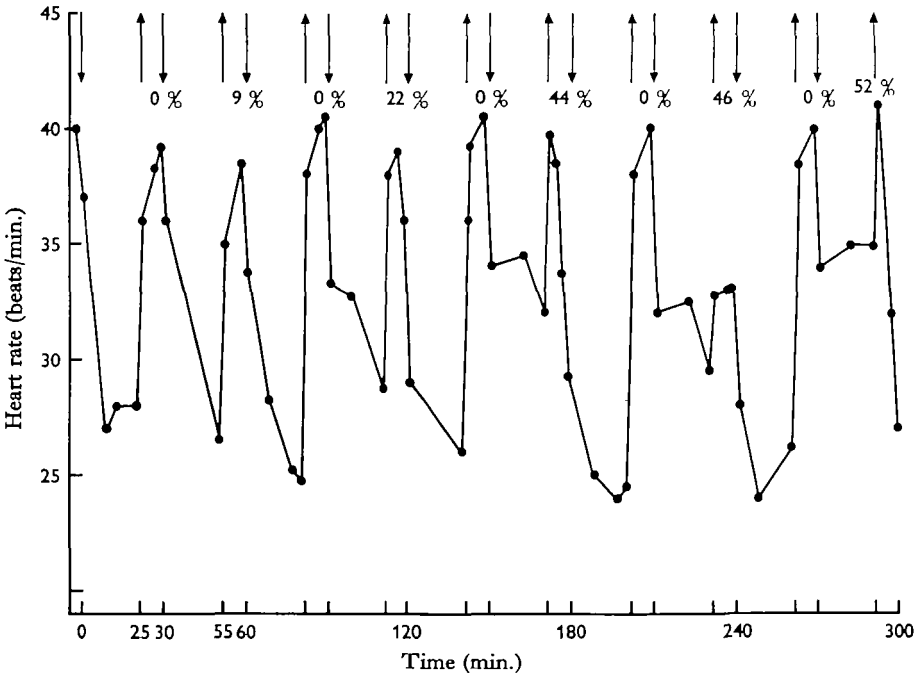

Fig. 2. The effect of allowing a frog to surface into increasing concentrations of carbon dioxide in air. Arrows indicate emersion and submersion as in Fig. 1. The frog spent 5 min. at the surface each time and on the graph these periods have been extended in relation to the time spent submerged. Percentage carbon dioxide concentration is shown beneath the arrows.

When animals were raised into increasing concentrations of carbon dioxide in air no effect on the recovery response occurred at low levels; but after exposure for 3–4 min. a small fall in heart rate usually occurred. This reduction in heart rate was associated with a decrease in frequency of lung ventilation. Above 10% carbon dioxide the response was variable, but even at very high concentrations (40–50% CO_2) complete recovery sometimes occurred (Fig. 2). Despite variability in the recovery response, bradycardia, which developed on resubmersion, was always more pronounced after exposure to carbon dioxide than after surfacing into air alone. Complete

failure to recover from bradycardia only occurred when the animal surfaced into 40–100% carbon dioxide and at lower levels if the carbon dioxide content of the water was increased correspondingly. This effect was probably due to narcotization of the animal for on surfacing the animal often failed to make respiratory movements, and, in these cases, there was no increase in heart rate. Free-choice experiments confirmed the above results. Recovery to the normal heart rate occurred on surfacing except when the levels of carbon dioxide in the air over the water were very high. The animals generally spent periods of less than 5 sec. at the surface and frequently were moribund at the end of the experiment.

Table 1. *Effect of allowing* Rana pipiens *to surface into different gas concentrations. Average results of five experiments. Heart rate developed on surfacing expressed as a percentage of the normal surface heart rate*

Gas mixture into which animal surfaced	Exposure time to gas mixtures		
	15–90 sec. (%)	120–210 sec. (%)	240–300 sec. (%)
Air	93	—	96
Air + 5% CO_2	94	91	87
Nitrogen	97	58	46
Nitrogen + 5% or 10% carbon dioxide	82	55	41
Nitrogen + 10% carbon dioxide + 5% oxygen	103	101	95

In further experiments *R. pipiens* was allowed to surface into a series of different gas mixtures, including mixtures calculated to maintain both hypoxia and hypercapnia on surfacing. The average results of five experiments are shown in Table 1. The recovery response of *R. pipiens* was similar to that of *R. temporaria* when allowed to surface into air, into air with 5% carbon dioxide, or into nitrogen, except that prolonged exposure to nitrogen caused a much greater fall in heart rate in *R. pipiens* than in *R. temporaria*. In anuran amphibians the lungs are filled by movements of the buccal floor. Two types of movement were performed by the resting animal; one corresponding to buccal ventilation and the other to exchange of gas with the lung. All the buccal movements were of the 'lung filling or emptying' type after surfacing into air (Fig. 4*b*(i)). These movements are larger than those of solely buccal ventilation and are characterised by an extra-downward movement of the buccal floor as gas is withdrawn from the lung. Prolonged exposure to nitrogen altered the normal post-dive ventilation pattern in both *R. temporaria* and *R. pipiens*. In the former, lung ventilation persisted but the frequency was reduced, whereas in the latter 'lung filling and emptying' buccal movements ceased, being replaced by those of solely buccal ventilation, and the heart rate fell rapidly. Occasionally *R. pipiens* made short bursts of lung ventilations, and during these movements heart rate increased. The most potent effect both on the recovery response and on respiratory movements was caused by mixtures of 5 or 10% carbon dioxide in nitrogen. Respiratory movements usually ceased after about 15–30 sec. exposure and heart rate decreased. On one occasion a frog performed lung ventilations after 4 min. exposure and in this period the heart rate doubled. But a mixture of 5% oxygen, 9% carbon

dioxide and 86% nitrogen had no effect on recovery and little on the performance of respiratory movements except in causing a reduction in frequency of lung ventilation.

In order to study the recovery response more closely and to eliminate effects of variations in respiratory movements, the lungs were inflated during submersion. The volume of gas used was determined prior to the experiment by deflating the lungs (after the animal had filled them by its own respiratory movements) and measuring the amount removed. In the majority of experiments the lungs were inflated in one movement and deflated after 2–3 min. but in ten experiments ventilation was performed rhythmically, at the normal post-dive rate of lung ventilation, for periods up to 2 min. In both cases the results were similar. Since it has already been shown that anoxia of the heart is the major cause of the development of diving bradycardia (Jones & Shelton, 1964) lung inflations were made either with air, to release the anoxia, or nitrogen to maintain it.

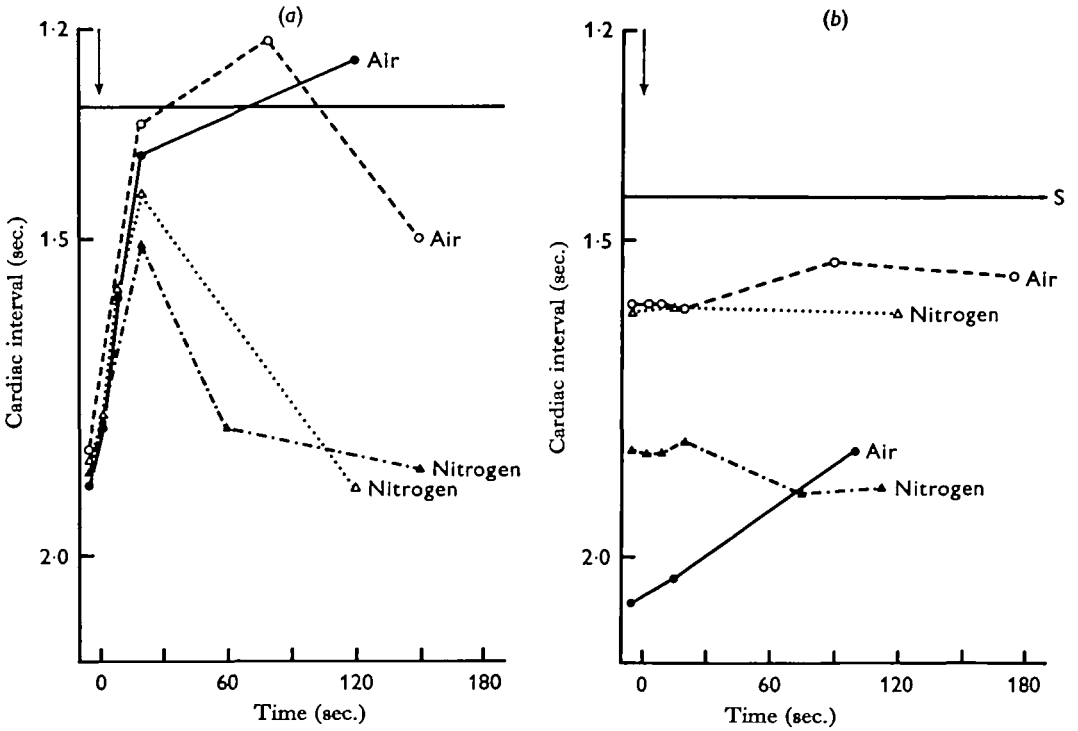

Fig. 3. Increase in heart rate caused by artificial inflation of the lungs with air and nitrogen during submersion (*a*) before, and (*b*) after bilateral vagotomy. Downward pointing arrow indicates inflation. S = average cardiac interval at surface. ○, ●, two inflations with air; △, ▲ two inflations with nitrogen.

In the majority of experiments heart rate increased immediately after inflation, and for about 20–30 sec. there was no difference between the responses to air and to nitrogen (Fig. 3*a*). Following an inflation with air, after the initial rise, heart rate continued to increase, and often complete recovery from bradycardia occurred; whereas with nitrogen heart rate declined and often reached a lower level than was present before inflation. (Fig. 3*a*). In these circumstances deflation was usually accompanied by an increase in heart rate until the 'normal' bradycardial level was

attained. Following inflation with air the collapse of the lungs was associated with a return to the heart rate present before inflation, although this was occasionally delayed for as much as 15 min. The animals never struggled after inflation with air, but occasionally did so after inflation with nitrogen and exhaled some of the gas. In a few cases the cardiac interval was increased during the actual period of inflation, but this was immediately followed by a rise in heart rate. When the animal struggled heart rate changes were slight and usually only lasted for 5–10 sec. after cessation of activity.

(2) *Effect of nerve section and drugs*

Bilateral section of the vagus, in the angle of the jaw, did not prevent the development of bradycardia but prolonged the recovery when the animal surfaced into air. In the normal frog, heart rate usually returned to within 5% of the surface rate in 20 sec., whereas after vagotomy re-establishment of the pre-dive rate took at least 5–10 min. Bilateral vagotomy, besides isolating the heart from the nervous system, affected the performance of respiratory movements, which have already been shown to be essential to a rapid recovery from diving bradycardia (Jones & Shelton, 1964). Recordings from the flank of a frog show two kinds of deflection associated with lung ventilation (Fig. 4*a* (i)). The slow deflection represents total change in lung volume, whereas rapid movements superimposed on this mark periods when the larynx is open and the lungs are in contact with the buccal pump. In the resting intact frog the lungs were filled by a series of buccal movements and remained inflated for several seconds, being emptied by a further series of buccal movements. After a dive the frequency of lung ventilation increased and heart rate rapidly returned to the normal level (Fig. 4*a* (ii)). Bilateral vagotomy reduced the frequency of buccal movements, the frog was unable to close its larynx, and all buccal movements, even those of the buccal ventilation type, were followed by a rapid inflation and deflation of the lungs (Fig. 4*a* (iii)). On many occasions the decrease in the number of buccal movements was not so great as occurred in the experiment shown in Fig. 4. However, during submersion the buccal floor was usually pressed against the roof of the buccal cavity, and in this position the larynx must have remained closed because artificial inflations of the lungs were maintained. Following inflation there was no immediate increase in heart rate (Fig. 3*b*). A small rise (2/3 beats/min.) usually occurred following inflation with air, but took 1–2 min. to develop, and a fall in heart rate of the same order frequently followed an inflation with nitrogen. There was no increase in cardiac interval during the actual period of lung inflation, as sometimes occurred in the intact animal. Following vagotomy, surface heart rate was reduced and the cardiac interval was more regular, any variation between ventricular contractions being 0·5–1%, whereas before vagotomy variation was as much as 5%.

Injection of yohimbine hydrochloride also prolonged recovery on surfacing. Before injection, recovery from bradycardia was complete in under a minute; after injection, complete recovery took about 15 min. But, as was the case with bilateral vagotomy, yohimbine hydrochloride also affected the performance of lung ventilation. When the normal animal surfaced buccal movements were usually the 'lung filling and emptying' type and recovery was rapid (Fig. 4*b*(i)); after yohimbine injection only about one-fifth of the normal number of buccal movements were made and recovery was slowed (Fig. 4*b*(ii)).

Since it is likely that both both the sympathetic and parasympathetic innervation to the heart run in the vagus, and since yohimbine injection slowed the rate of recovery while not having as marked an effect on lung ventilation as vagotomy, it seemed possible that the sympathetic nervous system was involved in the rapid recovery response. In order to test this, artificial inflations were performed after destruction of the sympathetic innervation of the heart.

There is some doubt as to the exact path by which the sympathetic innervation of the heart runs in frogs (Bidder, 1866; Dale & Mines, 1913; Holmes, 1927). Conse-

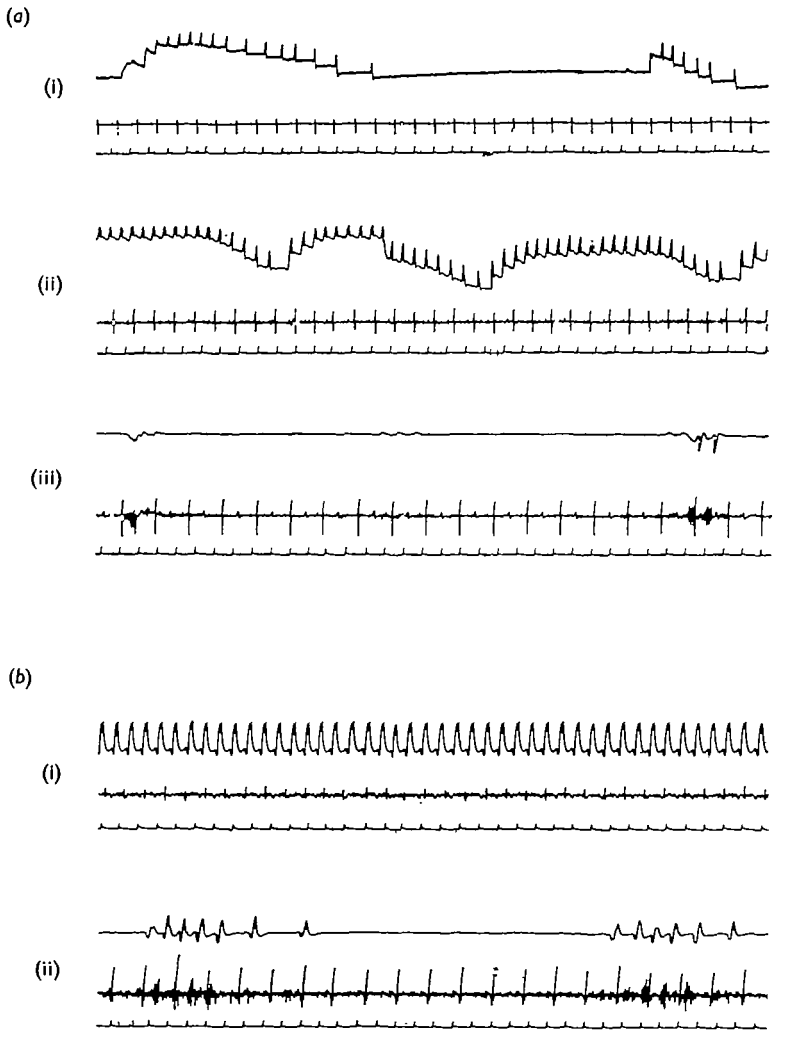

Fig. 4. (*a*) Effect of bilateral vagotomy on lung ventilation in the frog. Upper trace, movement of the flanks (up on trace = emptying of the lungs; for further explanation see text). Middle trace—e.c.g. Lower trace—time (sec.). (i) normal frog before submersion; (ii) normal frog 3 min. after emersion; (iii) vagotomised frog 2 min. after emersion. (*b*) Effect of injection of yohimbine hydrochloride on the performance of 'lung filling and emptying' buccal movements. Upper trace—movements of the floor of the buccal cavity (down on trace = expansion of the buccal cavity). Middle trace—e.c.g. Lower trace—time (sec.). (i) normal frog, 10 min. after emersion, (ii) following yohimbine injection, 10 min. after emersion.

quently, in the present series of experiments not only was the sympathetic chain cut between the vagus and first sympathetic ganglia, but also in a series of fourteen experiments the first, second, third and fourth ganglia were progressively removed bilaterally. None of these stages had any substantial effect on the initial rapid recovery phase which followed inflation (Fig. 5). But after removal of the first four sympathetic ganglia complete release of bradycardia did not occur (Fig. 5*b*). This is probably more a reflection of the condition of the animal after the lengthy operative procedures than of elimination of the sympathetic connexion to the heart. Surface heart rate fell after removal of the sympathetic ganglia but was unaffected by further bilateral vagotomy; following this lung inflation caused little or no change in heart rate.

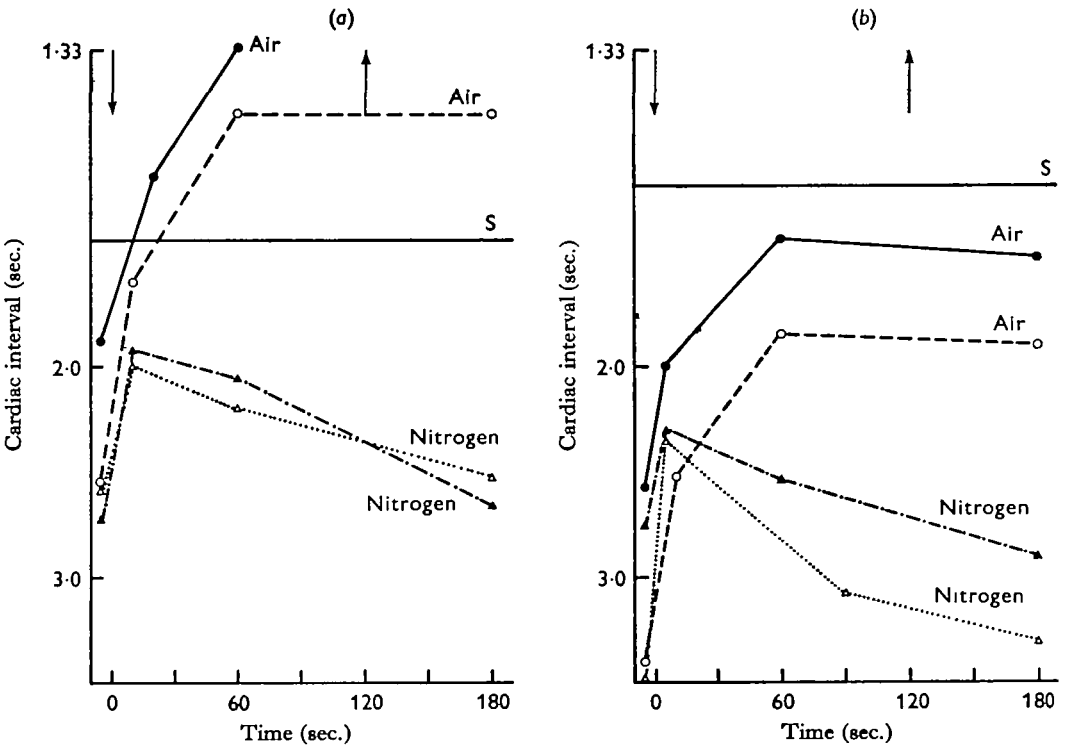

Fig. 5. Increase in heart rate produced by artificial inflation of the lungs with air and nitrogen during submersion after bilateral removal (*a*) of the first, and (*b*) of the first four sympathetic ganglia. Arrows indicate inflation (downwards) and deflation (upwards). S = average cardiac interval at surface. ○, ●, two inflations with air; △, ▲, two inflations with nitrogen.

(3) *Recordings from the pulmonary and cardiac vagus*

When the lungs were inflated spike activity in the pulmonary branch of the vagus increased (Figs. 6 and 7). These impulses were afferent since after section of the nerve they were only recorded from the peripheral end. Activity was greatest during the actual period of lung inflation and subsided afterwards (Fig. 6). A comparison of proprioceptor discharges during cycles of inflation and deflation showed that the majority of proprioceptors respond, by increase in activity, to expansion of the lungs. The number of active propriocepters or their rate of discharge was related to the

degree of distension of the lung (Fig. 6). Increased proprioceptor activity coincided in time with the increase in heart rate which followed lung inflation during submersion (Fig. 7). It proved difficult to record successfully from the cardiac branch of the vagus. It has been claimed that the vagus is only active at certain periods of the year (Iriuchijima, 1959) and, on many occasions, little activity could be detected during submersion. In those instances when activity was recorded it ceased, either partially or completely, when the lungs were inflated (Fig. 8). The decrease in vagal activity

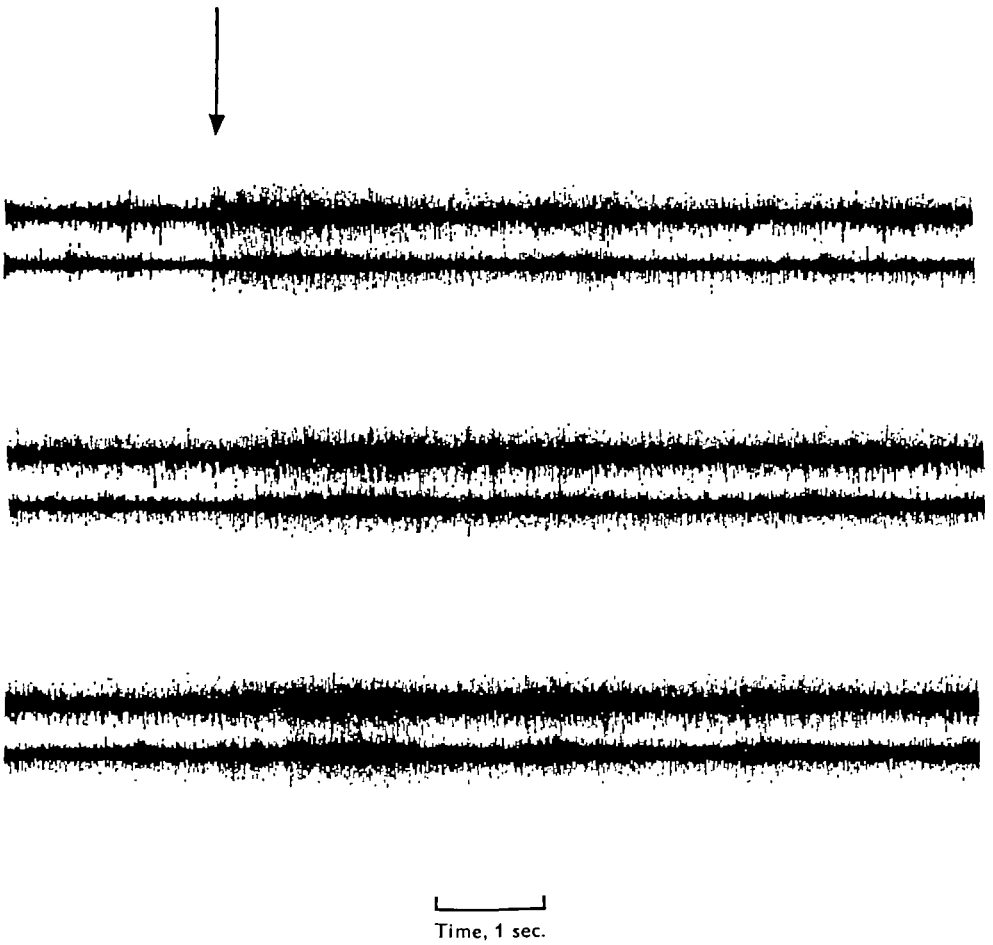

Fig. 6. Afferent spike activity in two branches of the pulmonary vagus. Continuous record showing the effect of increasing volume on the discharge of lung proprioceptors. Lungs inflated in three steps of 1 ml. each at the arrow.

was related to the increase in heart rate which followed inflation. Sometimes activity increased during the actual period of inflation (Fig. 8) and there was no increase in cardiac interval. This may be afferent activity from presso-receptors in the walls of the atria or great veins. Unfortunately a number of attempts to record from the peripheral end of the cardiac vagus proved unsuccessful. The decrease in activity in the cardiac vagus was directly related to the increased discharge in the pulmonary branch of the vagus.

In order to confirm the results of the nerve-transection experiments several attempts were made to record from the corrective between the first sympathetic ganglion and the vagus ganglion. No activity was recorded which could be associated with the increased heart rate following inflation of the lungs during submersion.

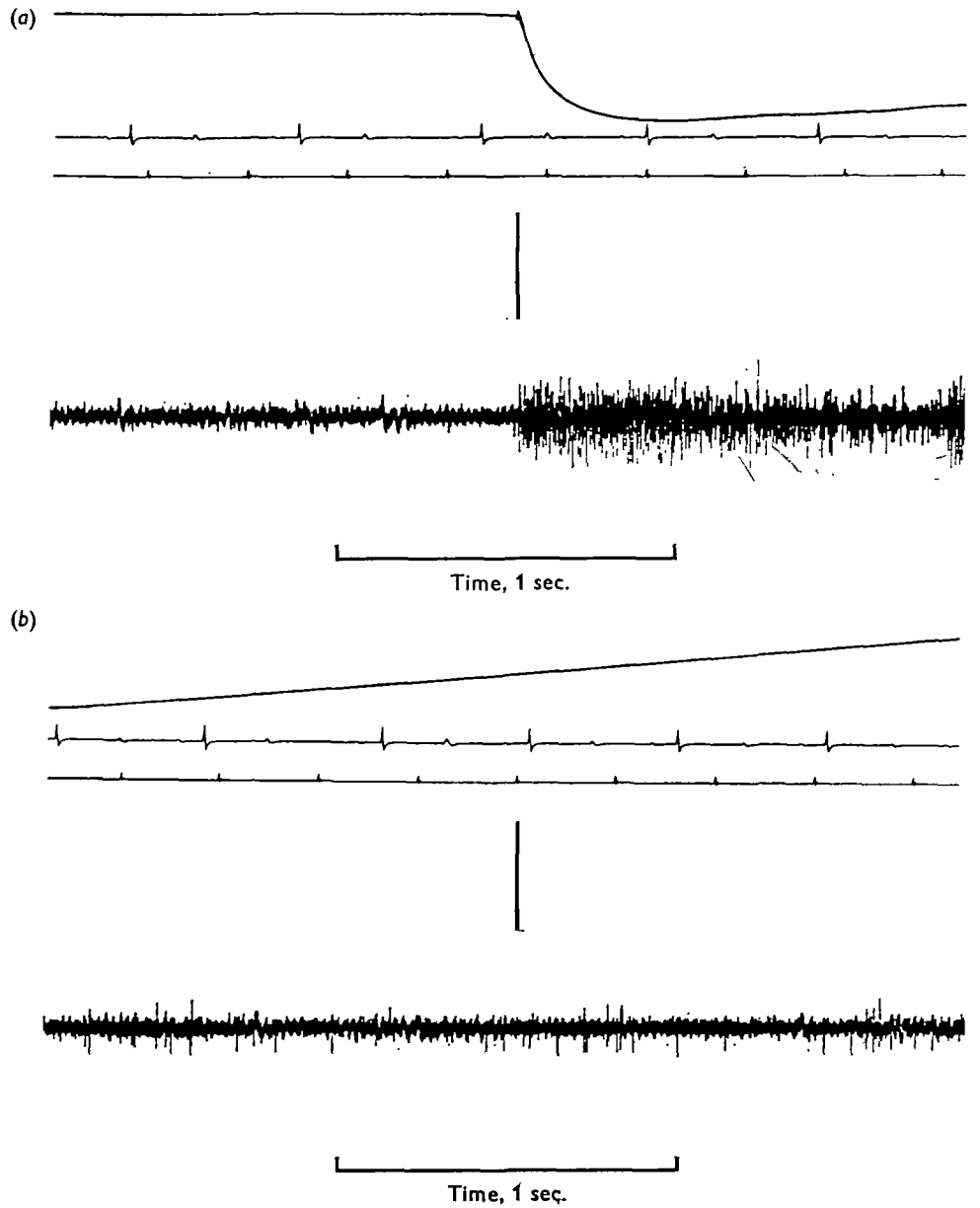

Fig. 7. The relationship between discharge of lung proprioceptors and heart rate during an inflation and slow deflation of the lungs. (*a*) Trace 1—lung volume (down on trace = increase in volume). Trace 2—e.c.g. Trace 3—time (sec.). Trace 4 selected portion of an oscilloscope recording of activity in the pulmonary vagus. This is taken on a different time scale as calibration shows. (*b*) Traces 1, 2 and 3 continue from (*a*) above. Trace 4—oscilloscope recording. Vertical lines link coincident points in time on the traces.

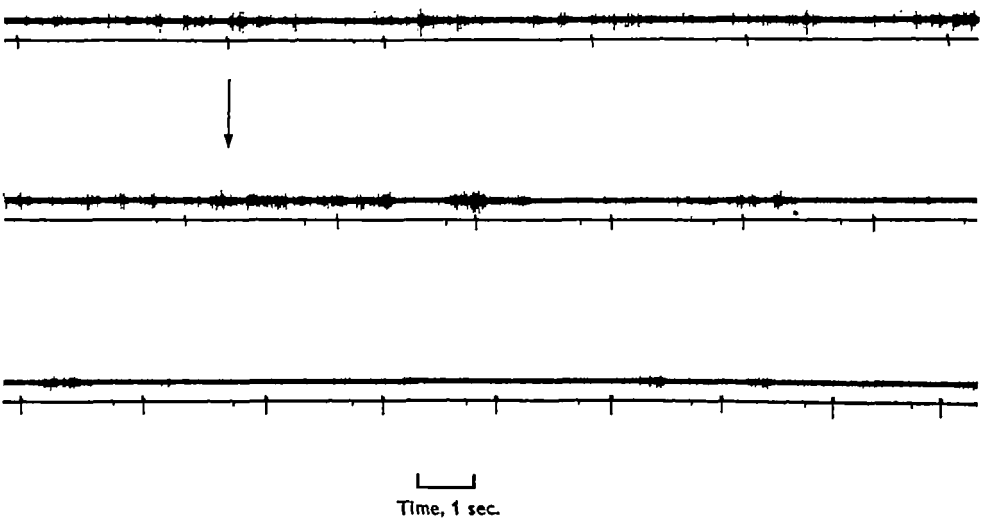

Fig. 8. Continuous record of activity in the cardiac branch of the vagus before, during and after lung inflation. Trace 1—activity in cardiac vagus. Trace 2—e.c.g. Arrow indicates start of period of inflation (inflation completed in 2–3 sec.) Time, 1 sec.

DISCUSSION

Neither high levels of carbon dioxide nor lack of oxygen in the air into which the frog surfaces are sufficient to prevent recovery from diving bradycardia. Provided that the animal ventilates its lungs heart rate rapidly returns to the pre-dive level. Although anoxia is the major cause of development of bradycardia the initial part of the recovery is independent of oxygen supply, but longer exposure (3–4 min.) to atmospheres of less than 3 % oxygen in air causes a decline in heart rate. The fall is greater in *R. pipiens*, which stops lung ventilation but continues buccal ventilation, than in *R. temporaria* in which the reverse is the case. The importance of lung ventilation in maintaining a high heart rate is also emphasised by the fact that, when *R. pipiens* is in nitrogen, heart rate increases after bursts of lung ventilation. Nitrogen inhalation is also important in breaking the diving adjustment of the seal, but within a short time bradycardia is re-established (Scholander, 1940; Irving, *et al.* 1941*b*). It is unlikely that the removal of carbon dioxide influences the release of bradycardia since it redevelops with continued breathing. Hypercapnia does not appear to be important in development of diving bradycardia by the frog (Jones & Shelton, 1964), and concentrations of the order of 5–20 % in air have little or no effect on the recovery. In any case, the accumulation of carbon dioxide is unlikely in the submerged frog, since carbon dioxide is readily eliminated through the skin (Krogh, 1904; Dolk & Postma, 1927) and is highly soluble in water. In fact, even prolonged submersion (90 min.) does not result in the concentration of carbon dioxide in the lungs exceeding 3 % (Winterstein, Alpdoğan & Başoğlu, 1944).

Only gas mixtures which stop lung ventilation rapidly have a marked effect on the recovery response. Complete release of bradycardia is rare when the frog surfaces into mixtures of nitrogen and carbon dioxide. However, addition of 5 % oxygen to a mixture of nitrogen and 10 % carbon dioxide allows complete recovery on

emersion and has little effect on heart rate during prolonged exposure. Furthermore hypoxia causes a greater fall in heart rate than hypercapnia at any given frequency of lung ventilation and, when lung ventilation stops, the decrease in heart rate is generally similar whether the frog surfaces into nitrogen or into nitrogen and carbon dioxide. These results suggest that the frog is more sensitive to oxygen lack both during development of bradycardia and prolonged recovery from it. The importance of release of anoxia for maintained recovery is shown by artificial inflations of the lungs during submersion. Although the initial reaction to air or nitrogen is the same, complete recovery from bradycardia only follows air inflation and release of annoxia.

Bilateral vagotomy, isolating the heart from the nervous system, does not prevent development of diving bradycardia but eliminates the initial rapid increase in heart rate which follows lung inflation. The aneural heart of *Myxine* and that of the hypothermic hibernating mammal *Erinaceus*, in which a nerve conduction is 'blocked' (Johansen, Krog & Reite, 1964), both show marked increases in heart rate as a result of an enhanced venous return. But a 'pressure sensitive' pacemaker cannot be involved in cardio-acceleration in the amphibian, since the linkage between heart rate and lung inflation is dependent upon the autonomic nervous system. The sympathetic system is usually assumed to be involved in any rapid increases in heart rate, so it appeared likely that the effect of bilateral vagotomy could be due to the removal of the sympathetic connexion to the heart. Elimination of the sympathetic innervation alone does not affect recovery; consequently the basis of the initial rapid response of the heart must lie on the para-sympathetic side of the nervous system.

There appear to be two possible ways in which lung inflation may affect the heart rate. The first is that the increased internal pressure, following lung inflation, increases venous return which affects the heart by means of a vagal reflex of the Bainbridge type. Presso-receptors in the walls of the pulmonary vein, inferior vena cava or atria (Neil & Zotterman, 1950), stimulated by the increased flow, may interact in such a manner as to reduce the parasympathetic discharges present during submersion. Certainly venous return from the lungs increases after inflation (Shelton & Jones, 1965). Recordings from the cardiac vagus often show an increase in activity during the actual lung-filling movement and for a short time afterwards. This may be afferent activity of the type required for a Bainbridge reflex. After inflation the parasympathetic discharges are reduced and the heart rate rises. The second possibility is that the increase in proprioceptor activity in the lungs upon inflation causes the fall in activity in the cardiac branch of the vagus. On the present evidence it is not possible to decide whether one of these mechanisms is more important than the other or that one in fact acts exclusively. Section of the pulmonary vagus alone, if this proves possible, will clarify the situation, as will stimulation experiments which are now in progress. A small number of the latter have been carried out and the results seem to indicate that the pulmonary branch of the vagus could be involved in the response. On several occasions increases in heart rate have followed pulmonary nerve stimulation during submersion, but they are not often as large as those seen after lung inflation. Undoubtedly the basis of the response of the amphibian heart to lung inflation is similar to that of the mammalian heart (Anrep *et al.* 1936). As in the mammal, vagal tone seems very important for the full expression of the response;

frogs displaying only a slight bradycardia, or a more pronounced one which has taken a long time to develop, do not show rapid increases in heart rate after lung inflation.

There is no evidence for the participation in recovery of either an 'anticipation of breathing' (Irving *et al.*, 1941*a*) or specific 'emersion reflexes' (Andersen, 1963; Feigl & Folkow, 1963). Respiratory movements in themselves are all important to the recovery. Several factors may stimulate respiration on surfacing apart from the need of the animal to obtain more oxygen. Endopulmonary pressure exerts a controlling influence on the respiratory movements of the frog (de Marneffe-Foulon, 1963); since this probably falls when the lungs collapse during submersion, it may explain why the first fillings of the lungs are invariably greater than normal. The peak systolic blood pressure, and sometimes also the diastolic blood pressure, declines during the development of bradycardia (Shelton & Jones, 1965). In the mammal a decrease in blood pressure stimulates respiratory movements and this also appears to hold for the amphibian, since, during experiments on cardiovascular dynamics, it was often noted that loss of blood increased both the amplitude and frequency of lung-ventilating buccal movements. Consequently the fall in blood pressure during submersion may help in increasing the drive towards breathing.

These experiments have also thrown some light on the control of the heart in the intact animal. Elimination of all nervous connexion with the heart slows the rate by an amount which is comparable to the reduction that occurs when the sympathetic connexion alone is cut. In fact, after section of the sympathetic, further removal of the parasympathetic innervation has little or no effect on heart rate. Hence it is apparent that sympathetic tone is more important in controlling the normal heart rate. Parasympathetic activity varies according to season (Iriuchijima, 1959), but even in summer, when most of these experiments were performed, it is probably exerting some measure of control on the heart since the regularity of the cardiac cycle is greatly increased after its removal.

SUMMARY

1. Recovery from diving bradycardia is not prevented by allowing a frog to surface into nitrogen or excess carbon dioxide.

2. Artificial respiration performed during submersion causes an immediate increase in heart rate whether the lungs are filled with air or nitrogen. The initial rapid increase in heart rate is prevented by bilateral vagotomy but is unaffected by section of the sympathetic connexion to the heart.

3. Spike activity in the pulmonary vagus increases as the lungs are filled. A burst of activity frequently occurs in the cardiac vagus during this period. The heart rate increases as the cardiac vagus becomes quiet. Activity continues in the pulmonary branch until deflation of the lungs.

4. The suggestion is made that the initial rapid increase in heart rate following artificial respiration or on surfacing is due to a decrease in parasympathetic inhibitory activity, by an interaction from either proprioceptors in the lungs or presso-receptors in the walls of the atria or veins.

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