Coordination of the Heart and Gill Rhythms in *Limulus*

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Summary. 1. When *Limulus* is exposed to hypoxia both heart rate and ventilation rate decrease together (Fig. 1, Fig. 2A). Hypoxia ultimately leads to cessation of ventilation and concomitant bradycardia.

2. When oxygen is reintroduced into an oxygen-free aquarium ventilation resumes rapidly, with a parallel increase in heart rate (Fig. 1, Fig. 2B).

3. Covariation of heart and gill activity similar to that in hypoxia experiments also occurs during the normal ventilatory behavior patterns of *Limulus*, such as intermittent ventilation, swimming, hyperventilation and gill cleaning.

4. The covariation of heart and ventilation rates is especially evident during transitions of intermittent ventilation (alternating periods of apnea and ventilation, Fig. 3).

5. Covariation is also evident during the large increases in ventilation frequency which occur during hyperventilation and swimming (Fig. 4).

6. Gill cleaning is a centrally determined motor sequence which consists of rhythmic flicking of the inner lobes of a gill plate between the book gill lamellae of the plate on the opposite side. During this behavior there is a marked slowing of the heart rate which is at least as great as the decrease in rate seen during periods of apnea (Figs. 5 and 6).

7. Changes in heart rate associated with ventilatory activity do not appear to be caused by the metabolic demand resulting from such activity (Fig. 7).

8. In addition to frequency covariation of the heart and ventilation rates, there can also be phase coordination of the two rhythms. When the two are close to the same frequency or to harmonic frequencies, the heart often maintains a phase preference with respect to the concurrent gill interval over a considerable period of time (Fig. 8).

9. These results suggest that there are common tonic inputs to both the cardiac ganglion and the central pattern generators for the various ventilatory behaviors, which modulate the frequencies of both simultaneously. Both the frequency covariation and phase communication between the two systems may serve to increase the efficiency of the respiratory-circulatory interactions.

Introduction

The respiratory behavior of *Limulus polyphemus* is a product of the coordinated activity of the five abdominal appendages and the neurogenic heart. Their combined activity is responsible for the efficient exchange of oxygen between the environment and the body tissues. The neural control of the ventilatory movements has been examined in detail (Hyde, 1893; Fourtner et al., 1971; Wyse, 1972; Knudsen, 1973; for recent review see Wyse and Page, 1976), as has the activity of the cardiac ganglion and its associated cardioregulatory nerves (Carlson, 1904, 1905; Patten and Redenbaugh, 1900; Pax and Sanborn, 1964; Pax, 1969; Bursey and Pax, 1970; Corning and Von Burg, 1969, 1970a, 1970b; Abbott et al., 1969). The interaction between these two systems has not been studied in detail. That there might be a close coordination between them is suggested by previous work with decapod crustacea (Larimer, 1964; McMahon and Wilkens, 1975; Wilkens et al., 1974; Young, 1973; Coyer, 1977a, b) and by work with *Limulus* itself, indicating that both heart and ventilation respond to the same stimuli. The ventilation rate of *Limulus* slows as the ambient oxygen concentration is lowered (Waterman and Travis, 1953; Page, 1973). The heart rate of *Limulus* is also sensitive to environmental oxygen levels (Falkowski, 1974). However, the simultaneous response of both systems to hypoxia has not been examined.

Heart and ventilation rates also fluctuate considerably during normal behavior of *Limulus*. Spontaneous alterations in ventilatory activity have been noted by a variety of authors, but very little data is available concerning changes in heart rate during these behaviors. In fact, there is only one case in which the heart rate was reported to change at all during normal behavior of the animal (Corning and Von Burg, 1970b).

In this paper we will demonstrate that in *Limulus polyphemus* there is a coordination of the gill and heart rhythm, both during normal activity, and in response to alterations in the ambient oxygen concentration. We will also discuss how these results may help us to more fully understand the neural control of respiration.

Materials and Methods

Specimens of *Limulus polyphemus* (6–10 in. carapace width) were obtained from the Marine Biological Laboratory, Woods Hole, Mass., and were maintained in a 150 gallon recirculating seawater system (15–19 °C).

Electric activity of ventilatory muscle was recorded chronically from intact animals with insulated 40 gauge stainless steel wires. The electrodes were inserted into muscle 20 (Lankester et al., 1885) through small holes in the overlying cuticle and cemented in place with Eastman 910 adhesive. Indifferent electrodes were placed in the opisthosoma near its lateral margin (Wyse and Dwyer, 1973).

Electric activity of the heart was recorded with 40 gauge stainless steel wires inserted through small holes along the dorsal midline of the opisthosoma, just over the cardiac ganglion. Electrical recordings from unrestrained animals during periods of vigorous activity were often quite noisy. Therefore, heart activity was recorded with a modified impedance plethysmograph (Coyer, 1977b). Amplified signals were tape-recorded (Philips Analog 7, FM) for subsequent photography and analysis.

Experiments were carried out at room temperature in a 15 gallon glass-bottomed aquarium, allowing direct visualization of the gill appendages at all times. Electrode leads were twisted together and attached to a sliding track above the aquarium so that animals were free to move about in the tank. Oxygen, air and nitrogen were introduced into the tank through an airstone located in one corner. Oxygen concentration in the seawater was monitored using a Yellow Springs Instrument Company Model 53 oxygen meter and an oxygen probe (Model 5311) located in an adjacent corner (see Page, 1973, for a more detailed methodology).

Filmed data were digitized (Coordicon Model T36/36–16 x-y coordinate digital converter attached to an IBM 526 keypunch) and computations were performed on a CDC Cyber 74 computer. Data were graphed on a Calcomp Model 563 drum plotter.

Results

Response of the Heart and Gills to Hypoxia

One way to determine if two rhythmic behaviors show a frequency covariation is to alter the frequency of one and look for a corresponding response in the other. The ventilation rate in *Limulus* is proportional
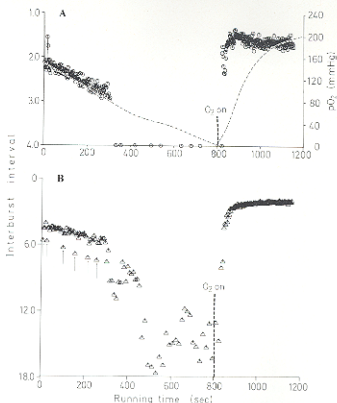

Fig. 1A and B. Changes in heart and ventilation rates in response to variation in the ambient pO_2. As in all succeeding graphs running time and interburst interval are expressed in s. Also Y-axis is reversed so that increases in rate (smaller interburst intervals) have a positive slope. Nitrogen was bubbled continuously to lower the pO_2; nitrogen was replaced by oxygen (O_2 ON) to increase the pO_2. Beginning and end of this experiment not presented. **A** Ventilation rate ($\circ$) and pO_2 (—). **B** Heart rate ($\triangle$). Arrows indicate some of the heartbeats which skipped a cycle of ventilation, thus maintaining a preferred phase of 0.5 with respect to next gill interval (see text). Time axis is identical but Y-axis is expanded to accommodate longer heart interburst intervals which occur at very low pO_2's. Note that ventilation and heart rates covaried throughout the experiment

to the ambient oxygen concentration, decreasing at lower pO_2 (Waterman and Travis, 1953; Page, 1973). Thus, by systematically altering the ambient pO_2 we were able to elicit changes in the ventilation rate and then examine the corresponding changes in the heart rate.

Figures 1A and 1B are graphs of such an experiment showing the changes in pO_2, ventilation, and heart rates during the middle portion of a typical one hour experiment. Figures 2A and 2B are graphs taken from the same data showing the transitions in ventilatory behavior in greater detail. As the pO_2 was lowered by bubbling nitrogen, both the heart and ventilation rates decreased in a nonlinear fashion. When ventilation ceased at low oxygen concentrations
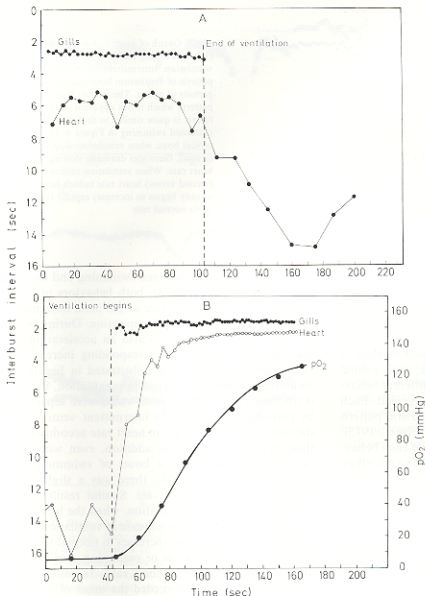
Fig. 2A and B. Changes in heart rate during transitions in ventilation, in response to alteration of ambient pO₂. Data taken from the same experiment shown in Figure 1A and B.
A. At pO₂ of approximately 40 mm Hg ventilation stopped and heart rate rapidly slowed.
B Oxygen was then reintroduced and after 23.5 s ventilation began and there was rapid six-fold increase in heart rate

there was a substantial and quite rapid decrease in the heart rate (Fig. 2A). Heart rate and amplitude continued to decline if hypoxia was maintained for long periods of time. When oxygen was reintroduced after a period of apnea, ventilation began within seconds, accompanied by a 3–5 fold increase in heart rate (Figs. 1, 2B). In some cases there was a slight increase in heart rate prior to the initiation of ventilation; whereas in others there was no change in heart rate until after the onset of ventilatory movements.

Thus, the heart and ventilation rates showed a strong tendency to increase and decrease together as the external oxygen concentration was systematically altered. Such a frequency covariation was found in all 24 experiments performed on 11 different animals.

This covariation was found to be dependent on the integrity of the cardioregulatory nerves. If these nerves were cut prior to altering the ambient oxygen concentrations the heart rate remained unchanged throughout the experiment, while the ventilation frequency varied as usual.

Covariation During Normal Behavior

Limulus has a limited but interesting repertoire of respiratory behavior patterns which includes hyperventilation, swimming, intermittent ventilation, and gill cleaning. Because results of the hypoxia experiments suggested that covariation of cardiac and venti-
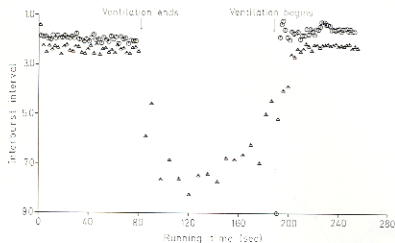
Fig. 3. Graph of heart (△) and ventilation (○) rates during one hour of intermittent ventilation. Intermittent ventilation is a pattern of ventilation bouts separated by periods of apnea. The overall long-term pattern, which is not apparent in this figure, is quite similar to that seen during patterned swimming in Figure 4. During a typical bout, when ventilation stopped (arrow), there was dramatic slowing of heart rate. When ventilation resumed (second arrow) heart rate (which had already begun to increase) rapidly returned to its normal rate

latory rhythms might be part of the normal respiratory behavior of the animal, we conducted a series of experiments designed to examine the relationship between these rhythms during all of the aforementioned behaviors.

In recordings made from unrestrained animals in well aerated aquaria for up to a week, each animal studied displayed at least three of the different behaviors at some time during the recording period. Each different behavior had its own characteristic pattern of electrical activity, or "ethogram" (Elsner, 1975). This enabled an accurate identification of each behavior so that constant observation during the recording period was unnecessary.

Intermittent Ventilation

Intermittent ventilation in *Limulus* is characterized by alternating periods of ventilation and apnea, similar, but not identical to the pattern seen in Cheyne-Stokes ventilation in vertebrates (Hyde, 1893). When ventilation stopped spontaneously during bouts of intermittent ventilation, there was a precipitous drop in the heart rate (Fig. 3), which was comparable to that seen during the hypoxia-evoked apnea in the previous experiments (cf. Fig. 3 and Fig. 2A). After a short period of apnea (typically about 2 min in duration) ventilation resumed and the heart rate returned to normal (Fig. 3). The increase in heart rate, however, did not follow the onset of ventilation; rather, it preceded ventilation in much the same manner as it did in some of the hypoxia experiments.

Swimming and Hyperventilation

The gill appendages of *Limulus* are often utilized for swimming as well as ventilation. Although there are

some basic differences between swimming and hyperventilation (Knudsen, 1973), both behaviors involve a dramatic increase in the rate of gill plate movement over that seen during normal respiration. During both of these behaviors there was also an acceleration of the heart rate during the corresponding increase in ventilation rate. This is best illustrated in Figure 4. Swimming resembled intermittent ventilation, in that it frequently occurred in bouts which were separated by periods of apnea. As in intermittent ventilation, the most dramatic changes in heart rate accompanied these periods of apnea. In addition, even when no period of apnea separated bouts of swimming (as in the beginning of Fig. 4), there was a slight, but consistent change in heart rate. Similar results were obtained during hyperventilation, when the legs were not active. Therefore, any increase in ventilation rate, whether due to slight rate increases in normal ventilation or to hyperventilation or swimming, was associated with an increase in heart rate. In most cases the heart rate increase preceded the onset of ventilation (Figs. 3, 4, 5).

Gill Cleaning

Gill cleaning behavior involves periodic medial adduction of gill plates across the midline, so that the gill plates of a pair can interact. The interaction consists of rhythmic flicking of the inner lobe of a gill plate between the book gill lamellae of the plate on the opposite side. Usually all gill plates engage in cleaning activity at the same time, for a period of about 10 s to a few min. This behavior is similar to the "cramp movements" described by Hyde (1893) and the "across rubbing" noted by Patten (1912); however, distinct cramping movements (in which the gill plates are held adducted across the
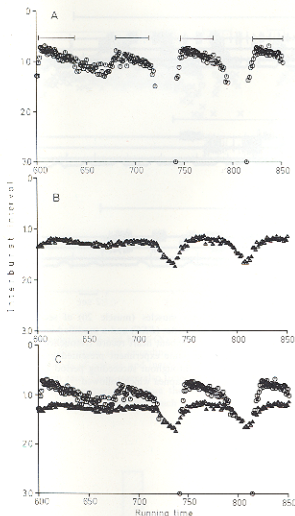
Fig. 4A–C. Heart and gill activity during intermittent swimming. **A** Ventilation rate. Bars: periods of swimming. **B** Heart rate. **C** Heart (△) and Ventilation (○) rates together. Like intermittent ventilation, swimming occurs in bouts separated by periods of apnea. Note the reduction in heart rate during these periods. Swimming bouts began with very rapid gill plate movements which gradually slowed until eventually there was transition from swimming to slow ventilation (during which operculum and legs were not active). Note that during changes in swimming, there were parallel changes in heart rate. Also note that when ventilation slowed but did not stop, between first two swimming bouts, reduction in heart rate was very small compared to decrease during apnea

midline without cleaning (flicks) usually occur only after damage to the gills or nerve cord.

We have recorded gill cleaning in intact and semi-intact animals, as well as in totally isolated opisthosomal nerve cords. Thus the basic pattern seems to be a centrally determined sequence whose pattern generator is located in the ganglia of the ventral cord. The motor pattern consists of prolonged activity in muscles 48 and 115 (which adduct the gill plates across the midline), along with brief rhythmic bursts in muscles 113 and/or 114, which flick the inner lobe. For experimental purposes it was sufficient to use the sporadic, large amplitude discharge from ventilatory muscle 20 to identify bouts of gill cleaning behavior (Fig. 5).

Characteristically, gill cleaning occurs as a component of intermittent ventilation. During periods of gill cleaning there was a rapid decrease in heart rate which was at least as great as that seen during apnea. In many instances the slowing of the heart rate was markedly greater than during apnea alone (Fig. 5C and the end of Fig. 6). Therefore, the reductions in heart rates seen during gill cleaning were not simply due to absence of ventilation, but probably involve additional mechanisms as well.

Effects of General Activity on Respiratory Behavior

From the evidence presented so far it is apparent that any change in the ventilation rate, whether experimentally evoked or endogenously activated, also will result in a change in heart rate. We were curious to see whether or not this was merely due to the increased metabolic activity associated with ventilatory movements, or whether it was specifically linked with ventilation apart from general metabolic effects.

To test these alternatives, recordings were made from unrestrained animals during periods of intermittent ambulatory activity. Recordings were made from leg muscle 25 (Wyse and Dwyer, 1973), as well as from the heart and gill muscles, so that constant observation was not necessary to determine times when such activity occurred. Leg movements were often correlated with increases in the ventilation rate, which made it difficult to separate the activity effects on heart rate, from the ventilation effects. We were fortunate, however, to obtain a number of records in which the animals were active both during ventilation and apnea (Fig. 7). It was clear from these records that changes in heart rate were always associated with changes in ventilation rate, regardless of the general locomotor activity of the animal. Both rates frequently increased in the absence of leg activity (Fig. 7, arrow), and simultaneous apnea and bradycardia, or gill cleaning and bradycardia, routinely occurred during periods of vigorous leg activity. Furthermore, those increases in heart and ventilation rate that were correlated with general ambulatory activity, usually started just before or just after the onset of activity. This suggests that they were not a result of any changes in the blood gas concentrations due to the increased metabolic demand of the tissues. These re-
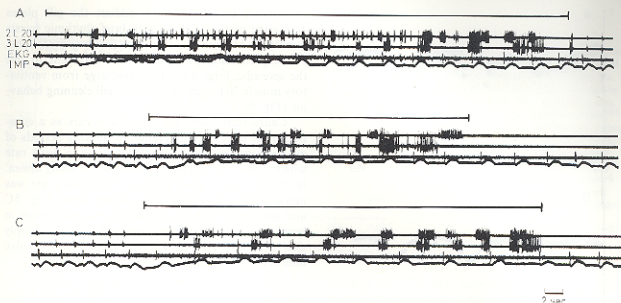
Fig. 5A–C. Heart and gill activity during gill cleaning. Top two traces EMG's from remotor muscles (muscle 20) of second left and third left gill plates, respectively. Traces 3 and 4 recordings of heart rate made with wire electrodes (*EKG*) and a modified impedance plethysmograph (*IMP*). Bars: periods of gill cleaning. The impedance device was utilized to supplement heart record (retouched), which was noisy during gill cleaning. **A, B,** and **C** not continuous, but successive records from the same experiment presented graphically in Figure 6. Note that heart rate slowed immediately during gill cleaning and remained slow throughout succeeding period of apnea. Furthermore, note that in **C** heart rate was much slower during gill cleaning than during period of apnea which followed
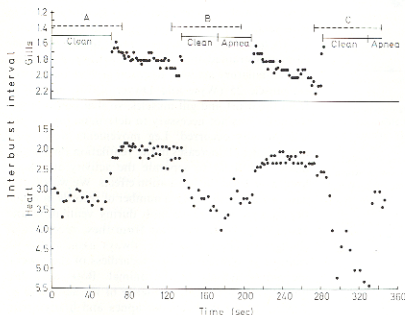

Fig. 6. Graph of rate changes in heart and gill rhythms during intermittent gill cleaning. Top graph: Ventilation. Bottom graph: Heart. Ventilation alternated with periods of gill cleaning and apnea during which heart rate slowed considerably. Dotted lines *A, B,* and *C*: portions of this experiment from which records were taken for Figure 5

sults of previous experiments, in which the heart rate was shown to increase prior to the onset of gill movements, indicate that although changes in heart rate are often correlated with increased metabolic activity, the metabolic demands resulting from such activity are not the only cause of these increases.

Phasic Coordination between the Gills and the Heart

Close examination of the film records indicated that in addition to a frequency covariation there was sometimes a phase preference of the heartbeat with respect to the concurrent gill interval (Fig. 8), such that the
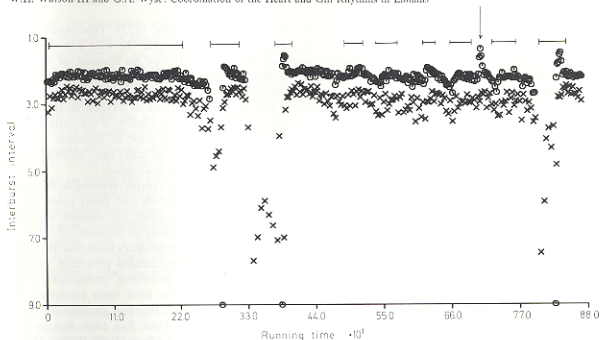
Fig. 7. Ventilation (0) and heart (X) rates during intermittent locomotor activity. Periods of general locomotor activity, as determined by direct observation and recordings of leg muscles, represented by solid bars at top of graph. Although there was some correlation between periods of activity and increased heart and ventilation rates, heart rate never changed unless there was a corresponding change in ventilation rate. There were also times when heart and ventilation rates increased or decreased independently of these activity periods (for example at arrow). Therefore the metabolic demand of increased activity is not the only cause of increased heart rates even though it is often associated with such increases
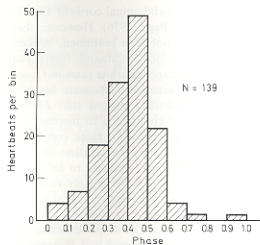

Fig. 8. Histogram showing phase preference of a cardiac ganglion burst with respect to concurrent ventilation cycle. Phase is calculated by dividing the *latency* (time between onset of a gill muscle burst and onset of cardiac ganglion burst) by concurrent interburst interval; (Phase=latency/interval). Number of heart beats of a particular phase were then totalled and a histogram was constructed. Heart beats per bin indicate number of events falling in each decile of phase, e.g., between 0.4 and 0.5. In this particular experiment there was strong phase preference of 0.5 (heart beating 180° out of phase with gills). Such strong phase preferences do not exist in all animals or in any one animal at all times. However, when phase preferences did exist they usually had a value near 0.5

heartbeat tended to fall near the midpoint of a gill beat cycle. Simultaneous analysis of frequency plots and sequential phase plots revealed that phasic coordination usually occurred during periods when the heart and ventilatory rhythms were at the same frequency or at harmonic frequencies. During periods of strong phase preference there were often instances when the heartbeat would skip whole cycles and return to the preferred phase in the next cycle. Such skips are evident during the first 300 s of Figure 1A (arrows). The strong phase preference and occasional cycle-skipping at this time resulted in periodic heart interburst intervals of approximately 6 s (3 gill intervals long, 3:1 coupling), rather than the usual 4 s intervals (heartbeat falling after every other gill burst, 2:1 coupling). This type of phase preference was found to occur in a number of animals, and in each case the preferred phase was around 0.5. However, it should be emphasized that although the preferred phase appears to be similar from animal to animal, the degree to which different animals maintain this coordination varies considerably. Whereas some animals were found to have little, if any, phase preference, one animal we examined maintained a particular phase preference throughout an entire day of experimentation.

Discussion

Although the cardiac ganglion and the pattern generators for ventilation in the abdominal ganglia are both capable of independent oscillation, changes in their activities in an intact animal are very closely coordinated. In fact, at no time have we ever witnessed a change in the heart rate which was not associated with a qualitatively similar change in the ventilation rate.

Limulus has been reported to have a cardiac response to hypoosmotic stress (Mangum et al., 1976), bradycardia in response to hypoxia (Falkowski, 1974), and cardioperiodicities (Corning and Von Burg, 1970b). Our data suggest that in these studies, both ventilation and heart rates changed together in a coordinated manner. It has also been reported previously that the heart rate of *Limulus* changes little over 24 hours (Pax and Sanborn, 1964). However, we find that in intact animals under normal conditions, the ventilation and heart rates are quite variable. The heart rate can change 3–5 fold from one minute to the next (see Figs. 3, 4, 6, 7), depending on the ventilatory activity of the animal. The coordination of the heart and gills, as well as the short-term variability of both rates, should be taken into account when interpreting the effects of environmental stress or other stimuli on the respiratory behavior of *Limulus*.

In *Limulus* the cardiac and the ventilatory responses to hypoxia are coordinated. This has also been found to be the case in decapods (Larimer, 1964; McMahon and Wilkens, 1975; Wilkens et al., 1974; Coyer, 1977a). In the case of decapods, it is not clear whether the responses to changes in ambient pO_2 are mediated via external receptors, internal receptors, or direct effects of oxygen on the central nervous system. McMahon and Wilkens (1975) hypothesized that the response of decapod crustacea to changes in ambient pO_2 is a result of changing internal oxygen levels directly affecting both cardiac ganglion pacemaker cells and oscillator cells (Mendelson, 1972) that are responsible for generating the scaphognathite rhythm. For *Limulus* there is considerable evidence that the responses are mediated predominantly by external oxygen receptors. First, external oxygen receptors on the gill lamellae and the intercoxal cuticle of *Limulus* have been identified which appear to be responsible for mediating the behavioral response of *Limulus* to changes in ambient pO_2 (Page, 1973; Crabtree and Page, 1974; Thompson and Page, 1975). Receptors which increase their rate of firing in response to hypoxia have also been identified on the gills of *Orconectes* (McMahon and Wilkens, 1975). Second, ventilation resumes in *Limulus*, following the reintroduction of oxygen, with a latency as brief as

5 s (Waterman and Travis, 1953). This resumption is rapid, despite the fact that the gills are clamped shut and thus allow little or no transfer of oxygen across the gill surface. Third, animals which have been bled prior to experimentation respond to hypoxia and the reintroduction of oxygen in the same manner as control animals. Finally, if the cardioregulatory nerves are lesioned prior to exposure to hypoxia, there is no change in heart rate, although the ventilatory system responds as usual.

What is the neural mechanism responsible for conjoint regulation of ventilation and heart rates? It is likely that similar mechanisms are utilized whether the changes are in responses to hypoxia, or due to spontaneous changes in respiratory behavior. Presumably, the heart rate is modulated primarily via the 14 cardiac nerves (see preceding paragraph). The properties of these nerves have been examined by a number of investigators, utilizing stimulation techniques (Pax and Sanborn, 1964; Pax, 1969; Bursey and Pax, 1970; Corning and Von Burg, 1969, 1970a). However, very little is known about the role of these nerves in the normal behavior of the animal, except for one report (Corning and Von Burg, 1970b) that cardioperiodicities were dependent on input from nerves 7 and 8. Ventilation rate is probably modulated by command fibers, as well as by phasic sensory feedback resulting from gill movement (Wyse and Page, 1976). Command fibers which affect ventilation rate have been localized in isolated abdominal cords of *Limulus* (Wyse, 1972; Wyse and Page, 1976). However, their effect on heart rate has not been examined. Wilkens et al. (1974) have examined command fibers that affected either heart or scaphognathite rates in *Cancer magister*. Of the fibers sampled, 68% were bivalent, affecting both rates. Our data suggest that *Limulus* may also utilize bivalent commands to increase and decrease the heart and ventilation rates conjointly.

In addition to a frequency covariation between the heart and gills, there also appears to be a type of phasic coordination. This most closely resembles the relative entrainment seen between bilateral scaphognathites in decapod crustacea (Wilkens and Young, 1975), and between the scaphognathite and cardiac ganglion in *Cancer magister* and *Cancer irroratus* (Coyer, 1977b). In all these cases there appears to be sufficient phasic communication between the two systems to enable a certain phase relationship to be maintained when they are near the same frequency or harmonic frequencies. However, the weakness of the coordination results in a very narrow range of entrainment (Wendler, 1974; Wilkens, 1976), and therefore, in any one animal, periods of tight coupling, relative entrainment, and independent drift are possible, depending on the relative frequencies of the gill and heart pattern generators.

Both the frequency and the phasic coordination may be important in maintaining efficient circulatory-respiratory interactions. By matching the circulation of the hemolymph with the activity of the gill plates necessary for oxygenation of the hemolymph, energy is not wasted, for instance, in pumping deoxygenated blood throughout the body during apnea. There are mechanical considerations as well. The ventilatory movements of the gill appendages may be utilized not only to aid in the oxygenation of the hemolymph, but also to pump the blood, through the branchial canals, to the pericardial cavity (Lochhead, 1950). If gill ventilating movements also serve as accessory hemolymph pumps then both a phase preference of the two pumps, and a frequency covariation between them, would function to coordinate heart output with venous return from the gills.

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