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Circulatory regulation in an open system: *Haliotis corrugata* (Mollusca, Gastropoda)

by

Allan Charles Roth

A Dissertation Submitted to the

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For the Graduate College

Iowa State University
Ames, Iowa

1977

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GENERAL INTRODUCTION

Objective

The main function of any circulatory system is to deliver nutrients to the tissues for metabolism, growth, and repair and to carry away waste products. To explain how the elements of the circulatory system perform this function requires the analysis of the anatomy of the system and an understanding of the modes of action of each circulatory component. Only after each element of the system has been analyzed can the interrelationships of these elements be examined. This interaction is the mechanism which regulates the distribution of blood under different physiological conditions. One way to study the extent and effectiveness of regulatory mechanisms is to observe the response of a system to a disturbance.

For the most part, the major research effort in circulatory regulation has centered on vertebrates, in particular mammals. The closed system of pumps and tubes of vertebrates has led to the development of several measuring instruments and analytical techniques. However, another major category of circulatory systems, the open circulatory systems, do not consist solely of pumps and closed tubes. The hemolymph of these systems passes through walled arteries into the tissue and then into large venous sinuses, returning to the heart by way of the veins. In addition, arthropods, molluscs, and ascidians which have open systems are usually small and have shells or exo-skeletons which make peripheral circulatory measurements difficult. Therefore, the regulatory mechanisms of open systems are poorly understood.

The objective of this study is to examine the regulatory mechanism of the pink abalone by first isolating the regulation of the heart and the peripheral system, and then observing their combined effects in an unrestrained animal. The effect of venous return pressure on heart function will be examined in Part I. The pressure-flow characteristics of the peripheral vascular system will be examined in Part II. In Part III the circulatory responses of the intact animal to light and temperature perturbations will be used to observe how the heart and periphery interact to control blood distribution during activity.

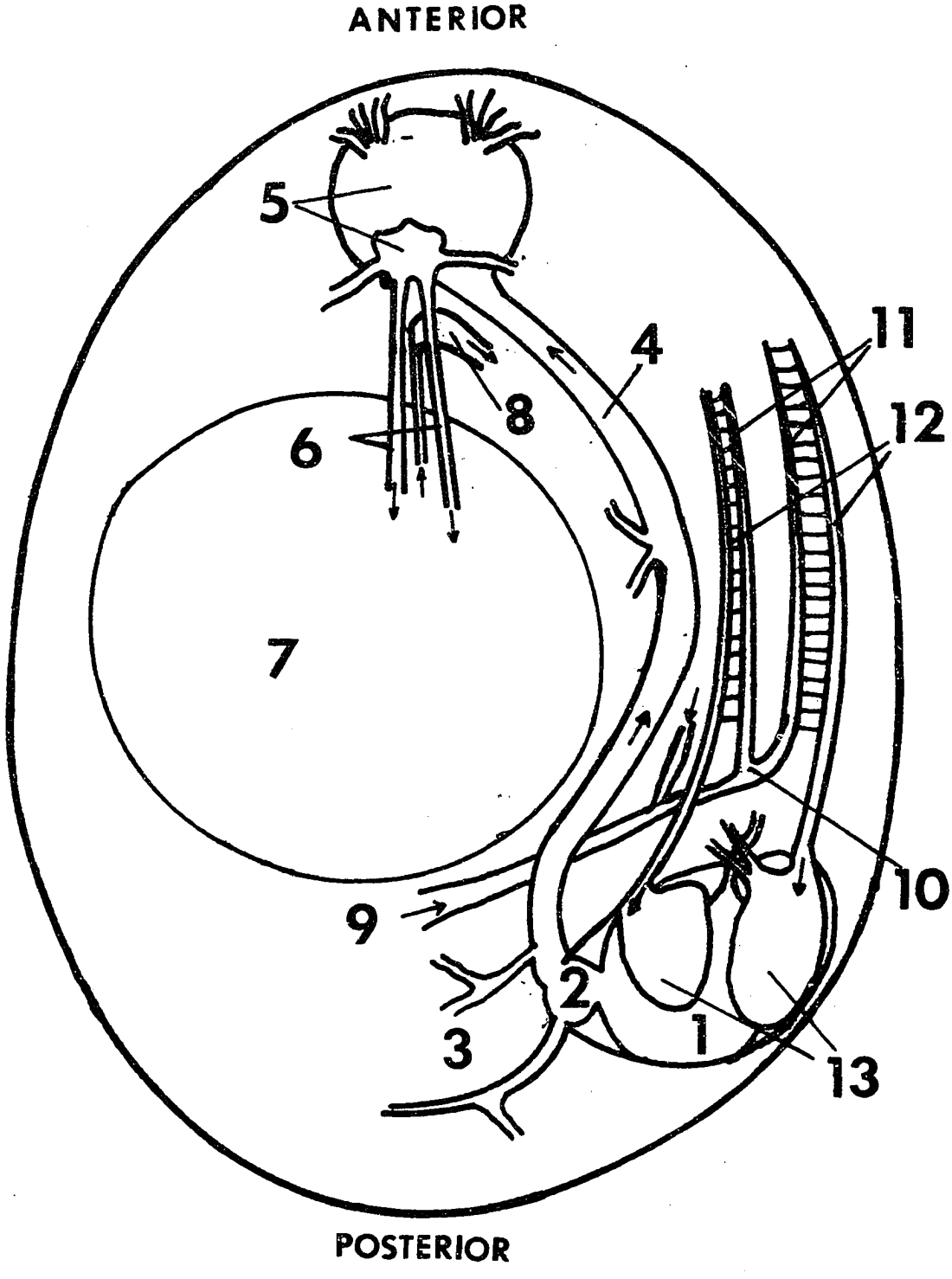
The Experimental Animal and General Preparation

The experimental animal used in this study was the pink abalone, Haliotis corrugata (Gray 1828), phylum Mollusca, class Gastropoda, subclass Prosobranchia, order Archaeogastropoda. This animal is large and the heart and many major vessels can be exposed by removing only a small portion of the shell with no surgery required.

The complete study of the anatomy of Haliotis tuburculata by Crofts (1929) and the recent work on Haliotis corrugata of Bourne and Redmond (1977a) give a complete description of the circulatory anatomy of Haliotis and permit the correct instrumentation of the heart and blood vascular system (Figure 1). In addition, Dr. James Redmond, who was consulted many times on this project, has considerable expertise with the pink abalone since it has been used in many respiratory and circulatory studies in his laboratory.

Figure 1. Diagram of Haliotis corrugata showing relevant circulatory anatomy (ventral view) (after Bourne and Redmond, 1977a).

1. Ventricle
2. Aortic bulb
3. Posterior visceral arteries
4. Anterior aorta
5. Cephalic arterial sinus
6. Pedal arteries
7. Foot muscle
8. Cephalo-pedal venous sinus
9. From right urocoel
10. Basibranchial sinus
11. Afferent ctenidial vessels
12. Efferent ctenidial vessels
13. Auricles


ANTERIOR
5
4
11
8
12
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10
9
2
3
1
13
POSTERIOR

The pink abalone used in this study were all obtained from Pacific Bio-Marine Supply Company, P.O. Box 536, Venice, California 90291. The animals were air-freighted to Des Moines, Iowa in iced bags of seawater. They were then picked up on arrival at the airport and transported to Ames, Iowa by car with total travel time kept under twelve hours. The abalone were then placed in a 150 gallon aquarium filled with 15°C Instant Ocean at 1.025 to 1.030 specific gravity. The animals were allowed to recover for at least two days before any experimental procedures were performed. The longest time from delivery to experimental use was approximately one month. All experimental measurements were conducted in a seawater-filled plexiglass aquarium with a capacity of approximately 10 gallons. This tank was inside a larger 50 gallon water bath which was kept at 15°C by a Blue M refrigeration unit.

To obtain access to the heart and blood vessels a portion of the shell had to be removed. A diamond drill bit fitted to a high speed drill was used to cut away a section, 12 cm x 3 cm from the left posteriolateral area of the shell. When this shell section was freed from the mantle membranes; the pericardium, both efferent ctenidial veins, the pallial artery, and a portion of the anterior aorta were exposed.

The animals were anesthetized in a bath of carbon dioxide-saturated seawater. Pieces of dry ice were added to seawater at room temperature and the water was considered saturated when its temperature reached 15°C. The abalone were then placed inverted in the bath and removed when "righting" movements had ceased or for no more than three minutes of submergence. The animals were then removed from the bath and placed in a shallow pan where the surgical procedures were performed.

Instrumentation

Blood pressure

Blood pressure was measured by Statham P23V strain gauge pressure transducers. For measurement of cephalic arterial sinus pressure in Part III a RP-1500 Narco pressure transducer connected to a Narco strain gauge coupler was used. Each transducer was fitted with Hamiton 3-way valves, and a length of polyethylene tubing connected to a small needle. The transducer-catheter-needle was filled with degassed distilled water and the frequency characteristics measured by a step input or "pop" test (Shirer, 1962). Catheters were composed of 60 cm of PE 90 tubing (I.D. = 0.86 mm) and a 3 cm portion of 23 gauge hypodermic needle. This combination had a natural frequency of approximately 11 Hz and a damping factor of .45. This level of response was considered sufficient for the low frequency pressure pulses produced in the abalone.

The Statham pressure transducers were connected to a Beckman 411 Dynograph with the strain gauge couplers. A U-tube water manometer system was used to statically calibrate the transducers to 2 cm H_2O pressure per 1 cm chart deflection. For some animals with high blood pressures the sensitivity was reduced by a factor of two. After static calibration the needles were placed in the tank at the level of measurement and the base line record zeroed by using a rack and pinion device to raise or lower the transducers.

Blood flow

Absolute blood flow and relative changes in blood velocity were measured with two different types of transducers; an electromagnetic flowmeter

and a continuous wave Doppler flowmeter. The electromagnetic flowmeter used was a Biotronix Model BL-610 which uses a square wave excitation. A good discussion of the advantages and disadvantages of this type of flowmeter can be found in Wyatt (1977). A cannulating type In Vivo Metric Systems 2 mm internal diameter flow probe was connected to the Biotronix flowmeter and the whole unit patched into the Beckman Dynograph. This unit was calibrated by pumping seawater through the probe and measuring flow using a stop watch and collecting flask.

For the unrestrained whole animal preparation a Parks Electronics Lab directional doppler flowmeter Model 806 fitted to a 15° infant-sized transcutaneous probe was used. The doppler flowmeter was used only to obtain a measure of changes in velocity and not absolute flow levels. It must be stated that the doppler flowmeter does have limitations. First, if the exact angle of the reflected ultrasonic beam is not known, no exact velocity can be determined. However, if the angle is maintained constant then percent changes in velocity can be monitored. Secondly, no qualitative flow can be measured if the diameter of the vessel at the sampling point is unknown. Thirdly, if the velocity profile is parabolic then the portion of the vessel scanned by the ultrasonic beam determines the accuracy of the velocity measurement (McCarty and Woodcock, 1974; Welkowitz and Deutsch, 1976). However, in the present case, the velocity is measured in the aortic bulb and the assumption of a flattened velocity profile (due to entrance effects) and linearity of measurement is a reasonable assumption. The flow probe was attached to an articulating arm which could be screwed into the shell to permit stable positioning of the probe over the measurement site (Figure 2).

Figure 2. The Doppler flowmeter probe (A) attached to the fabricated articulating arm (B).

PART I. HEART REGULATION OF THE PINK ABALONE

LITERATURE REVIEW

To attempt to understand the regulatory mechanisms of any circulatory system requires a close examination of each of the elements of that system. The heart is one of the critical elements of most circulatory systems, both open and closed, and has been studied extensively in numerous species. Two groups of factors influence the output of the heart and thus affect its role in circulatory regulation. These factors are: (1) chemical changes (nervous and humoral); and (2) mechanical perturbations.

Investigators have used two general types of heart preparations for their studies of the above mentioned factors. The first of these preparations involves the "isolation" of the heart with most of the inputs to the heart artificially controlled and the outputs measured. The "isolated organ" preparation has the obvious advantage of ease of control and measurement of many variables. However, in these experiments critical elements of the organ's environment may be missing and the results may not be representative of normal animal physiology.

The second preparation is the "intact organ" experiment where some of the heart inputs and outputs are controlled and measured but the environment of the organ is supplied by the animal tissues. In the "intact organ" preparations variables are more difficult to control, measure, and interpret but will be more physiological.

When analyzing any control system one must define the characteristics of each subsystem and component, the structure of communication between these components, and the nature of the input signals to these components (Milsum, 1966). This type of analysis can be applied to biological

systems but the distinction of subsystems and controllable inputs may be very difficult.

Nervous Control of the Heart

There are extensive data on the effect of certain neurohormones on isolated molluscan hearts, but only limited information is available on the *in vivo* nervous control of these hearts. Carlson (1905a,b) first identified many cardioregulatory nerves in molluscs. He specifically demonstrated an acceleratory response in the heart of *Haliotis* when the visceral ganglion was stimulated.

Krijgsman and Divaris (1955) reviewed extracardiac regulatory and pacemaker data on molluscs. They concluded that there could be no doubt of the existence of extrinsic regulation of the molluscan heart by branches from the visceral ganglia in most species. It was noted that, as in the vertebrate heart, acetylcholine (ACH) was inhibitory, presumably by acting on the diffuse pacemakers. They also reported that atropine did not antagonize this inhibition but curare and curare-like drugs did.

Welsh (1956) found the cardio-inhibitor ACH in the nervous system of *Buccinum* and also reported the presence of the cardio-excitor 5-hydroxytryptamine (5-HT). Hill and Welsh (1966) found ACH in the cerebral ganglion of *Haliotis* but found no trace of 5-HT. They noted that in many molluscs ACH elicits a biphasic response, being inhibitory at low concentrations and excitatory at higher levels. In the isolated heart, 5-HT produced an increase in amplitude and frequency.

Greenberg (1969) studied the pharmacology of many bivalve hearts. He classified the ACH receptors as "nicotinic" and described the blocking of

ACH by curare-like drugs. MacKay and Gelperin (1972) studied the heart pharmacology and reflex response of Limax maximus and found that tactile stimulation to the head decreased heart rate and touching the tail increased the rate. Once again, they found 5-HT to be a cardio-excitor and ACH a cardio-inhibitor. Martin (1974) completely reviewed invertebrate circulatory systems and noted the extremes of heart control in molluscs. He reported that disturbing the animal can stop the heart for as much as two to three hours with little effect on the animal.

The Origin of the Heartbeat

Most authors agree that molluscan heart muscle is myogenic but they disagree on the underlying mechanisms for the initiation of the heart beat. Krijgsman and Divaris (1955) found no evidence for a conducting system in the molluscan heart and claimed that there was a diffuse chemosensitive pacemaker responsive to a substance produced by the heart. The chemical threshold was lowered by stretching the muscle fibers, thus the frequency of the heartbeat would be influenced by internal ventricular pressure. Hill and Welch (1966) suggested a similar system where an agent in the blood sensitized the heart to stretch and thus lowered the threshold. They also reported an unidentified chemical (substance X) that was cardio-excitatory and found in many molluscan hearts and blood extracts.

Greenberg (1969) suggested that substance X may play a role in the initiation and maintenance of cardiac rhythmicity in bivalves and other molluscs. Sommerville (1973b) commented on the failure of isolated Helix

hearts to beat unless five percent of snail blood was present in the perfusion fluid. She comments that rhythmical beating in heart preparations of Helix may be ". . . possible only if a small volume of recirculating perfusion fluid is used . . . (where) . . . some essential factor has been carried over with, or manufactured by the preparation."

Mechanical Factors of Heart Regulation

The majority of data related to the mechanical factors affecting the normal heart have been acquired with vertebrate heart preparations, but principles and techniques discovered in these experiments have not been widely and successfully applied to invertebrates.

Knowlton and Starling (1912) reported one of the first successful isolated heart preparations. They found that the dog's heart rate was related to temperature but not to venous pressure or arterial resistance. They also reported that cardiac output was proportional to venous input pressure. Patterson and Starling (1914) continued these experiments and found that the input pressure distended the ventricle and this caused increased output up to a maximum. They also noted that as arterial resistance was increased above normal levels, the output of the heart was reduced. The above two papers led to the now-famous Linacre lecture on The Law of the Heart by Starling given at Cambridge in 1915 (published in 1918).

Guyton, Jones, and Coleman (1973) reviewed the literature on factors controlling heart output in vertebrates. They concluded that the most useful technique to measure the control of the heart is by creating

cardiac function curves (i.e., cardiac output or stroke volume versus right atrial pressure).

Randall (1970) stated that the fish heart appears to obey Starling's Law (relating cardiac output to heart muscle tension) and he cited the papers of several authors where increased venous input pressure caused increased heart output. Unfortunately, there are no reports of the effects of input pressure changes on cardiac output in Chondrichthyes which have a rigid pericardium and aspirate blood back to the heart.

Several authors have investigated molluscan heart function with the pericardium removed. Straub (1904) measured the pulse volume in the isolated Aplysia heart and found a maximum output when input pressure was at 2 cm H_2O. Biering (1929) opened the pericardium and isolated the heart of Helix, controlling input to the auricle. He found a small dependence of stroke volume on input pressure; heart rate and minute volume were directly related to venous input pressure with a minimum output near 1 cm H_2O and a peak at approximately 8 cm H_2O. Hill and Schunke (1967) externally stretched the isolated ventricle of Aplysia and measured active tension. They found that the Aplysia ventricle was more plastic than vertebrate hearts and exhibited a poor relationship between isometric tension and length. However, the slow onset and decay of contractility were similar to that of vertebrate hearts. Hill and Irisawa (1967) internally pressurized the isolated ventricle of the marine gastropod Rapana thomasiana and measured output while changing the perfusion pressure. The ventricular output increased as head pressure was raised from 25 cm H_2O to a plateau at a head of 85 cm H_2O which, after a pressure drop in the

tubing, corresponded to an internal perfusion pressure of about 40 cm of H_2O.

Sommerville (1973b), in a series of experiments, controlled input pressure and output pressure of the isolated (pericardium removed) ventricle of Helix and recorded the heart rate and output. She found that the heart rate and output were increased with increasing auricular pressure and that heart rate was insensitive to arterial pressure when the aortic valve was competent. Herold (1975) removed the heart of Helix from its pericardium and measured its work output by pressure flow calculation and its heat output by calorimetry. The efficiency of the Helix heart was found to increase as internal pressure was increased. Also, low oxygen tension did not seem to affect efficiency of the heart although work and total energy were lowered.

Only a few investigators have examined the mechanical factors controlling heart function in molluscs with "intact organ" preparations with a competent pericardium. Schwartzkopff (1954) cannulated the input and output of the heart of Helix and measured the effects of changes of input and output pressures on stroke volume, heart rate, and work output. Unlike Sommerville, he found that frequency and output varied inversely with arterial pressure and peripheral resistance. He also reported that stroke volume, heart rate, and cardiac output increased with increasing input venous pressure when arterial pressure was maintained at zero, a non-physiological condition. When venous pressure was increased and the arterial venous pressure difference held constant there was little variation in heart rate and the stroke volume increased only when the venous

pressure became very large (out of the physiological range). Very little data were presented with auricular pressure held at 5 cm H_2O and no data at less than 5 cm H_2O. The physiological range of auricular pressure in the nondisturbed Helix can be expected to be from 4-8 cm H_2O as reported by Jones (1971). The possible importance of the pericardium in the control of the heart has recently been realized with the confirmation of constant volume filling hypothesis in the molluscan heart. Ramsay (1952) proposed that the negative pressure set up in the fluid filled pericardium by the contractions of the ventricle might aid in filling the auricles. Krijgsman and Divaris (1955) proposed a similar mechanism and used data gathered by previous workers to support their hypothesis. Jones (1970) stated that in the Patella heart during the auricular systole the ventricle is filled by: (a) differential between ventricle and auricular pressure; and (b) the lower pressure in the pericardium which "sucks" the ventricle open. In 1971 Jones also found the pericardial pressure to be reduced during auricular and ventricular systole thus causing suction to fill these chambers in Helix.

Florey and Cahill (1977) reported a negative pressure of 2 cm H_2O in the pericardium during ventricular systole in 3 bivalve molluscs which they claimed was sufficient to explain auricular filling. Bourne and Redmond (1977a) used a pulse wave analysis of simultaneous aortic and mirror image auricular pressure waves and found them ". . . entirely consistent with a constant volume theory of cardiac filling in the pink abalone."

Civil and Thompson (1972) used an artificial pericardium in their experimental preparation. In pericardium-free hearts they demonstrated a direct relationship between auricular pressure and heart output. However, when pressures were varied in the artificial pericardium the relationship of auricular pressure and stroke volume was dramatically altered. They also suggested that the opening and closing of the reno-pericardial canal by a muscular sphincter could control the pericardial pressure and thus control heart function.

The following describes an "intact organ" preparation of the heart of Haliotis corrugata where the pericardium is intact. Mechanical factors affecting the function of this heart are measured and some comments are made concerning nervous and humoral control.

MATERIALS AND METHODS

A portion of the shell over the heart was removed and the animals were left to recover overnight. The following day the animals were anesthetized with carbon dioxide as described in the introduction. The anterior aorta was cannulated with PE 240 tubing (1.68 mm I.D.) at a point approximately 4 cm from the aortic bulb with the cannula tip pushed toward the heart approximately 2 cm, and sutured in place with size 000 silk suture. The first branch of the visceral artery was ligated when it was prominent. The right and left efferent ctenidial veins were cannulated with PE 160 tubing (1.14 mm I.D.) at a point approximately 2 cm from the junction with the auricles and the tip pushed toward the heart approximately 1 cm and secured with sutures.

Several preliminary experiments were performed with an "open circuit" preparation where the auricular cannulae were connected to a constant head tank and the aortic cannula was open to the environmental tank. In the "open circuit" set up there was no recirculation of the perfusion fluid, and there was no regular heart beat. Therefore, this approach was abandoned, and "closed circuit" preparations were used to obtain all the data reported here.

Both sets of cannulae were connected to constant head tanks as shown in Figure 3. The tank connected to the aorta was positioned so as to maintain aortic diastolic pressure at 5.5±1.0 cm H_2O as measured by a pressure tap in the cannula. This pressure is near the mean physiological pressure as reported by Bourne and Redmond (1977a) and serves to close the aortic valve between beats and keeps afterload on the heart constant. A

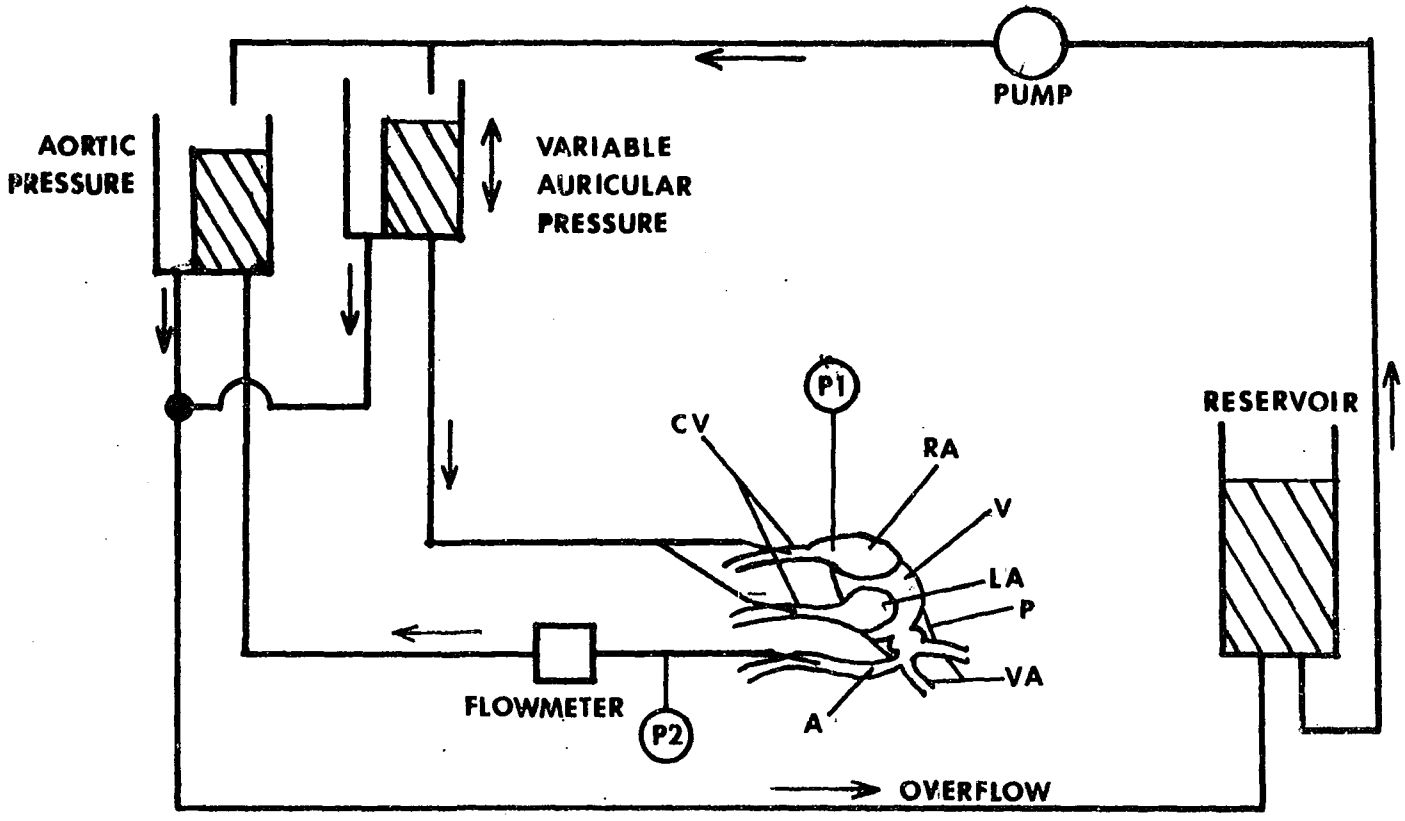


Figure 3. Schematic of flow system to measure heart output while auricular pressure is varied. P1, auricular pressure; P2, aortic pressure; CV, efferent ctenidial vessels; RA, right auricle; V, ventricle; LA, left auricle; P, pericardium; VA, visceral artery; A, anterior aorta.

2 mm diameter cannulating type electromagnetic flowmeter was placed in the circuit between the aorta and the constant head tank to measure the instantaneous flow in this external circuit. This measured flow is probably a good estimate of heart output when the resistance of this circuit is kept low, but as resistance is increased, flow from the heart probably will follow alternate pathways such as through the second visceral artery or the first visceral branch if ligation was not complete. Therefore, flow through this circuit can be considered only a relative measure of total cardiac output.

The tank connected to the efferent ctenidial veins was attached to a Narco-Bio-Systems Myograph Tension Adjuster so that smooth changes in height of the tank would create corresponding pressure changes that were measured by a pressure tap in the auricle close to the cannula.

The constant head tanks were connected to an overflow tank which recirculated the perfusion fluid back to the tanks thus creating a closed system. The perfusion fluid consisted of Instant Ocean made with doubly distilled water and neutralized with 1 N HCl. For the first two animals an ACH antagonist (Gallamine) was added to the perfusion fluid to attempt to block any inhibiting neurotransmitter release. However, this practice was discontinued when results revealed virtually no effect on heart rate when Gallamine was added to an already beating heart preparation. The closed perfusion system allowed the build up of any metabolic products from the heart that may help in the initiation of the heart beat as suggested by several authors (Sommerville, 1973b; Jullien et al., 1959; Schwartzkopff, 1954).

A stabilization time of approximately 1 to 3 hours was required before the heart was observed to beat at constant rate and strength when the auricular pressure was at a constant 3 cm H_2O. After stabilization the auricular pressure was varied by adjusting its constant head tank, and the change in stroke volume through the anterior aorta was recorded. Stroke volume was computed by a triangulation of the output records and heart rate computed from the average period from at least three heart beats. Auricular pressure was measured at the level on the recording just prior to the initiation of the beat.

RESULTS

There were several difficulties associated with obtaining data from the hearts cannulated *in situ*. The surgical procedures were very critical to the success of the experiment and the successful cannulation of the membranous vessels required many preliminary experiments to develop the proper technique. In addition, the maintenance of a steady heart beat was difficult. None of the "open circuit" animals developed a sustained beat but most of the properly prepared "closed circuit" experiments exhibited a steady heart beat after the stabilization period although the heart rate was lower than normal.

The anatomy of the aorta as it leaves the ventricle makes it difficult to accurately measure heart output. The cannulation of the aorta is distal to two major branches of this vessel, the first and second visceral arteries. It was not possible to successfully ligate both of these vessels because of their location deep in the tissue. Therefore, a fraction of the cardiac output would pass through these visceral arteries and not be measured by the aortic cannula. The resistance of the cannula was kept as low as possible to receive the largest fraction of the output and to minimize the effects of changes in peripheral visceral resistance on aortic flow. Therefore, the aortic flow is only an approximation of cardiac output. However, the measurement of changes in aortic cannula flow will still accurately indicate changes in heart output caused by input pressure changes. Unfortunately, the presence of these visceral arteries also prevented the measurement of the effect of increased load on heart output. If a resistance load such as a constriction were placed on

the aortic cannula, the reduction in aortic flow might only indicate that the cardiac output had taken collateral pathways through the visceral arteries and not that the heart output had decreased. Also, if a pressure load was placed on the heart by increasing the height of the aortic constant head tank, the auricular pressure would increase to a point beyond normal physiological pressure. This auricular pressure rise was due to an increased venous return from several small vessels (e.g., pallial and renal veins) which were not ligated due to inaccessible location. Even with the above mentioned limitations the measurements of aortic flow allowed a reasonable assessment of the effect of changes in auricular input pressures on heart function.

The results of five animal experiments are presented here and a sample of an output record is shown in Figure 4. The anterior aortic cannula flow pulse associated with each heart beat approximated the stroke volume and was graphed as aortic stroke volume (ASV). Figure 5 presents the data from each animal showing the variation in ASV as auricular pressure is varied from approximately -1 to 4.5 cm H_2O with aortic diastolic pressure kept constant at approximately 5 cm H_2O. As can be seen from the data, in general there is little variation of stroke volume as auricular pressure is varied. The exception appears to be animal 32 which had few points plotted and exhibited wide variation. This animal had a very erratic heart beat even after a 2 hour stabilization period and died after 3 hours, thus, the lack of data points. These results are summarized in Figure 6. As can be seen the mean ASV is relatively independent of auricular pressure and has a value between 0.3 and 0.4 ml. Therefore, it

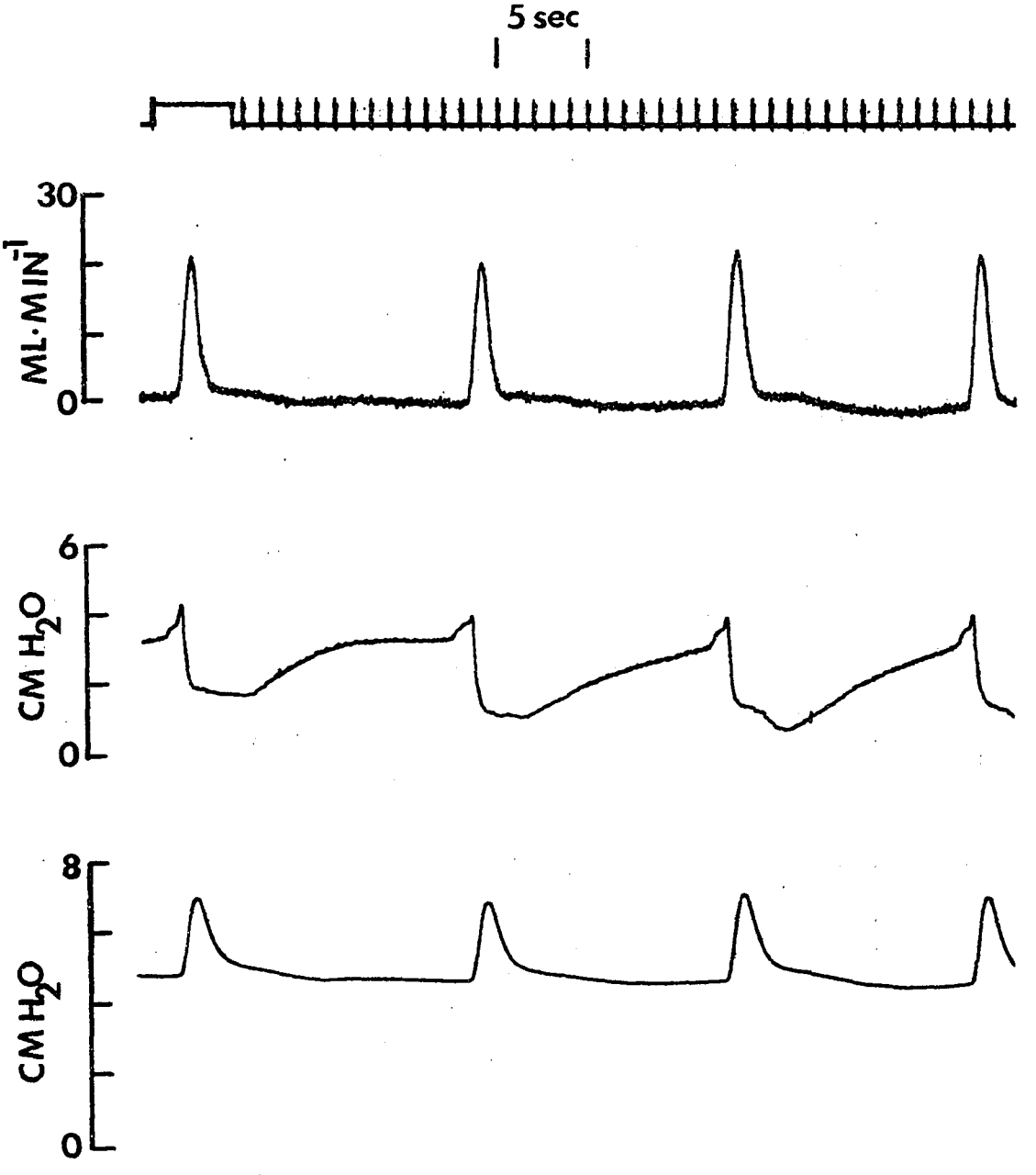


Figure 4. The upper trace is a recording of aortic flow of Haliotis corrugata #33. The middle trace is right auricular pressure and the lower trace is the aortic pressure.

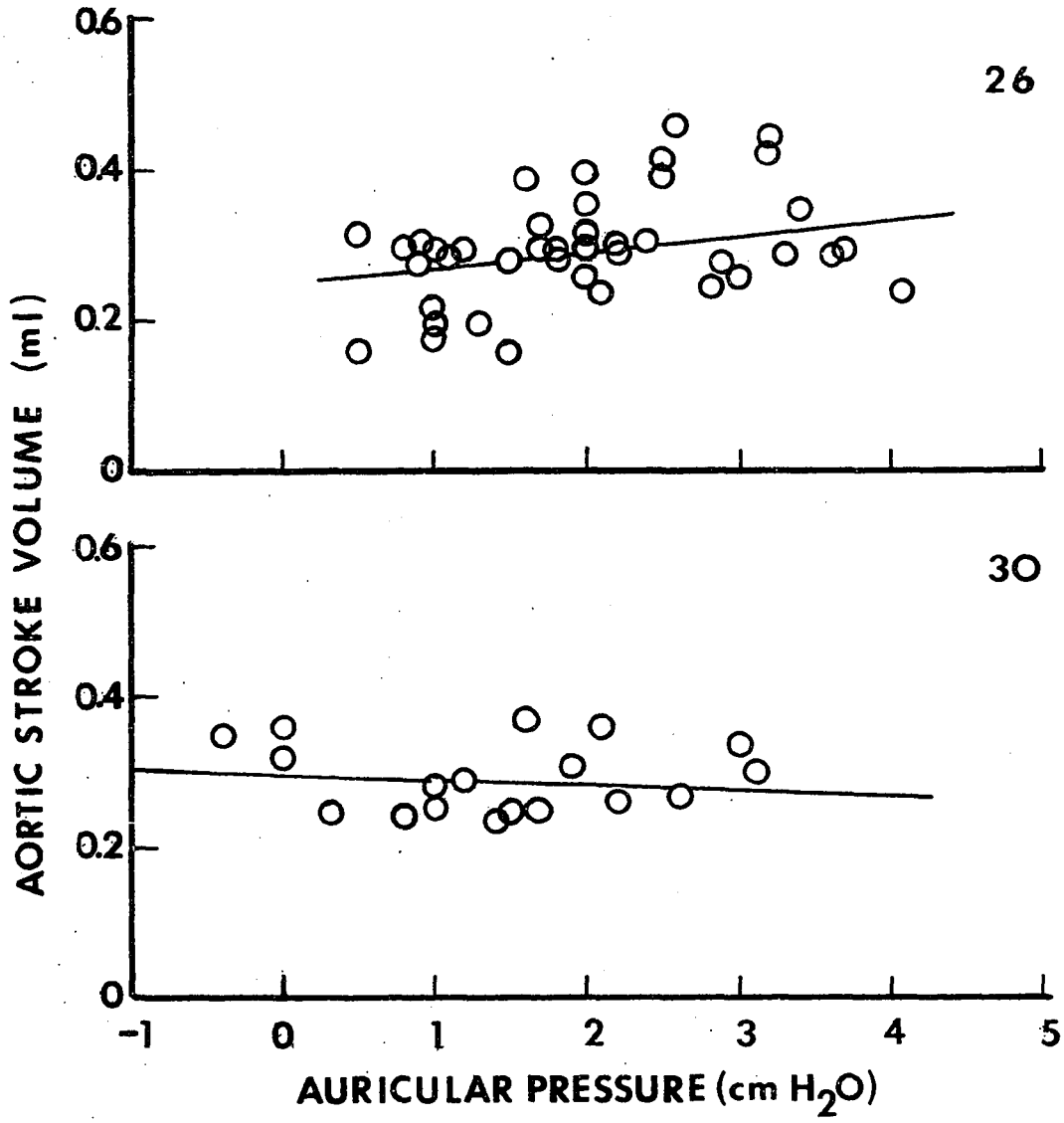


Figure 5. Variation in aortic stroke volume with auricular pressure in Haliotis corrugata (#26, 30, 31, 32 and 33). Aortic diastolic pressure at 5.5±.5 cm H_2O. Straight lines are fitted by the least squares method.

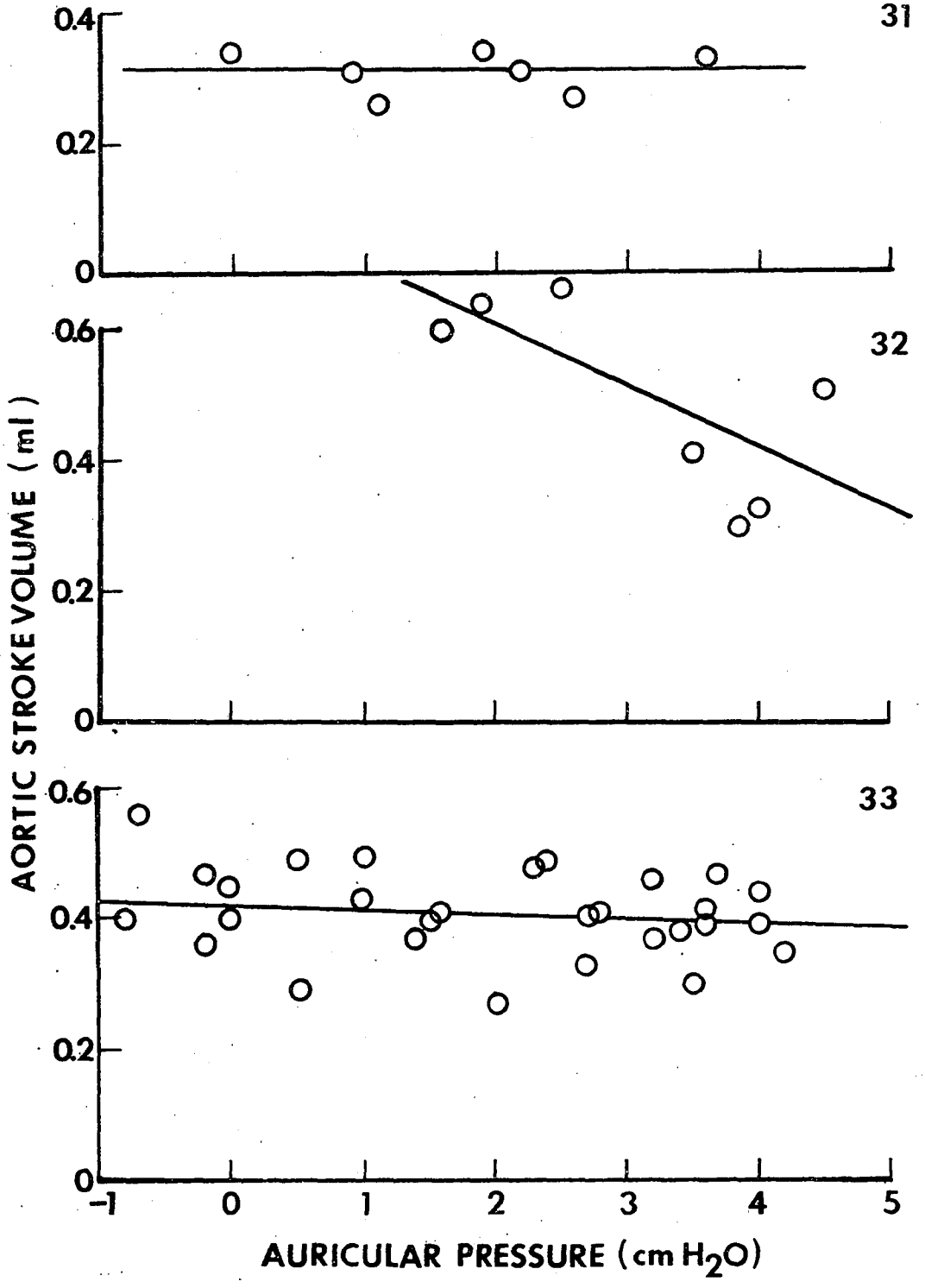


Figure 5. (Continued)

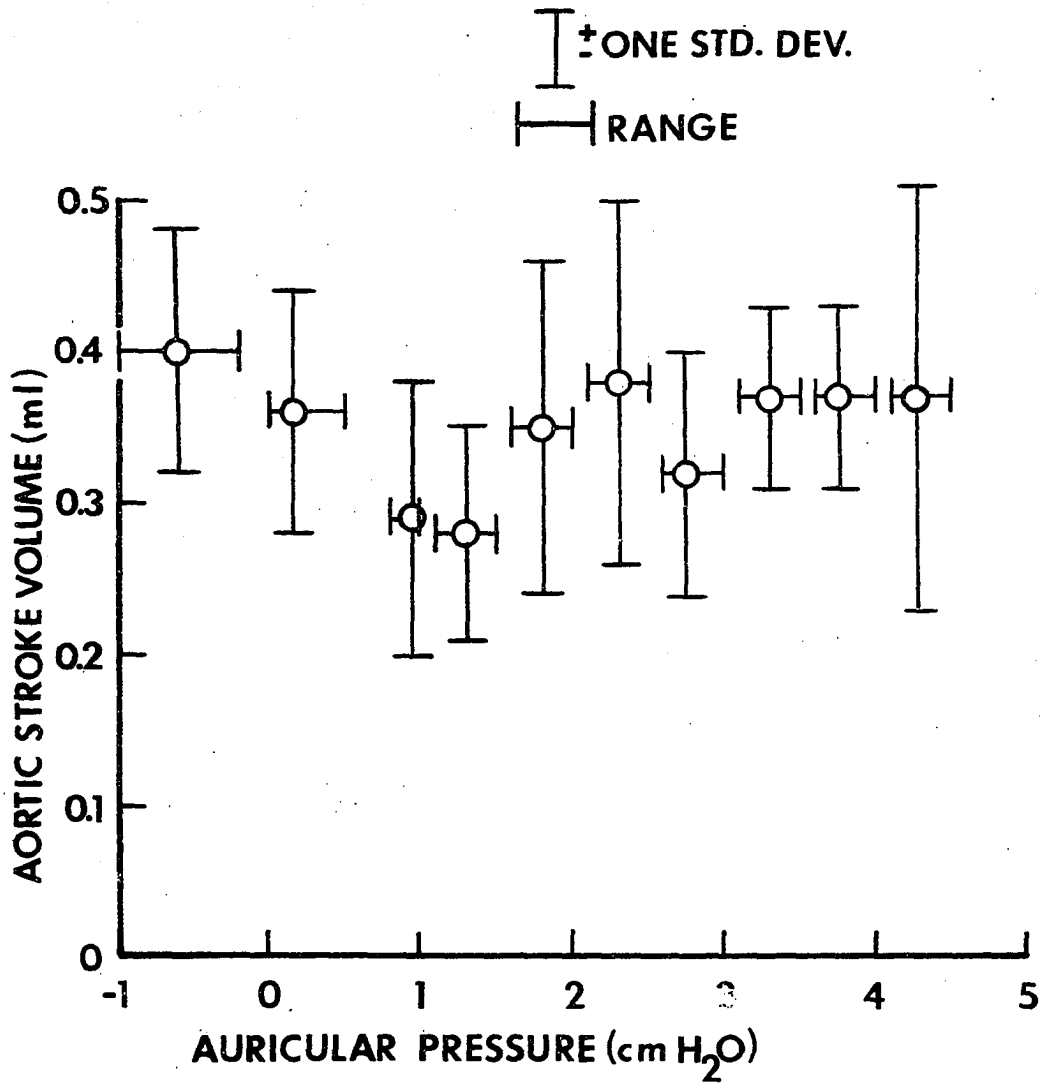


Figure 6. Variations in aortic stroke volume with auricular pressure. Aortic diastolic pressure at 5.5±.5 cm H_2O.

can be assumed that the heart stroke volume is relatively constant and has a value somewhat greater than 0.3 ml of blood.

Figure 7 presents the data showing the effect of auricular pressure changes on heart rate. The very nature of this in situ preparation leads to much variability in heart rate due to little control of nervous input and, possibly more importantly, the effect of humoral elements in the perfusion fluid (constantly changing in a "closed circuit" preparation) is hard to predict. Consequently, the low and variable heart rate reported here as compared with that of a nondisturbed animal (Bourne and Redmond, 1977a,b) was not entirely unexpected. Animal 26 had heart rates that were higher than the other animals, and there were "bursts" of rapid heart beat between 0 and 2 cm H_2O auricular pressure. It is possible that there may have been "bursts" of nervous activity in this animal. These data are summarized in Figure 8 which illustrates a low (compared to normal animal) heart rate, a slight peak at 1 cm H_2O input pressure, and a wide variation in measured heart rates. Even so, it appears that the parameter of heart rate is relatively insensitive to changes in auricular pressures.

Figure 9 shows the product of heart rate and ASV which is the aortic minute volume. It can be seen that the bursts of high heart rate by animal 26 as shown in Figure 7 has affected the graph minute volume by giving it a negative slope. Animals 30, 31, and 32 also have a slight negative slope. Animal 33 does not show this negative slope. To lessen the effect of heart rate variations due to variation in each animal preparation as mentioned above, the aortic flow of each animal was normalized to the peak flow observed during the experiment. This procedure more

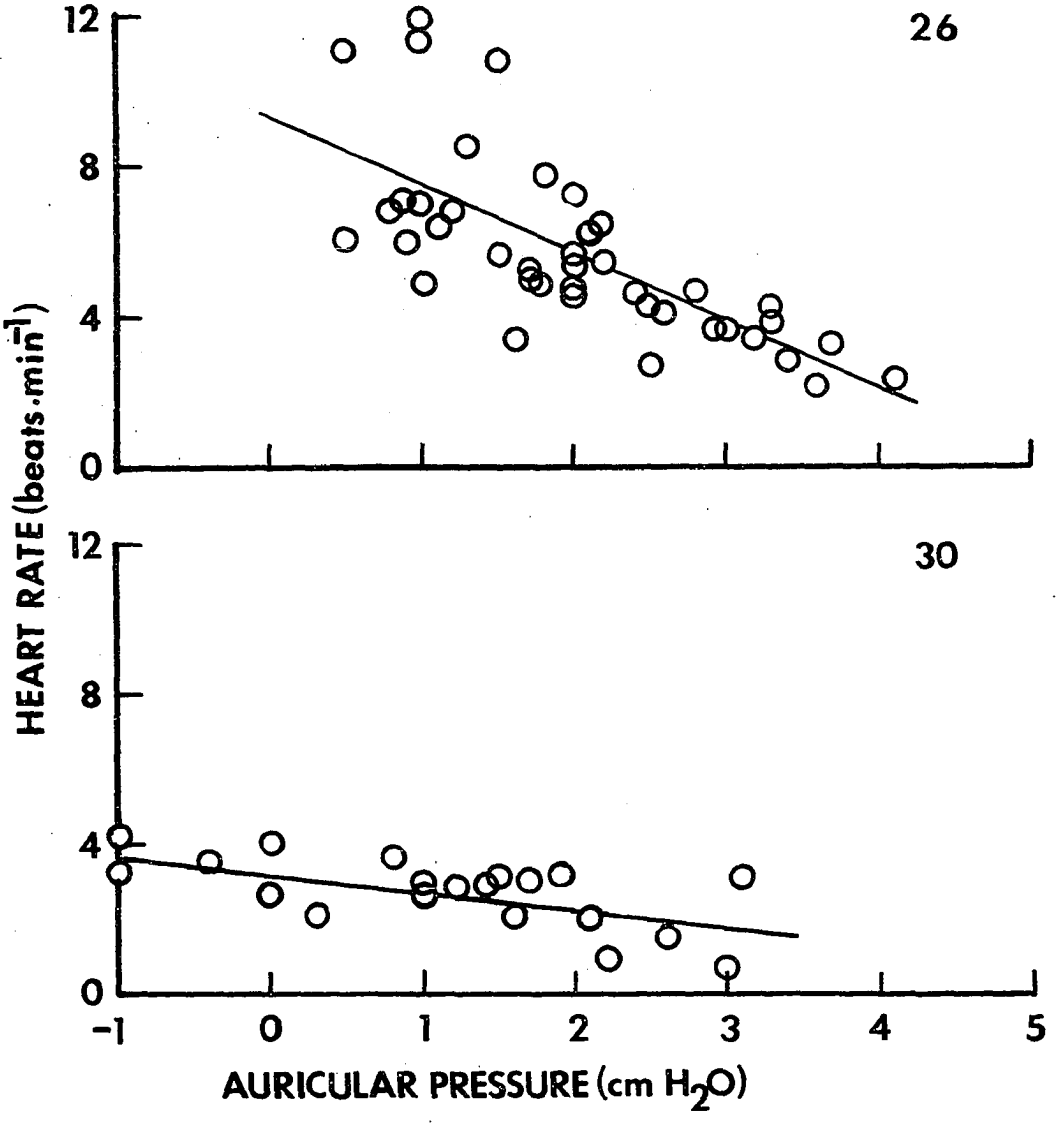


Figure 7. Variation in heart rate with auricular pressure in Haliotis corrugata (#26, 30, 31, 32, and 33). Aortic diastolic pressure at 5.5±.5 cm H_2O. Straight lines are fitted by the least squares method.

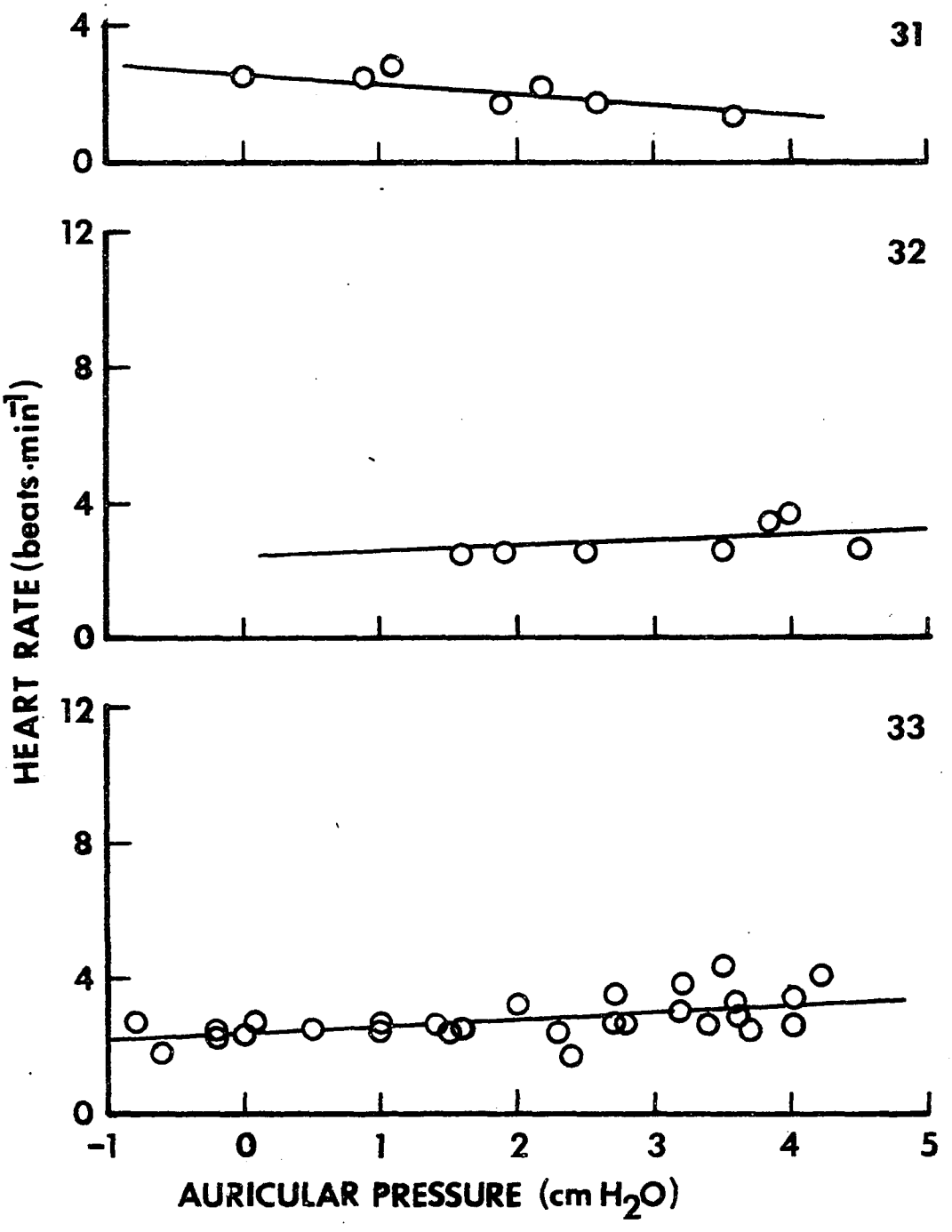


Figure 7. (Continued)

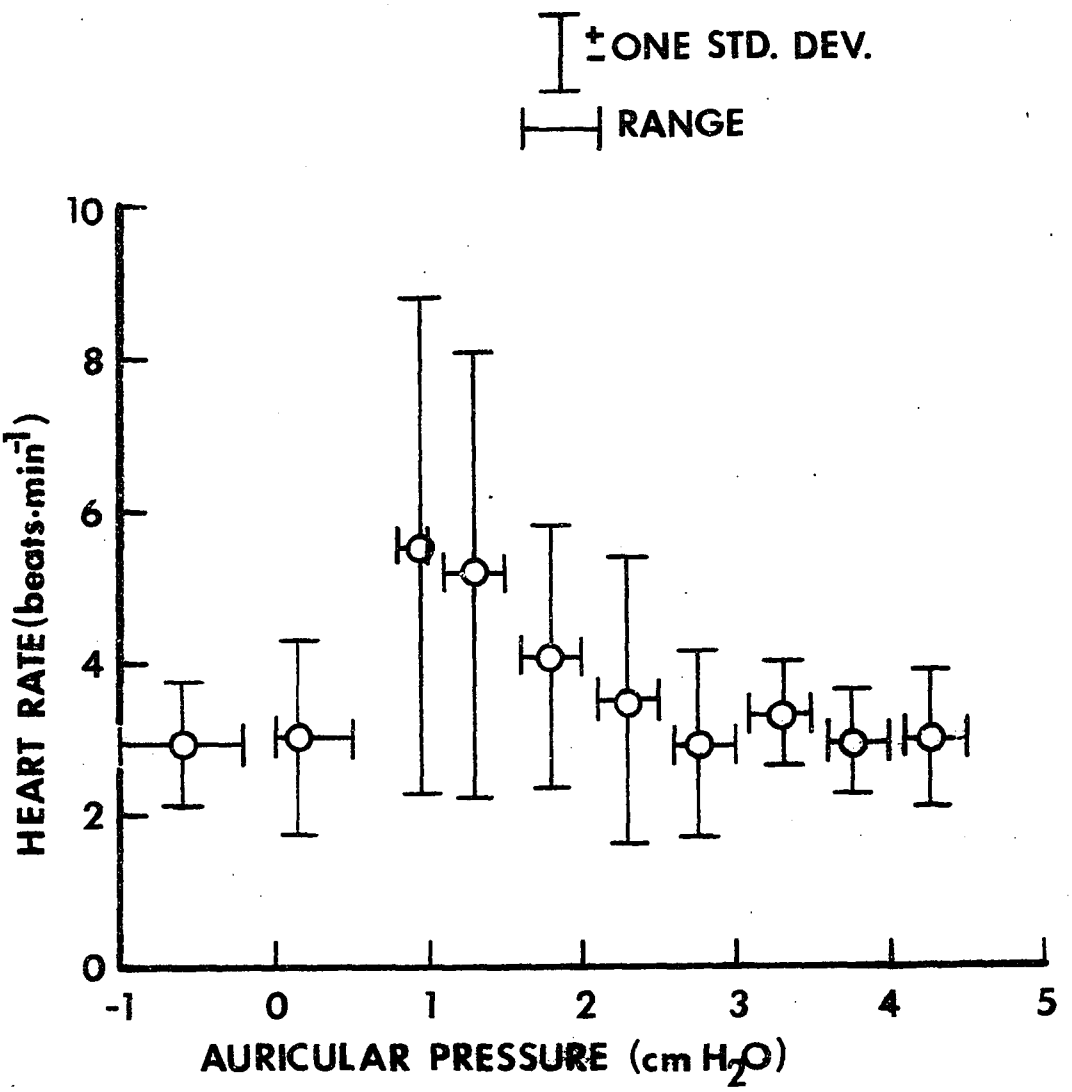


Figure 8. Variations in heart rate with auricular pressure. Aortic diastolic pressure at 5.5±.5 cm H_2O.

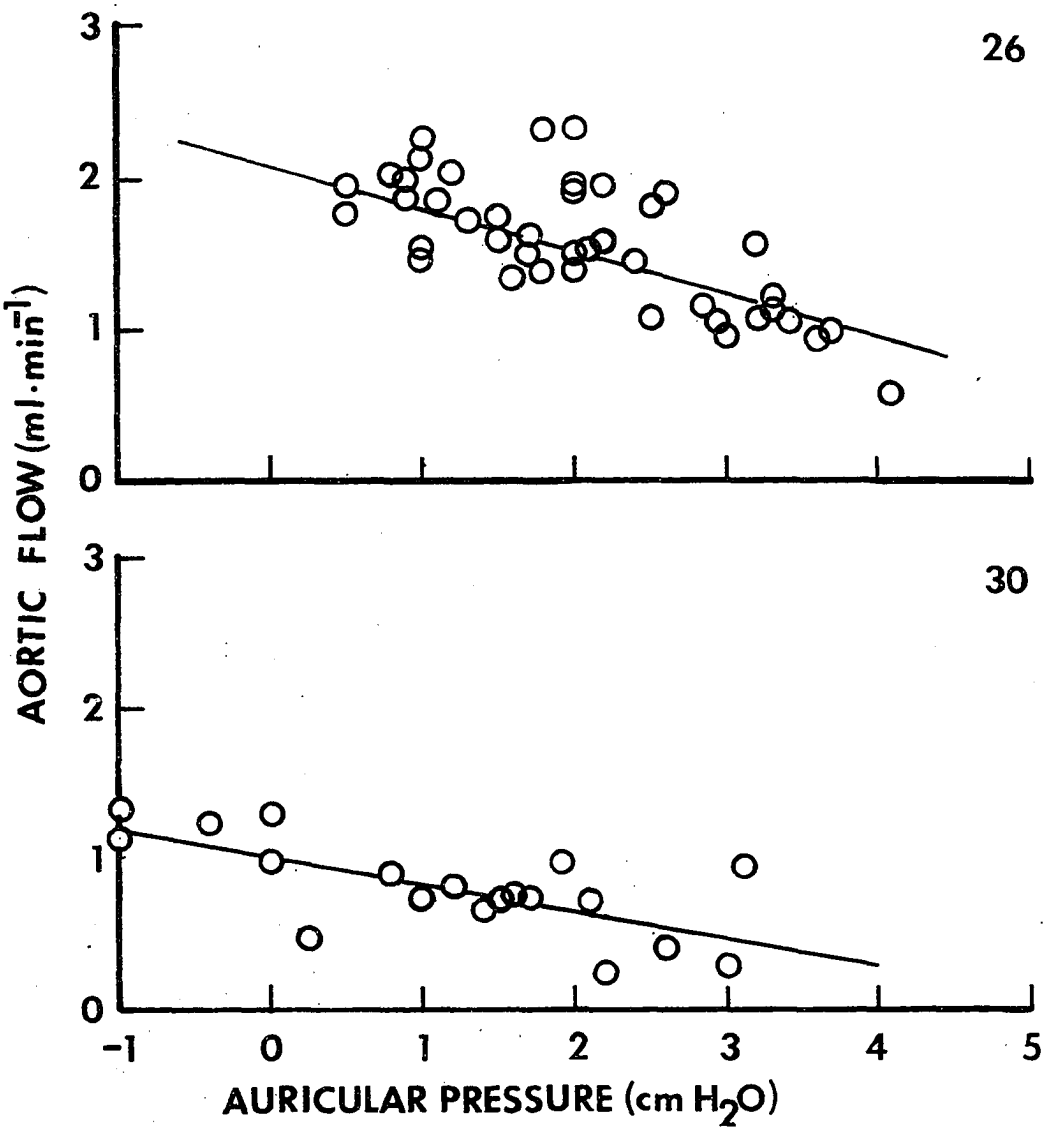


Figure 9. Variation in aortic flow with auricular pressure in Haliotis corrugata (#26, 30, 31, 32, and 33). Aortic diastolic pressure at 5.5±.5 cm H_2O. Straight lines are fitted by the least squares method.

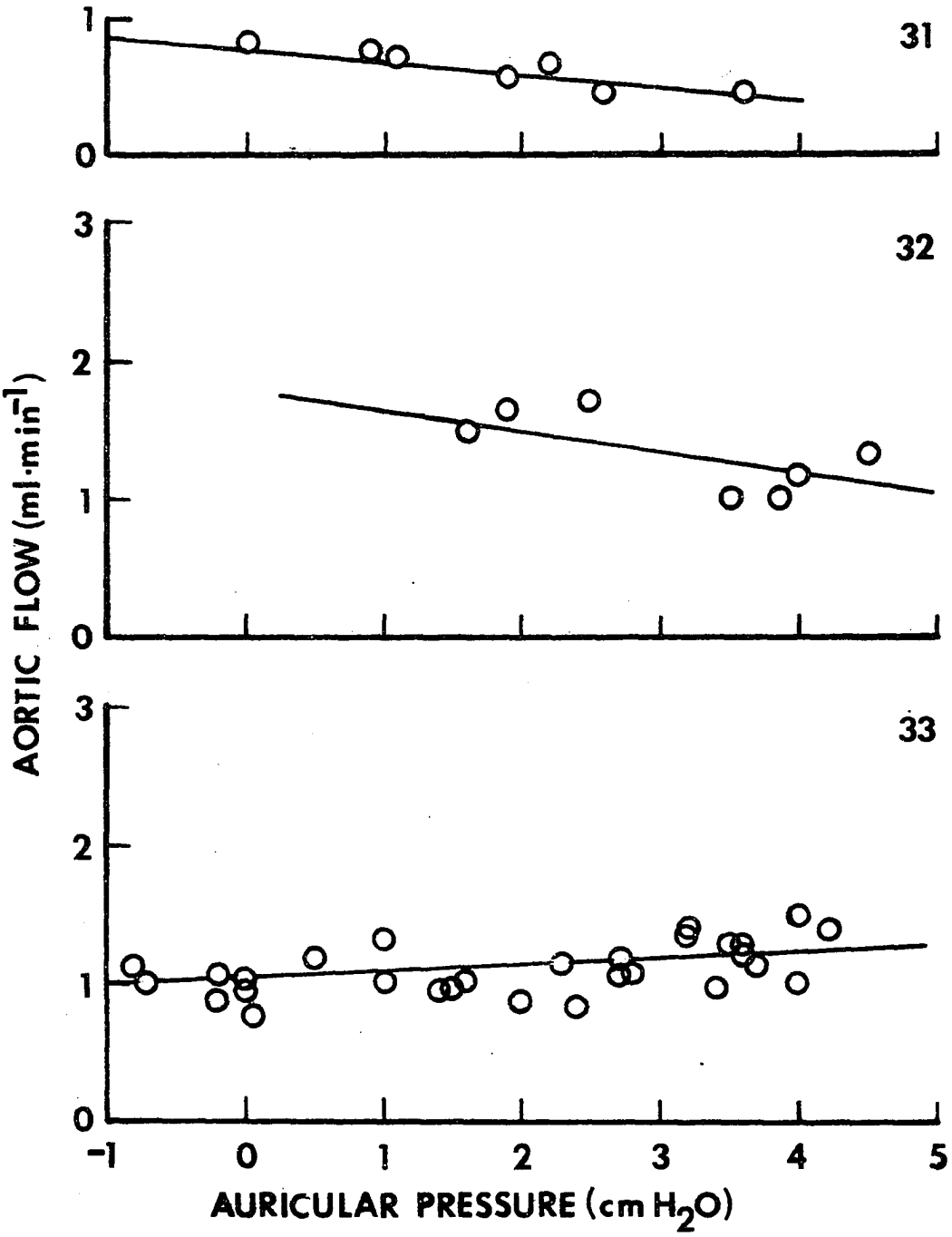


Figure 9. (Continued)

clearly demonstrates any changes in heart output caused by changing auricular pressure. The normalized flows for all experiments are summarized in Figure 10. This graph illustrates a very slight decrease in heart output as auricular pressure is increased within physiological limits and as aortic back-load is held constant.

The regression statistics from the above data are summarized in Table 1. The statistics indicate that the stroke volume is independent of auricular pressure and the negative slope of the heart rate and output curves is significant but very small.

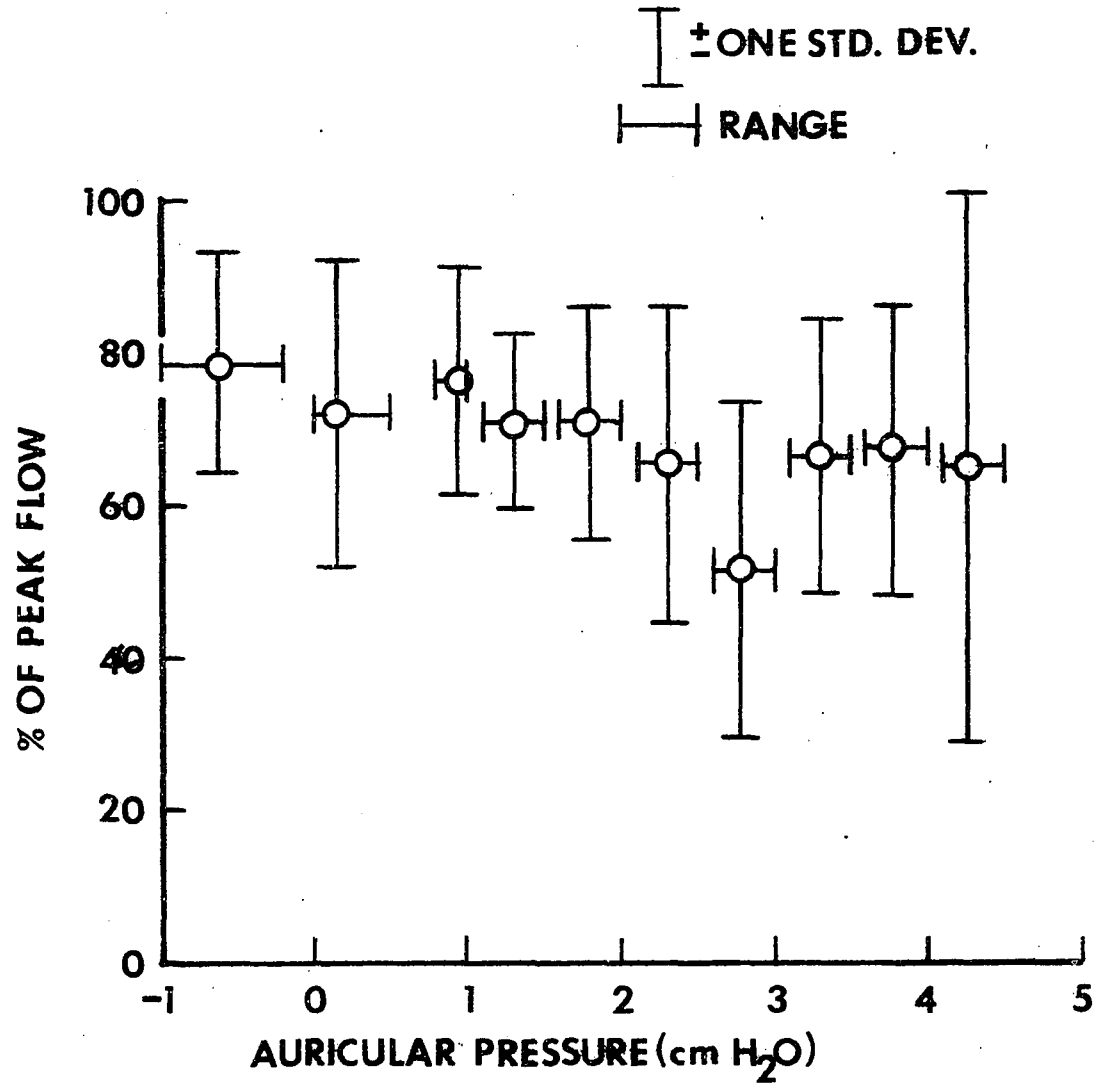


Figure 10. Variation in aortic flow (normalized to the peak flow of each animal) with auricular pressure. Aortic diastolic pressure at 5.5±.5 cm H_2O.

Table 1. Significance table with the slopes (± the standard error of the estimate) of the regression lines from five Haliotis corrugata (+ indicates significance at the 5% level of t test, - indicates no significance)

Animal No.	Aortic Stroke Volume vs. Auricular Pressure	Heart Rate vs. Auricular Pressure	Aortic Minute Volume vs. Auricular Pressure	% of Peak Aortic Flow vs. Auricular Pressure
26	0.026±0.01 (+)	-1.812±0.19 (+)	-0.326±0.04 (+)	-13.91±2.12 (+)
30	-0.008±0.01 (-)	-0.480±0.22 (+)	-0.179±0.04 (+)	-13.16±2.45 (+)
31	-0.001±0.02 (-)	-0.371±0.40 (-)	-0.119±0.081 (-)	-14.20±4.41 (+)
32	-0.099±0.02 (+)	0.224±0.42 (-)	-0.180±0.09 (+)	-10.42±4.67 (+)
33	-0.010±0.007 (-)	0.212±0.14 (-)	0.057±0.03 (-)	3.85±1.54 (+)
Weighted Mean	-0.005±.006 (-)	-0.442±0.12 (+)	-0.102±0.02 (+)	-5.38±1.34 (+)

DISCUSSION

These studies were undertaken to measure some of the mechanical regulatory factors of the heart of the pink abalone, Haliotis corrugata. Although complete elucidation of these factors has proved difficult, the results reveal that the heart physiology of the abalone is quite different from vertebrate hearts.

Several limitations of these experiments must be emphasized before drawing conclusions from the results. There was no control of nervous regulation during the experiments. However, since data were gathered after a long stabilization period and auricular pressures were changed quickly as output and heart rate were monitored, the effect of short term nervous regulation on these results was minimized. Also, the low heart rate observed in these experiments may be due to the absence of some factor in the circulating fluid that facilitates the initiation of heartbeat. The fact that the "open circuit" preparation did not beat and the recirculating preparations did, indicates that there is some metabolite required by the heart to beat that is being added to the fluid. Finally, due to the physical arrangement of the visceral arterial branches, it was not possible to measure exact cardiac output and to increase the resistance load on the heart to confirm the results reported by Schwartzkopff (1954) who claimed that the heart frequency and output varied inversely with arterial pressure and peripheral resistance. However, an assumption of reduced output with increased load is reasonable based on energy requirement considerations.

The Effect of Auricular Pressure on Stroke Volume

The measured aortic stroke volume can be considered a constant fraction of heart stroke volume and was indicative of changes in ventricular stroke volume. Figure 6 summarized the relationship between auricular pressure and aortic stroke volume. As can be seen from this figure, there is very little correspondence between this variable and stroke volume; therefore, stroke volume can be said to be nearly independent of venous input pressure. This result is quite different from results from vertebrate preparations (Guyton et al., 1973; Randall, 1970) and those molluscan experiments where the pericardium is opened (Straub, 1904; Biering, 1929; Sommerville, 1973b). The data also differ from that reported by Schwartzkopff (1954) in the cannulated *in situ Helix* heart. However, it must be remembered that Schwartzkopff had no back pressure on the heart for some of his experiments which would lead to a direct pressure gradient across the heart where venous pressure was the driving pressure. Also, in experiments where there was an aortic back pressure, auricular pressures applied were much higher than those found in the intact animal.

It may be hypothesized that the maintenance of the stroke volume within narrow limits may be an advantage in that the heart of the abalone, and other gastropod molluscs, may not be able to adjust its tension to fiber length changes (Starling's Law) within a broad range (Hill and Schunke, 1967). It is probable that the fluid-filled pericardium with its limited volume is the physical cause of this stroke volume regulation.

The Effect of Auricular Pressure on Heart Rate

The heart rate was easily monitored by records of auricular and aortic pressure and aortic stroke volume. It was expected that heart rate would be more variable than stroke volume because of the many factors that can change heart rate and are difficult to experimentally regulate. Unfortunately, it was not determined what kept heart rate so much lower than normal. It is very possible that the ionic balance of the perfusion fluid was not optimal and future studies might be needed to determine a more physiologic perfusion fluid. Figure 8 summarized the results obtained from five animal experiments; and indeed, does show great variability of heart rate, especially near 1 cm H_2O. This peak at 1 cm H_2O may be indicating an input pressure where the heart pacemaker may be the most sensitive or it may be just an instance of a burst of nervous stimulation in animal 26 (Figure 7) at a specific point in the experiment. However, taken as a whole the heart rate of the pink abalone in an intact pericardium with constant aortic diastolic pressure seems nearly independent of input auricular pressure within physiological values. Once again the constant volume pericardium would seem to act as a hydraulic isolation unit when changing vascular conditions change venous return input pressures. Therefore, the prime regulators of heart rate of the pink abalone may be nervous and humoral input and not venous return.

The Effect of Auricular Pressure on Cardiac Output

The ultimate measure of the significance of venous return input pressure as a regulator of the heart is to determine its ability to change cardiac output. Figure 10 summarized all changes in cardiac output for the experimental animals due to varying auricular pressure. Using a normalized scale for cardiac output removes some of the variation between animals which might have been at a slightly different nervous state. This figure shows a slight negative slope due to the heart rate changes with auricular pressure. This result is different from all reported data and suggests that venous return does not play a major role in the regulation of heart output of the pink abalone.

A possible explanation of the above conclusion is suggested by the constant volume filling hypothesis for molluscan heart, and specifically, by the data of Civil and Thompson (1972) with the Helix heart in an artificial pericardium. It may be hypothesized that the constant volume pericardium prohibits quick muscle fiber length change by compensating to internal pressure changes. As auricular internal pressure increases, due to increased venous return, the walls of the auricle would attempt to expand; however, the surrounding pericardial fluid could not pass through the reno-pericardial canal quickly enough to accommodate the increasing auricular volume and thus, the pericardial pressure rises to match this auricular internal pressure. Thus, no further change in auricular volume occurs. Therefore, during auricular systole a constant volume of blood is injected into the ventricle and the stroke volume of the ventricle remains relatively constant as indicated by Figure 10. Further evidence for a

pressure compensation in the pericardium is found in the data of Bourne and Redmond (1977a). They show the pericardial pressure to follow fluctuations in auricular pressure exactly, and to be at a measured pressure only slightly less than auricular. Also, the data from Schwartzkopff (1954) can be explained with this hypothesis. As he increased the auricular pressures to supraphysiological values, the pericardial pressure would also have risen dramatically. These extremely high pericardial pressure would have greatly increased flow through the reno-pericardial canal into the left urocoel (at low physiological pressures); therefore, the volume of the pericardial fluid would decrease with time and auricular and ventricular volume would increase; thus, a larger stroke volume.

The above hypothesis suggests an additional potential controlling element for molluscan heart output that had been mentioned earlier by Civil and Thompson (1972). The reno-pericardial canal guarded by a muscular spincter may actively control the pericardial fluid volume and pressure, and thus, control the stroke volume.

In review, it has been seen that in the intact heart of the pink abalone, the output is not controlled by the venous return pressure, and suggests the following variables as candidates for the primary regulators of cardiac function:

1. Nervous inputs.
2. Humoral inputs.
3. The volume and pressure in the pericardium and the condition of the reno-pericardial sphincter.
4. The backload on the heart.

Future studies should attempt to examine the above factors.

PART II. THE PERIPHERAL CIRCULATION OF THE PINK ABALONE

REVIEW OF LITERATURE

In comparison with the information available and research effort applied to studies of the heart, the literature on the properties of the peripheral vascular systems of invertebrates is meager. This lack of information is understandable because of the inaccessibility, complexity and diffuse nature of the peripheral system. However, the elucidation of some of the peripheral properties is extremely important because it is this system that controls the distribution of nutrient flow to the organs of the body, including to some extent the heart itself. Since it is extremely difficult to separate the individual elements of the peripheral system, it is useful to consider it as a system of lumped components and to measure the input-output characteristics of these lumped elements.

Some of the more useful techniques for studying the peripheral circulation have been developed with the vertebrate's closed system of "pipes, elastic chambers, and pumps." Guyton et al. (1955) were among the first investigators to suggest that the condition of the peripheral vascular system may play a most important role in the regulation of cardiac output. They measured the effect of increased blood volume on the venous return in the dog. To accomplish this they by-passed the heart with a pump and measured the venous return flow and simultaneous right atrial pressure while varying the blood volume. Increased blood volume increased the mean circulatory filling pressure thus increasing the vis a tergo (force from behind) and, therefore, increased the venous return at a given auricular pressure.

A more sophisticated technique for determining the factors that influence venous return of the dog was described by Guyton et al. (1957). They bypasses the heart with a pump as in the earlier paper; however, they were more precisely able to control the auricular pressure by the addition of a vertically moving section of collapsible tubing to maintain a pressure head on the right atrium and to isolate the atrium from the suction of the pump. Guyton et al. (1957) also suggested equating venous return curves (venous return flow versus right auricular pressure) with cardiac output curves, as described in part one, to find the common point of the curves which they defined as the operating point of the circulatory system.

Guyton, Jones and Coleman (1973) defined the factors that influence venous return in vertebrates. They emphasized the vis a tergo concept which involves the driving pressure gradient but discounted the importance of the vis a fronte (force from in front) which would involve suction of blood by the heart and collapse of veins at the heart which impedes return to the heart. They concluded that the two major parameters of the peripheral vasculature of vertebrates which influence venous return are: vascular capacitance as indicated by the mean circulatory filling pressure (MCFP), and the viscous resistance changes caused by vessel caliber variations (nervous or autoregulatory) and shunts.

The techniques developed by Guyton have not yet been successfully applied to nonmammalian vertebrates and invertebrates. Most reported data on the peripheral systems of nonmammals have been concerned with the measurements of the pressure gradient causing circulatory flow, or in some

instances when flow and pressure gradient were measured simultaneously, the resistance was calculated.

Randall (1970) reviewed circulatory data for fish, which usually have the gills in series with the rest of the peripheral circulation. The capillary beds of the gills represent a considerable portion of the total vascular resistance. Total peripheral resistance decreases during swimming and increases during hypoxia. Also, in the hagfish several accessory hearts help regulate venous flow. Teleosts possess venous valves as do other vertebrates.

Jones (1971) measured pressures in Helix and reported a pressure gradient between aorta and pulmonary vein of 19 cm H_2O produced by a systolic pressure of 24 cm H_2O in the ventricle and diastolic auricular pressure of 5 cm H_2O. Sommerville (1973c) measured pulse pressures and venous return flow (using a differential pressure transducer to record flow) in Helix but did not know the relationship to atmospheric pressure so no comparison to Jones' values were made and no pressure gradient calculated. However, she did claim that increased frequency and amplitude of heart beat was associated with increased venous return. However, no calibration of the pressure-drop flowmeter was ever successful to verify flow data. Sommerville (1975) also reported pulse pressures of the auricle and ventricle of the swan mussel Anodonta cygnea, but once again no pressure gradient values were reported.

Bourne and Redmond (1977b) reported efferent ctenidial venous and aortic pressures (and pressure gradient) and simultaneous aortic flow of Haliotis corrugata. While recording flow, the mean aortic pressure was

between 3.9 and 4-0 cm H_2O and efferent ctenidial pressure was approximately .6 cm H_2O. The measured flow was approximately 3 ml/min and, thus, the measured peripheral resistance was approximately 7 x 10^4 dynes•sec•cm^{-5}. Several limitations of the flow measurements techniques were mentioned and the flow values reported were probably less than normal. The reported peripheral resistance was noted to be one order of magnitude higher than the resistances reported for mammals.

It can be expected that the open systems of the molluscs have different mechanisms for peripheral regulation than the better known vertebrate systems. Some of these differences may be suggested by examining the general and specialized anatomical structures of the molluscan circulatory systems. Martin et al. (1958) measured the blood volume of many different molluscs, using a combination of T1824 dye and inulin to separate total blood volume and extracellular space. He reported that in all molluscs except Cephalopoda, the blood volume was essentially extracellular volume indicating the lack of separating membranes between circulatory pathways and tissue.

Martin and Johansen (1965) reviewed the literature on invertebrate circulation and summarized the general anatomical features of molluscan circulation. For prosobranch gastropods the following general features exist:

1. No endothelium in blood sinuses and lacunae.
2. The veins are generally rhythmically contractile.
3. Blood is filtered from the ventricle into the pericardium and from there into the left kidney.

Hill and Welsh (1966) also reviewed molluscan circulation and confirmed many of the anatomical characteristics reported above. Johansen and Martin (1962), in what was perhaps the most complete analysis of the circulatory systems of a cephalopod mollusc, pointed out the high level of system development in Octopus dofleini. This nearly closed system has six identifiable elements: the active elements of ventricle, branchial hearts, rhythmically propulsive vessels, the passive forces such as respiratory movements, and the windkessel of the larger arteries and afferent branchial vessels.

The most complete description of circulation in Haliotis is found in the work of Crofts (1929). She pointed out the following anatomical features of the circulation which could have a bearing on the peripheral vascular regulation:

1. The cephalic aorta has a contractile orifice at the point it becomes the cephalic arterial sinus.
2. The epipodial and pedal arteries have complete walls.
3. The venous pedal sinuses have no true endothelium, but the neighboring muscle and connective tissues arrange themselves to make muscular walls to propel blood.
4. The flow of blood into the epipodial and pedal arteries is controlled by a valve at the exit from the cephalic sinus which prevents back flow into cephalic region.
5. The arrangement of muscle layers at the outflow from the cephalopedal venous sinus limits outflow from the foot when the shell muscle is contracted.

Considerable work, especially with bivalves, suggests that body movements combined with vascular valving may play a large role in peripheral circulation regulation. Jullien and Ripplinger (1953) studied the circulatory system of Helix and recorded high venous pressures associated

with body movement. However, no data were given on the pressure gradient created by activity. Trueman (1966) studied the effect of body movement on blood pressure and distribution in two bivalve molluscs, Mya and Margaritifera. Trueman measured blood pressures from various parts of the circulation during various activities such as siphon extension, burrowing and valve contraction. He found that during pedal retraction in Margaritifera there exists a pressure gradient between foot and gill, and the blood flow is controlled by the flap-like Keber's valve at the exit from the foot. Also, adduction produces high pressures in the mantle cavity and blood is shunted to the siphon to produce hydraulic extension. It was concluded that "higher pressures derived from the body musculature make an important contribution to movements of the blood." As Ramsay (1968) stated "in the snail, for example, the movements of the blood which are brought about when the animal retires into its shell are very much more extensive and violent than anything which the snail's heart can produce."

Chapman (1967) reported studies made by tracing the blood of the South African gastropod Bullia with a radiopaque dye, "Thorotrast." On retraction of the foot, blood from the pedal sinus flowed into the visceral sinus via the cephalopedal vein. The heart output was unchanged during this movement but blood did not flow in the anterior aorta until the foot retraction was completed. Brand (1972) measured the pressures in various parts of the bivalve, Anodonta anatina. Activity pressure peaks were transmitted equally in the peripheral circulation but were of a longer duration in the pedal haemocoel thus producing a pressure gradient

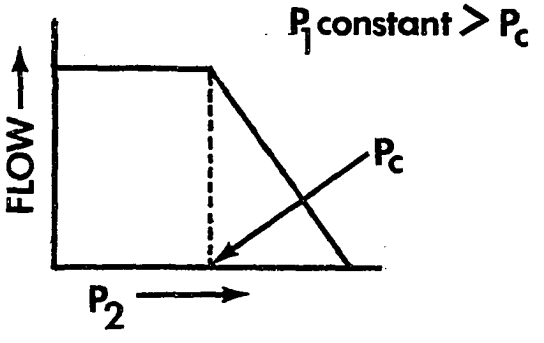
for blood movement out of the foot. He also proposed that Keber's valve may limit outflow from the foot during burrowing so that venous return pressure does not rise excessively. Therefore, it can be concluded that body movements may assist blood flow in parts of the peripheral circulation, but certain structures help isolate the heart from pedal haemocoel pressure surges.

Dale (1973) measured pressures in parts of the peripheral haemocel of Helix. A minimum venous pressure of 8 cm H_2O was required to overcome the resistance of the branchial plexus, and blood pressure in the visceral and subrenal sinus barely exceeded this value. Blood pressure in the cephalopedal sinus and general foot haemocoel was much higher than this, nearly 16 cm H_2O. Also, it was suggested that a flap of the aortic valve occluded the visceral artery during ventricular systole, and visceral flow only occurred during diastole. Thus, blood distribution was somewhat controlled by the systolic/diastolic ratio (heart rate).

Sommerville (1973a) observed the heart of Helix through a plastic-covered opening made in the shell and mantle. She made subjective judgments on heart and pericardial chamber volumes. She stated that the heart showed changes in the degree of filling indicating an increased blood flow to the heart from the haemocoel when the mantle cavity floor was depressed. However, Bourne and Redmond (1977a) reported simultaneous and equal pressure surges in the aorta and efferent ctenidial veins of Haliotis corrugata during activity. Therefore, there would be no pressure difference change to cause an increase in venous return. In summary they stated, ". . . body activity . . . is probably more important in the

regional translocation of hemolymph than in significantly increasing cardiac output due to increased venous return."

A complicating situation exists in the pedal circulation of molluscs where there are vessels with endothelium such as pedal and epipodial arteries completely surrounded by muscle tissue. In this situation the possibility exists for vascular waterfalls. Permutt and Riley (1963) described the phenomenon of vascular waterfall as the condition when the driving pressure for flow through vessels is not the difference between input and output pressure, but is rather the difference between input pressure and the pressure surrounding the collapsible vessels (total tissue pressure). Holt (1969) and Conrad (1969) performed model studies with a collapsible tube in a chamber where the pressure (P_c) could be altered, the upstream pressure (P_1) held constant, and the downstream pressure (P_2) was changed. He found that when $P_1 > P_c > P_2$, the flow through the collapsed tube became independent of downstream pressure as shown below.



Guyton, Taylor and Granger (1975) clarified the concept of total tissue pressure. They defined this pressure as the sum total of the forces of interstitial fluid pressures and the pressure exerted by solid

structures on collapsible tubes. They stated "a blood vessel can be caused to collapse as a result of either solid structures pressing against it or fluid molecules acting on the outer surface of the vessel." Leith (1976) reviewed some of the literature of the vascular waterfall and concluded that the exact physical mechanisms are as yet incompletely described. This phenomenon may be present at times in cerebral, renal, coronary, pulmonary, retinal, erectile, urethra, and, perhaps, the lymphatic systems.

The major objective of this study is to determine the resistance characteristics of the peripheral circulation of Haliotis corrugata. Some measurements were made to determine if a vascular waterfall phenomenon exists in this system.

MATERIALS AND METHODS

Several attempts were made at setting up the closed system apparatus for determination of the venous return curves as described by Guyton et al. (1973). However, due to the membranous collapsible nature of the circulatory pathways, the large and nonstable capacitance of the abalone, and the inability to plug all potential hemorrhage areas of this animal created by suturing cannulae, these techniques proved impractical. Therefore, a compromise system was developed to measure the resistance characteristics of the peripheral circulation. Fluid was supplied to the circulatory system at a constant pressure and the effect of the changing auricular pressure on venous return was measured.

A portion of shell over the heart was removed and the animals were anesthetized with carbon dioxide. The anterior aorta was cannulated with two sections of PE 240 tubing, one placed approximately 4 cm from the heart and the tip was pushed anteriorly, and the second placed approximately 2 cm from the heart and pushed posteriorly. Both were sutured in place. The second cannula supplied the visceral circulation and the first the pedal and cephalic circulation. Both ctenidial veins were cannulated by suturing around PE 160 tubing that had been inserted into auricles and pushed into the veins away from the heart. The aortic and ctenidial vessels were connected to two constant head tanks as shown in Figure 11. The tank connected to the aorta was kept at a constant height that maintained a perfusion pressure of 5.5±.5 cm H_2O into the aorta. This pressure is near the mean diastolic pressure for the pink abalone as measured by Bourne and Redmond (1977a).

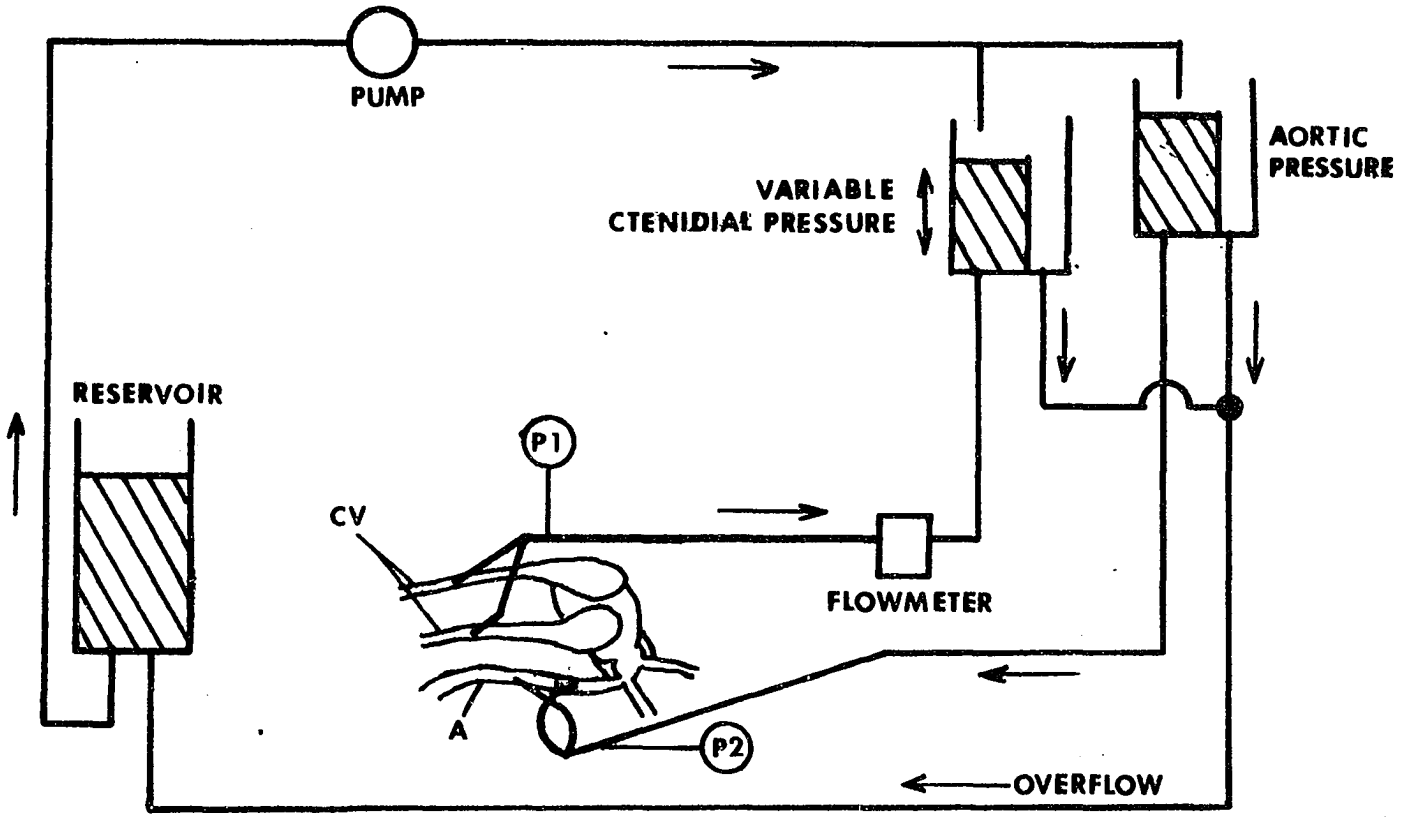


Figure 11. Schematic of flow system to measure venous return while auricular pressure is varied. P1, auricular pressure; P2, aortic pressure; CV, efferent ctenidial vessels; A, anterior aorta.

An electromagnetic flowmeter was connected between the ctenidial vein cannulae and the constant head tank to record the flow through the peripheral circulation (the venous return). The ctenidial constant head tank was attached to a Narco Biosystem Myograph Tension Adjuster to allow continuous variation of downstream pressure (auricular pressure), and therefore, determine the pressure-flow resistance characteristics of the peripheral circulation.

The constant head tanks were connected to an overflow system which kept the perfusion fluid recirculating and free of contamination. The perfusion fluid was Instant Ocean made from distilled water and neutralized with HCl.

A short stabilization period was allowed where the auricular pressure was kept low to allow continued flow through the animal to completely fill the circulation which may have emptied during the surgical procedures. It was likely that some of the circulatory pathways may have opened as the muscles of the animal relaxed and the resistance decreased with time throughout the experiment.

The cannulae were checked by adding a blue vegetable dye to the aortic constant head tank, and if excess leaks were noticed, the experiment was terminated. After checking for leaks the auricular constant head tank was raised slowly so that flow in the cannula was reduced, and the decreasing flow was recorded until auricular pressure was near 5 cm H_2O, and a zero flow baseline was established.

After a series of normal measurements were made a few of the animals were placed on small petri dishes, smaller than the shell's diameter.

Then one weight or a series of weights were placed on top of the shell. The weights were made from plastic bags filled with lead shot and were able to conform to the shell shape to remain on top of the animal. The net force of these weights were determined by weighing the lead-filled bags and the weight of the water displaced by them was subtracted. This had the effect of slightly compressing the animal. It was felt that since the foot muscle was unusually flaccid after the surgical procedures, a slight compression might simulate the conditions within the pedal muscle when a living animal was attached to a substrate. After the animal was compressed another series of flow-pressure drop measuresments were made. Also, on one animal the effect of adding a linear viscous resistance on venous return was measured by clamping the venous cannula and measuring the pressures and flows.

An attempt was made to measure the capacitance of the animal by recording the pressure-volume relationship. On one animal the aortic and ctenidial vein cannulae were connected together with a 3-valve and a syringe attached, thus making a closed circuit. Fluid was injected into this circuit and the pressure recorded. Ideally, the capacitance could be estimated from this data by the relationship $\Delta V/\Delta P$ (Rothe and Drees, 1976).

RESULTS

The purpose of this study of the peripheral circulation of Haliotis corrugata was to determine the resistance characteristics of this system when supplied by a constant source. The parameters of venous return flow and the auricular pressure (the same as efferent ctenidial vein pressure) were plotted as suggested by Guyton et al. (1973). This graphical technique allows the resistance of the peripheral circulation to be represented by the slope of the least squares fitted line. To compute peripheral resistance with C.G.S. units, the slope $\frac{(\Delta Q)}{(\Delta P)}$ was measured and divided into $-\rho 980(60)$ where:

ΔQ was the change in venous return flow ($ml \cdot min^{-1}$).

ΔP was the change in downstream pressure ($cm\ H_2O$).

ρ was the density of Instant Ocean in $g \cdot cm^{-3} = 1.025$.

980 was the gravitational acceleration in $cm \cdot sec^{-2}$.

60 was the seconds per minute to convert ΔQ into $ml \cdot sec^{-1}$.

Therefore, the expression for resistance becomes -6.03×10^4/slope $\frac{(\Delta Q)}{(\Delta P)}$ ($dyne \cdot sec \cdot cm^{-5}$). The resistance of the cannulae (2.81×10^4 $dyne \cdot sec \cdot cm^{-5}$) was subtracted from the calculated value to obtain the resistance of the abalone circulation.

An unexpected result occurred when the animal was compressed under selected weights. The slope of some of the resistance lines exhibited a dramatic change such that below a given auricular pressure the venous return flow was nearly independent of downstream pressure. This result suggested the possible existence of a vascular waterfall in the peripheral circulation of the abalone.

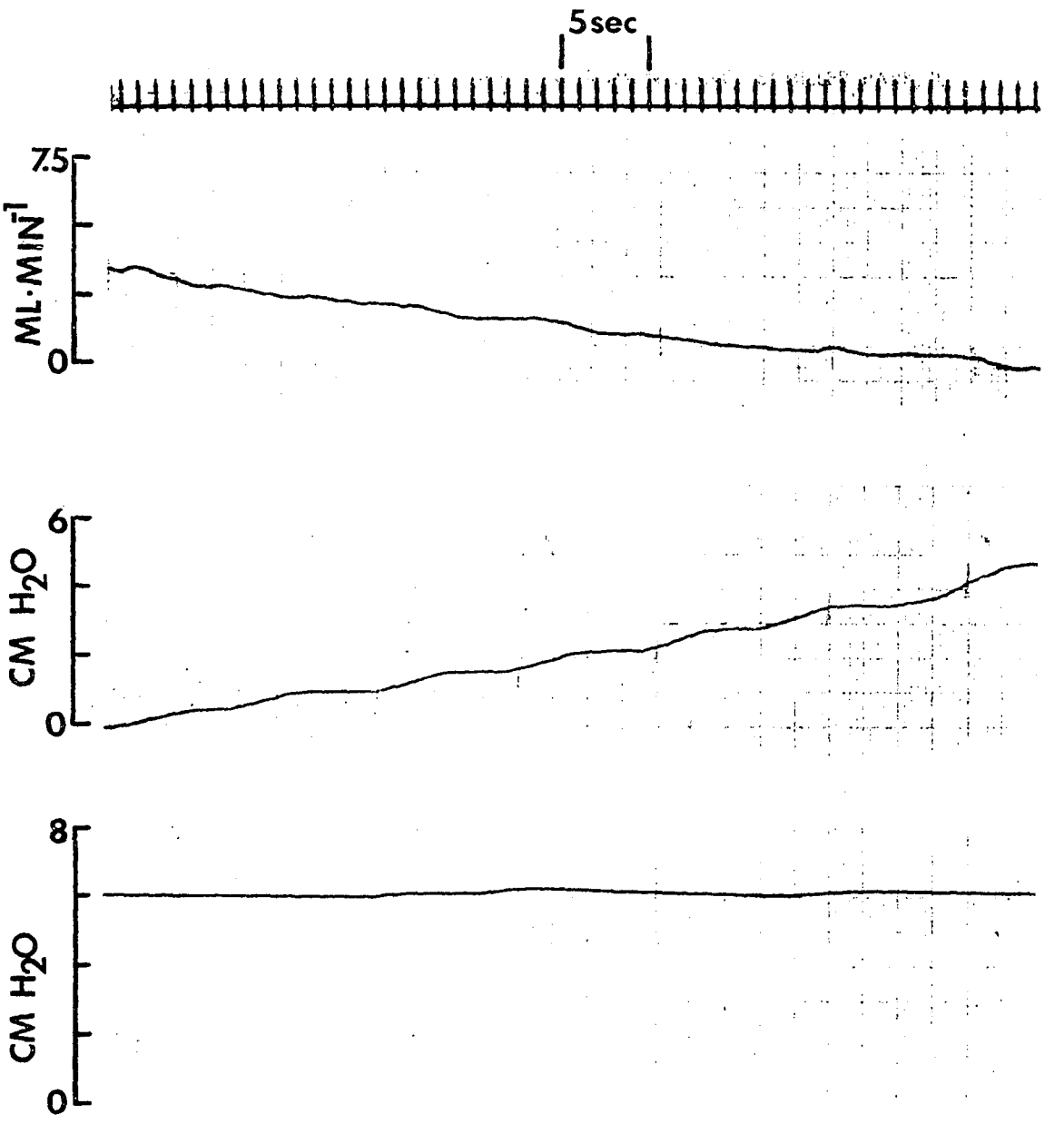


Figure 12. The upper trace is a recording of venous return of Haliotis corrugata #24. The middle trace is auricular pressure and the lower trace is the aortic pressure.

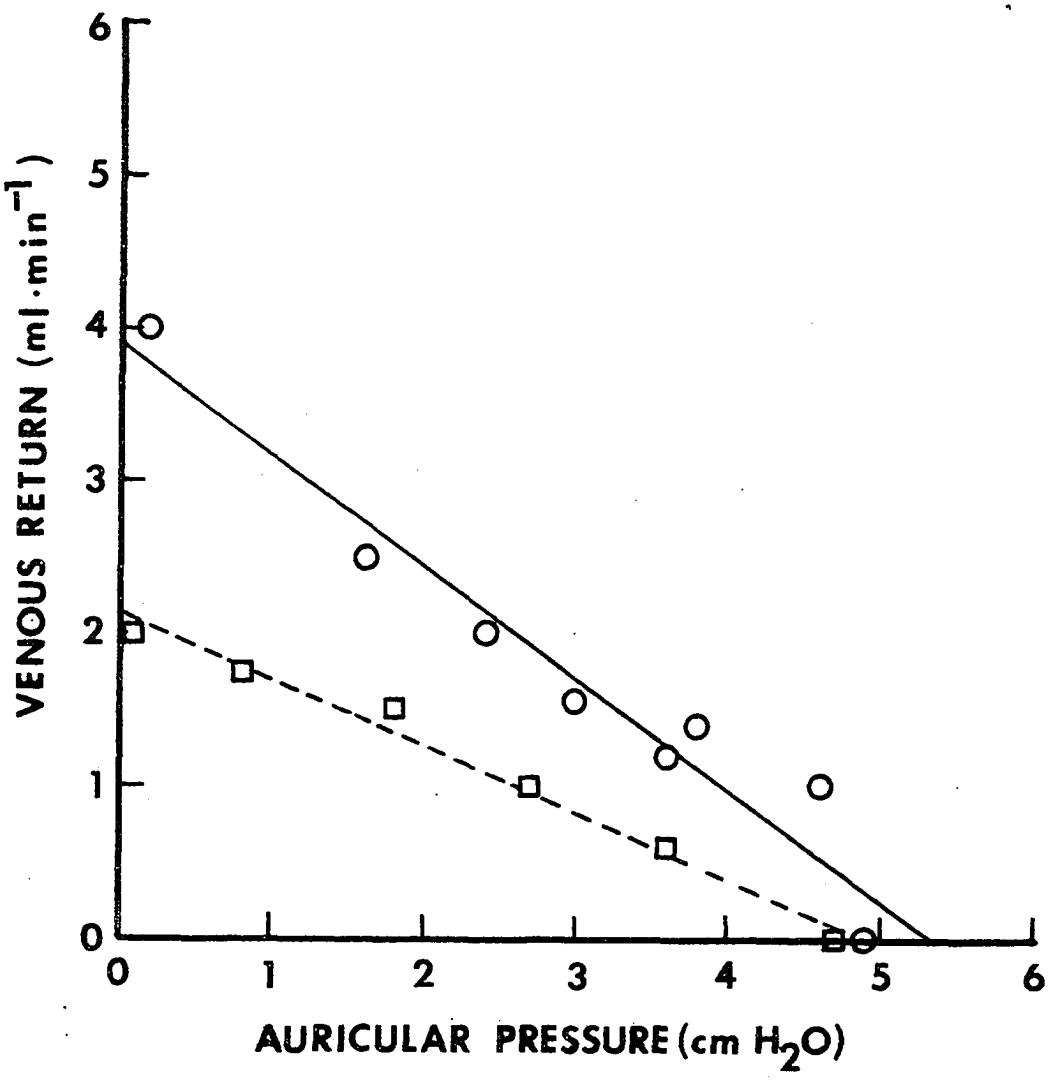


Figure 13. Variation in venous return with auricular pressure in Haliotis corrugata #21. Aortic pressure at 5.5±.5 cm H_2O. O—O, no load; □--□, increased compressive load.

A sample record of the dynograph recordings is shown in Figure 12. The graphed results from animal 21 are shown in Figure 13. The uncompressed animal had a resistance of 6.08×10^4 dyne•sec•cm^{-5} which is linear and when the animal is compressed the resistance remained linear but increased to 11.84×10^4 dynes•sec•cm^{-5}. This change could be easily interpreted as a reduction in caliber of circulatory pathways causing an increase in resistance predicted by the Hagen-Poiseuille equation. Another more plausible explanation would be that several circulatory pathways had collapsed under the increased pressure, and therefore, there was an increase in total resistance in the foot muscle. The experiment with animal 21 was done before the experimental procedure was well-established. Unfortunately, the force applied by the added weight was unknown and could only be expressed as somewhere between 0 g and 1000 g.

Figure 14 presents results from animal 22 which was the first animal discovered to exhibit a two-part resistance curve when compressed. When the animal was not compressed, the total peripheral resistance was 4.92×10^4 dynes•sec•cm^5, very similar to animal 21. When an 800 g compressive force was applied, the apparent resistance at auricular pressures greater than 2 cm H_2O was slightly higher (6.08×10^4 dynes•sec•cm^{-5}) than the uncompressed animal which is consistent with the results from animal 21. However, when the auricular pressure was decreased below 2 cm H_2O, the venous return flow stayed relatively constant. The technique for determining total peripheral resistance in this regime is very poorly understood and must be related to flow through a collapsible tube (Holt, 1969). In general, the resistance is related to upstream pressure and total tissue pressure with no consideration for downstream pressure.

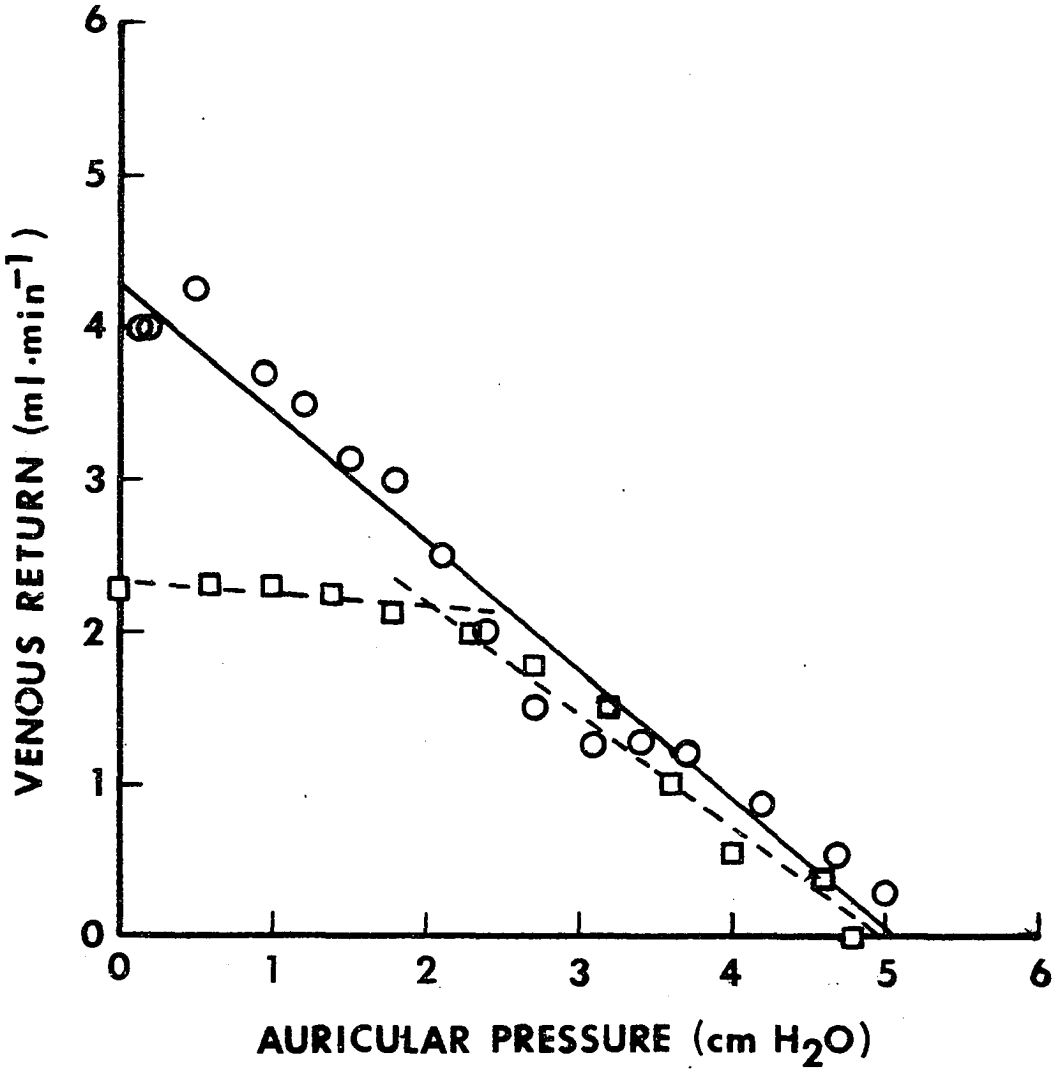


Figure 14. Variation in venous return with auricular pressure in Haliotis corrugata #22. Aortic pressure at 5.5±.5 cm H_2O. O—O, no load; □---□, 800 g compressive load.

Animal 24 (Figure 15) exhibited a much lower normal total peripheral resistance of 2.22×10^4 dynes·sec·cm^{-5}. When an 800 g compressive force was applied, the resistance did increase but it remained linear throughout, unlike animal 22. The total peripheral resistance of the compressed animal was 4.02×10^4 dynes·sec·cm^{-5}.

Figure 16 (animal 39) illustrates an example of the time dependent nature of the resistance characteristics during the experiment. The early normal resistance was 8.62×10^4 dynes·sec·cm^{-5} but decreased to 4.62×10^4 after 50 minutes had passed. Also, when a tubing clamp was then placed on the venous return line, the resistance was increased from the late normal value to 7.52×10^4 dynes·sec·cm^{-5} verifying that the experimental technique did measure a decrease in viscous resistance.

Animal 39 (Figure 17) illustrates the phenomena of the physiological waterfall. The resistance characteristics of the uncompressed animal indicated a linear viscous resistance of 3.73×10^4 dynes·sec·cm^{-5}. However, as weights were added to the animal the curves became nonlinear with the "critical pressure" increasing with increasing weight. It is evident that the total tissue pressure surrounding collapsible vessels in the circulation increased with more compressive force. Also, the resistance of the decreasing slope part of the curve is increased as weight is added indicating that many circulatory pathways could be blocked off completely. A summary of the resistance characteristics of the five animals is presented in Table 2.

The experiments to determine capacitance of the peripheral system were only partially successful in that the capacitance can be only

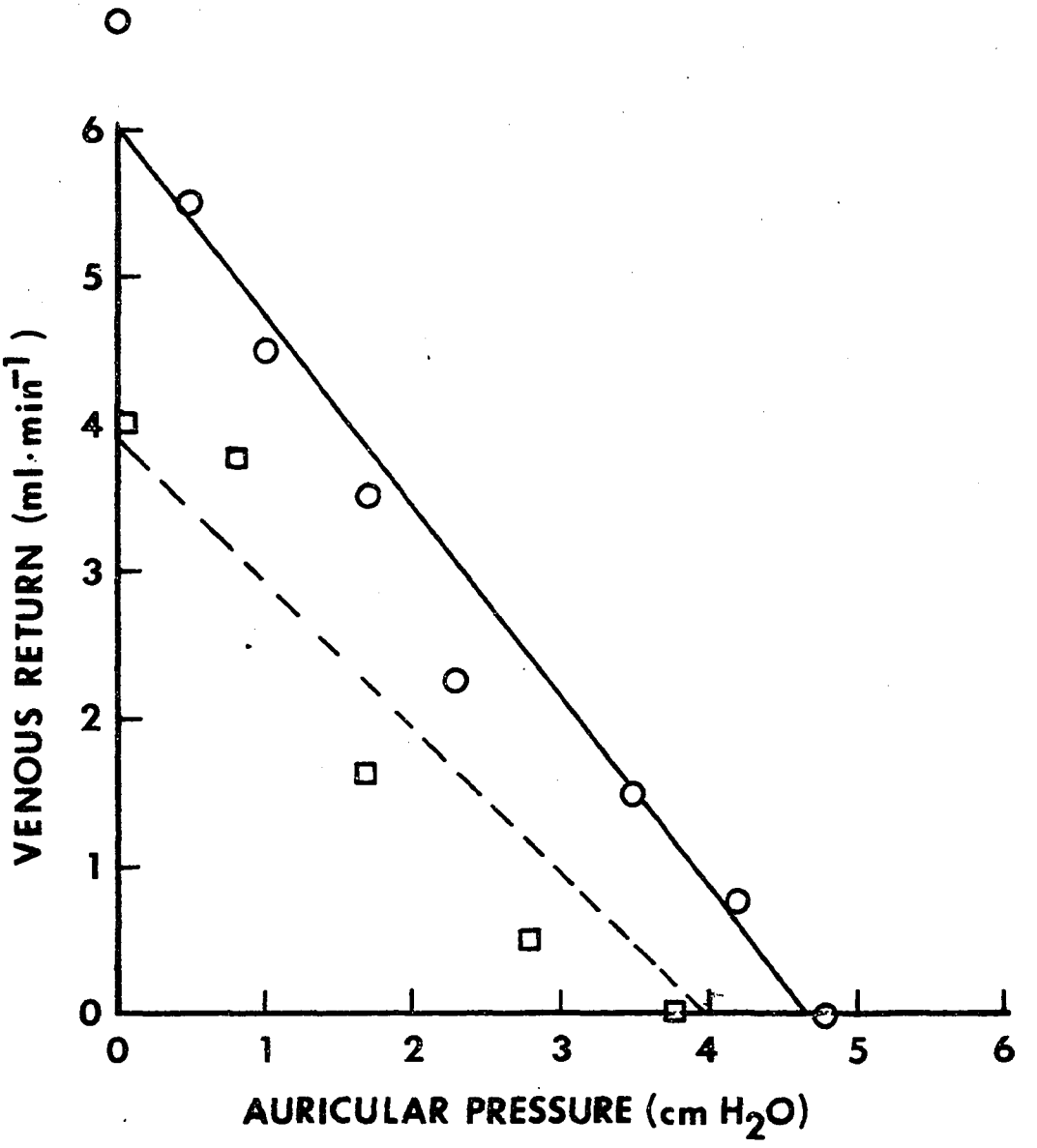


Figure 15. Variation in venous return with auricular pressure in Haliotis corrugata #24. Aortic pressure at 5.5±.5 cm H_2O. O——O, no load; □---□, 800 g compressive load.

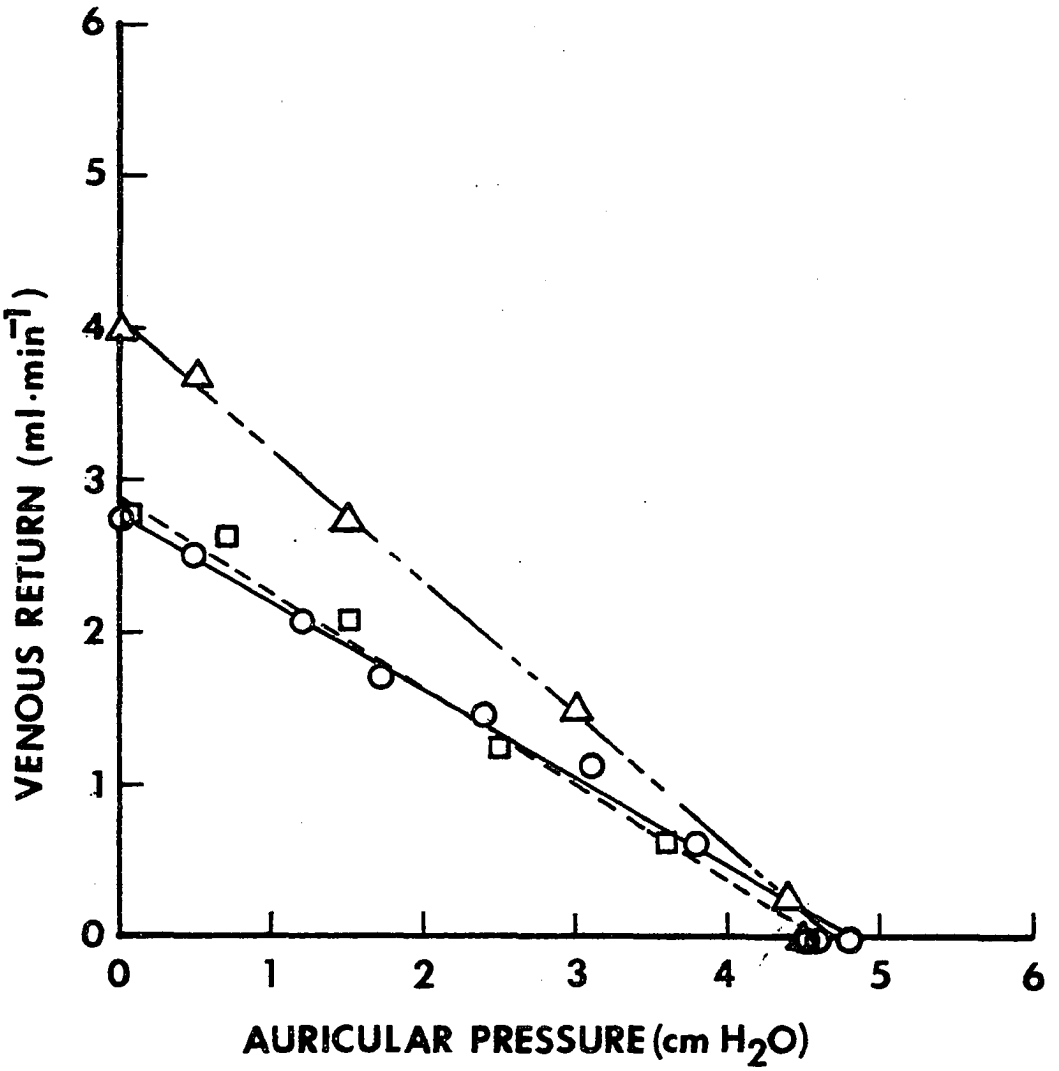


Figure 16. Variation in venous return with auricular pressure in Haliotis corrugata #36. Aortic pressure at 5.5±.5 cm H_2O. O——O, no load; □---□, increased resistance by clamping tubing; Δ——Δ, no load 50 min later.

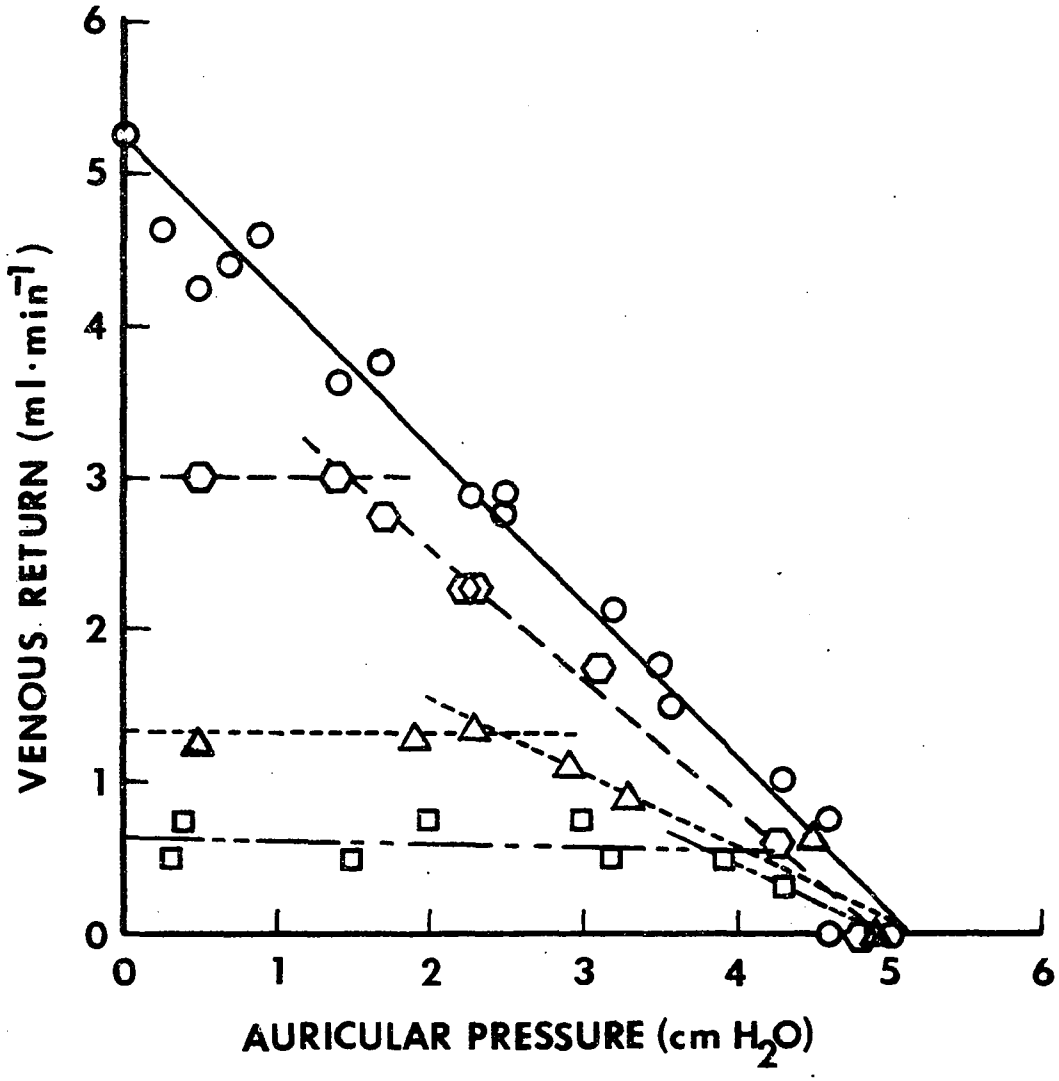


Figure 17. Variation in venous return with auricular pressure in Haliotis corrugata #39. Aortic pressure at 5.5±.5 cm H_2O. O—O, no load; ⬡---⬡, 2300 g compressive load; Δ---Δ, 2700 g compressive load; □----□, 3100 g compressive load.

Table 2. Summary of resistance values from 5 animals

Animal No.	Flaccid resistance dynes•sec•cm^{-5}	Compressed Weight gm	Compressed Resistance dynes•sec•cm^{-5}	Critical pressure cm H_2O
21	6.08×10^4	?	11.84×10^4	-
22	4.92×10^4	800	6.08×10^4	2.05
24	2.22×10^4	800	4.02×10^4	-
36	8.62×10^4			
	4.62×10^4			
39	3.73×10^4	2300	4.75×10^4	1.45
		2700	10.58×10^4	2.45
		3100	9.82×10^4	3.80
Mean	5.06×10^4			

estimated as being very large. There was virtually no change in closed system pressure with the injection of fluid volumes as high as 50 ml (approximately 50% of blood volume). The system would respond to the injection with a pressure surge but would quickly return to the previous level. A sample record of this response is shown in Figure 18.

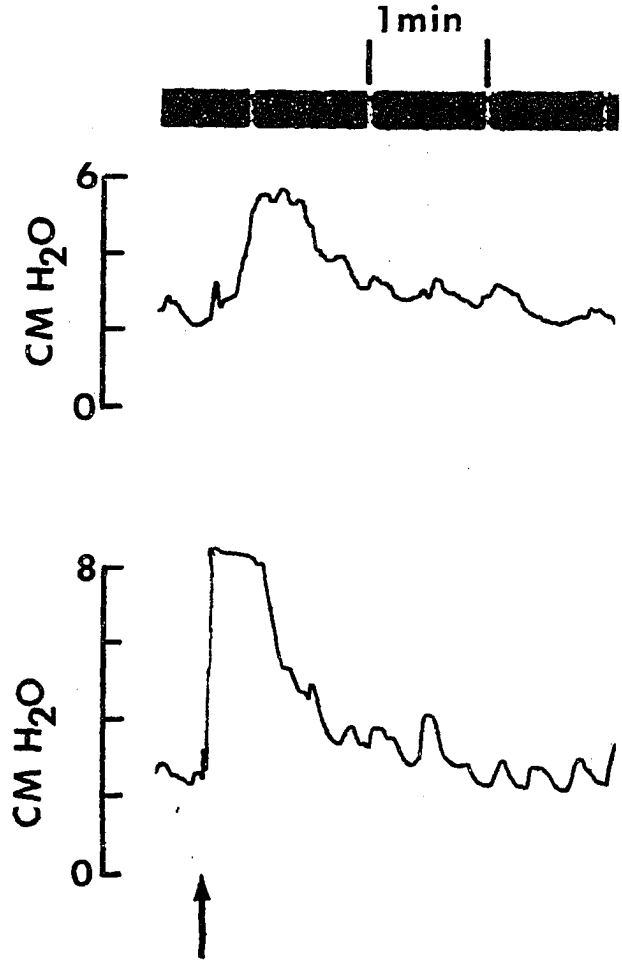


Figure 18. The effect on circulatory pressures of injecting fluid into the circulation of Haliotis corrugata. The upper trace is auricular pressure and the lower trace is aortic pressure. ↑, injection of 50 ml of fluid.

DISCUSSION

The preparation of an "isolated peripheral circulation" was undertaken to attempt to obtain values for peripheral resistance and capacitance. It became obvious, as shown in Figure 18, that the capacitance in this open circulatory system is so large as to be a difficult variable to measure and even harder to determine the effects of changes in vascular capacitance on the circulatory flow. Therefore, that variable will not be discussed further here except for suggestions for future studies.

The resistance characteristics, as summarized in Table 2, indicate that there is variability in resistance between different abalone, and also there is the ability for change within a particular animal with time as shown specifically in Figure 16. The measured resistance values are in the same range as previously reported for Haliotis corrugata (Bourne and Redmond, 1977b), the value calculated from pressures and cardiac output (Altman and Dittmer, 1971) for Octopus dofleini, and an order of magnitude higher than reported by McDonald (1960) for mammals. Direct comparison of the resistance of Octopus and mammals, both with closed circulatory systems, at first glance, may indicate some evolutionary difference in blood delivery between invertebrates and mammals since the Octopus circulatory system was secondarily derived from the open system of other molluscs. However, it must be pointed out that the octopus has auxiliary branchial hearts and, therefore, the relationship between the aortic and venous pressures will not be due solely to viscous resistance as is generally true in mammals. In addition, the possible existence of a vascular waterfall in the peripheral circulation of molluscs may make resistance

measurements computed on flow, aortic and venous pressures inaccurate. This is because in conditions with vessels collapsed, the resistance through those vessels is related to aortic pressure and the collapsing-tissue pressure, independent of the venous pressure. The peripheral circulation of Haliotis corrugata appears to exhibit this waterfall phenomenon at times as shown in Figures 14 and 17. Figure 17 indicates that an increased compressive load, such as during clamping movements, would be transferred directly to collapsible vessels in the circulation causing an increase in the "break" pressure (total tissue pressure), at which the flow becomes independent of downstream pressure.

Two potential pedal circuits appear to be susceptible to collapse during foot muscle contraction: (1) the epipodial and pedal arteries; and (2) the cephalo-pedal venous sinus. A plausible hypothesis can be made for the control of pedal inflow circulation by a vascular waterfall. Both of the arterial vessels have intact walls (Crofts, 1929) and they are completely surrounded by muscle fibers. During foot muscle contraction the interstitial pressure would rise and would be transmitted directly to the inside of the arteries, and thus, there would be no change in transmural pressure due to fluids. However, the solid pressure due to the contraction of muscle fibers would increase transmural pressure and cause collapse if it exceeded the downstream blood pressure. When the heart beats the pressure in the cephalic arterial sinus is generally kept higher than the generalized fluid pressure in the haemocoel. If this sinus blood pressure is greater than the total collapsing tissue pressure created by the foot muscle the blood will flow into the foot independent of haemocoel

blood pressure. Therefore, foot muscle tension and cephalic arterial sinus pressures (controlled by heart rate) would be regulators of pedal circulation.

A second more plausible hypothesis for waterfall control of the circulation involves the cephalo-pedal venous sinus. Crofts (1929) describes the walls of the sinus as made up of foot muscular sheets and bands, "These muscles grow out as a valve which must press against the large shell muscle, when the latter is in a state of tonus, so that blood is prevented from emptying from the foot." This valve may prevent flow into the left kidney when the foot muscle is strongly contracted and may act as a "choke point" (waterfall) when the foot muscle is moderately contracted as in creeping. During this moderate activity the valve would release blood, but the flow would not be dependent on downstream kidney or baso-bronchial sinus pressure and would be controlled by the increased pedal pressure created by the creeping movements.

In summary, it can reasonably be stated that during certain activities the peripheral flow may be regulated in part by muscular movements. Therefore, the peripheral circulation can be separated into two distinct different pathways: (1) the visceral pathways; and (2) the cephalo-pedal pathways. The visceral pathway is probably regulated by the pressure gradient between the aorta and the baso-branchial sinus and is a pure viscous resistance. The pedal circulation carries a large share of the blood from the heart and is a much more complicated circuit. Under certain conditions this pathway may be represented as a group of lumped resistances (some viscous, some waterfall type) in series, with movements of

the foot actually acting as a pressure source (approximating an auxiliary heart) for movement of blood from the foot into the right urocoel independent of the pressure in that sinus. The blood is then moved across the high resistance ctenidia by a pressure gradient created in part by auricular suction.

Much additional investigation would be required to accept or reject the circulatory scheme proposed here. Specifically, more pressure and flow measurements need to be taken on living animals to determine the resistance characteristics between various points in the circulation especially in the pedal circulation during various activities. Just such a study is described in Part III.

PART III. THE EFFECT OF CHANGES IN LIGHT AND TEMPERATURE ON THE OPEN CIRCULATORY SYSTEM OF THE PINK ABALONE

REVIEW OF LITERATURE

After the input-output characteristics of the heart and peripheral vascular system are reasonably well-analyzed, the next logical step is to investigate how they interact in the intact animal. This problem requires the utilization of instrumentation that minimizes the disturbance to the animal. Therefore, after complete instrumentation the animal can be perturbed to upset the circulatory balance and then the adaptive changes that follow can be observed and analyzed. These results will help to describe the three critical elements of the circulation in invertebrates as defined by Hoar (1975): (1) cardiac output regulation; (2) peripheral resistance changes; and (3) blood movements caused by body activity.

Chronic Circulatory Measurements in Intact Invertebrates

Crustacean circulatory studies have contributed a great deal to the understanding of the mechanisms of open circulatory systems. However, only recently have investigators attempted to measured both flow and pressures in the intact animal. Redmond (1955) studying the oxygen carrying properties of hemocyanin used the Fick principle to estimate the minute volume and stroke volume of Panulirus. Belman (1975) in a very complete study of the high pressure crustacean circulation measured both circulatory pressures and velocity in the several parts of the circulation of Panulirus interruptus. He claimed that "the ventricular pulse is sufficient by itself to circulate blood from arteries, through the tissues, sinuses and gills," although he did state that there could be an accessory heart to overcome high cerebral ganglion capillary network resistance.

Belman (1976) recorded the blood pressure in relatively nondisturbed specimens of nine species of Crustacea and found very high values. He pointed out that these high pressures contrasted with the view of Prosser (1975) that the open circulation has low pressure and is sluggish.

Several species of molluscs have had circulatory parameters monitored in the intact animal. Most of the studies have involved measurements of pressures only and were discussed in Part II (Trueman, 1966; Brand, 1972; Dale, 1973; Sommerville, 1973c). Sommerville (1973a) used cinematographic recordings to observe the effects of mantle cavity movements on the heart of Helix. She claimed that the total volume of the heart and pericardial cavity could be seen to change from diastole to systole. In a related paper Sommerville (1973c) measured pressure and approximate blood velocity in the intact Helix. She used both a manometer setup and strain gauge transducers to record pressures. She also used differential pressure measurements between pulmonary vein and pericardial cavity to estimate venous return. There were considerable problems with this technique both in theory and in practice and no satisfactory calibration was completed after data were taken.

Bourne and Redmond (1977a,b) recorded aortic blood flow and blood pressure in several parts of Haliotis corrugata. They recorded pressure in the ventricle, aorta, left epipodial artery, afferent ctenidial and efferent ctenidial veins, and pericardial chambers in the unrestrained animal. Also, blood flow was measured by a cannula loop in the aorta connected to an electromagnetic flowmeter and by doppler flowmetry. There was considerable surgery involved in the aortic loop and therefore, they claimed that their results for stroke volume and heart rate were sub-

normal. The noninvasive doppler ultrasonic flowmeter was used to record acute aortic velocity, cephalic arterial sinus and pedal artery flow. Unfortunately, there was some restraint during the doppler measurements.

Circulatory Stress Techniques

Bourne and Redmond (1977a) reported a dramatic change in blood pressure caused by turning off the room lights. They found the response to the "off" stimulus to be more consistent than the "on" response and no movement of the abalone was observed during this maneuver. It is possible that this response is a defense response simulating a predator approaching from above and blocking the light.

The circulatory response to temperature changes has formed a large body of literature in vertebrates but most invertebrate data have been limited to the heart rate changes with temperature. Segal (1956) measured the effects of increasing the environmental temperature on heart rate of Acmaea and found that colder acclimated animals were less temperature dependent than warmer acclimated animals. Trueman and Lowe (1971) warmed the bivalve Isogromun from 27-35°C and reported a Q_{10} for heart rate of 2.0 for this range. Widdows (1973) reported a Q_{10} of 2.71 between 15-20°C for Mytilus edulis adapted to 15°C. Although the above authors did not report on any circulatory variables other than heart rate it is clear that temperature changes are an environmental stress which require adaptation in the delivery of the blood by the cardiovascular system.

Circulatory Models

Complete models of molluscan circulation describing the sources of driving pressure and resistance characteristics are extremely scarce. Jullien and Ripplinger (1953) presented a hydraulic model of the circulatory system of Helix. Their model involved the major vessels from the heart and a sinus in parallel with the anterior aorta. From this sinus the blood enters the perivisceral sinus which is modelled as a larger reservoir with a piston structure where the pressure can be raised to force blood back through the gills into the heart again.

Bourne (1974) performed anatomical studies and pressure flow measurements of Haliotis corrugata and combined these with the anatomy results of Palmer (1907) and Crofts (1929) to create an electrical analog model of Haliotis circulation. His model consisted of a pair of parallel resistors representing the cephalic and visceral elements as one branch and the foot and epipodium as another branch. The ctenidial resistance was in series with the parallel pair. The heart is represented by a voltage in series with the resistance and the pedal generated pressure is modelled as a voltage across the foot resistance and is separated from the circuit by valves to hold pressure in the foot.

This study details pressures and flow measured in the unrestrained Haliotis. The response to light intensity and temperature changes are reported and discussed. Finally, a hydraulic model of the circulation is proposed.

MATERIALS AND METHODS

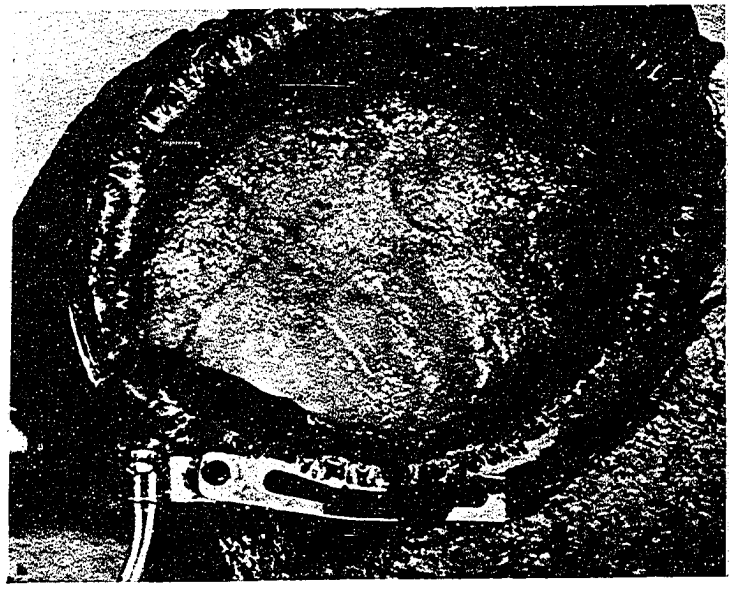
Light and Temperature Effects

A portion of the shell covering the heart and posterior ctenidia was removed and a hole for attachment of the doppler arm was drilled in the shell on the anterior ventral margin of the opening. The animals were allowed to recover overnight. They were then placed in carbon dioxide saturated seawater for a very short time to relax the foot muscle to permit the easy attachment of instrumentation. The doppler probe was attached to the shell and positioned near the base of the heart as shown in Figure 19a. A pressure catheter was then threaded through an anterior shell perforation and placed near the doppler probe arm. The folds of the pallium were reflected dorsally and a length of aorta exposed. The needle of the pressure tap was then pushed into the aorta and the pallium allowed to fall on top of the tubing, acting to obstruct the removal of the needle.

After the aortic pressure tap was flushed and normal readings (typical aortic shape with incisura) observed the doppler probe was pushed under the heart so that the crystals were positioned at the aortic bulb tissue (Figure 19b). No significant reverse flow was observed, consistent with the assumption of Bourne and Redmond (1977b). Therefore, the doppler probe was adjusted to act as a nondirectional flowmeter to reduce noise. If large, consistent flow traces were observed on the Dynograph, the doppler probe was screwed tightly to the shell to prevent large movement. If a satisfactory record was not obtained the probe was moved slightly in relation to the heart until a good record was obtained. The animal was

Figure 19a. Doppler probe attached to shell and positioned near the heart of the inverted Haliotis corrugata.

Figure 19b. Doppler probe placed over aortic bulb.



then placed on a plastic plate and the other pressure catheter was slipped into the efferent ctenidial vein so that the tip was just in the right auricle.

Therefore, the chronic instrumentation involved recording the simultaneous aortic and right auricular pressures and the changes in instantaneous aortic velocity measured by the doppler ultrasound flowmeter. These variables were recorded continuously while the environment was changed.

The light response, as described by Bourne and Redmond (1977a), was easily elicited by turning off the room lights for 10 minutes and noting circulatory changes. An "on" response also was recorded, but was less dramatic. Changes in environmental temperature were created by circulating hot tap water through a silicone-coated coiled copper tubing placed in the seawater tank. The environmental temperature was recorded by a Yellow Springs Instrument Company Tele-thermometer.

Recording of Sinus Pressures

On two animals the cephalic arterial sinus pressure was recorded in the nontraumatized animal. The animal, prepared as described above, was allowed to attach itself to a plexiglass plate with a V-shaped cut-out over the anterior end. Then, needles were inserted through the ventral surface of the foot along the centerline. As the needles were pressed into the foot, they reached into the lumen of the desired blood sinus and blood was observed flowing from the needle. The needles were then

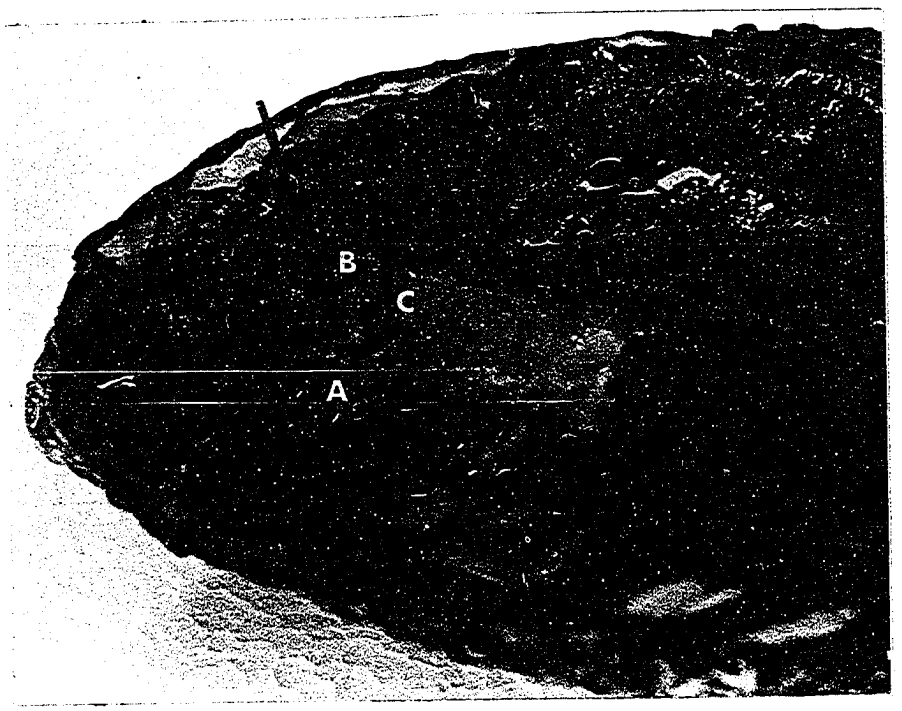
connected to pressure cannulae to record the sinus pressure. Records were made on the undisturbed animals and also the light and temperature responses were measured. In addition, one animal with shell intact was instrumented for simultaneous measurement of cephalic arterial sinus and cephalo-pedal venous sinus pressures and the response to the "light off" stimulus was measured. At the end of each experiment the placement of the needles in the sinuses was verified as shown in Figure 20.

Figure 20. Pressure catheter needles in position in cephalic sinuses (anterior ventral view).

A. Cephalic arterial sinus.

B. Needle track into cephalo-pedal venous sinus.

C. Needle into a pedal venous sinus (no data taken from this position due to large movement artifacts).


B
C
A

RESULTS

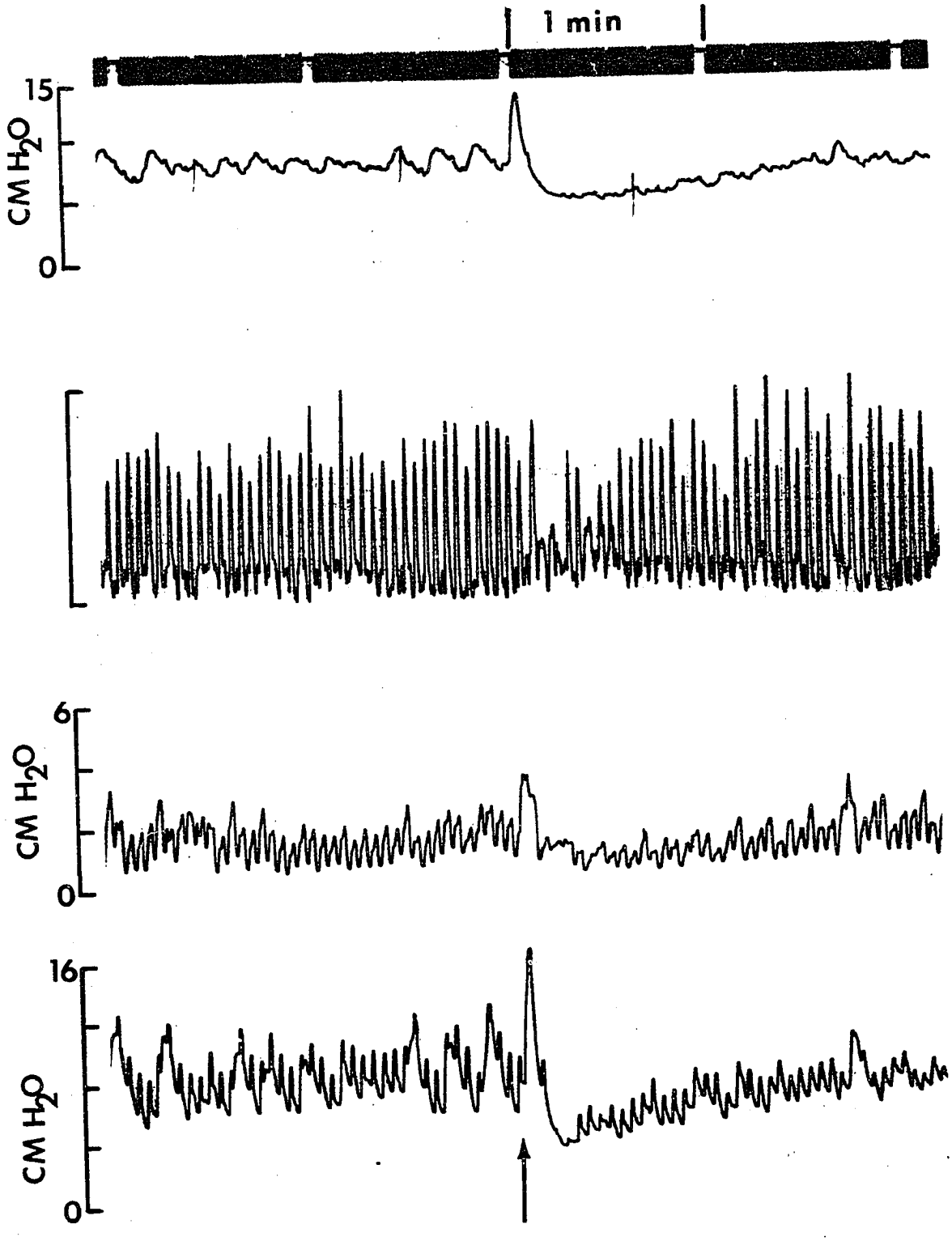
In an attempt to measure the circulatory responses to light and temperature changes, blood pressure was measured in the anterior aorta, right auricle, cephalic arterial sinus and cephalo-pedal venous sinus. Blood flow also was measured in the aortic bulb. Several limitations of the measurement and perturbation techniques must be stated before the results are presented and discussed. Unfortunately, there is no quantification of the change in light intensity since changes in the orientation of the animal in the tank would alter the relative light on the animal. Also, it was extremely difficult to control the rate of temperature change in the heat stress portion of the experiments. Therefore, some animals were stressed more than others when the temperature rose rapidly (Somero and Hochachka, 1977). Also, the doppler probe is very sensitive to position relative to the axis of flow. Therefore, comparisons of velocity output can only be made if the position of the probe relative to the aortic bulb is maintained constant and records are made in a short span of time.

Light Response

Sample recordings of pressures and heart output response to turning out the room lights are shown in Figure 21. Some of the interesting points that should be noticed from this figure are:

(1) The aortic pressure can rise without an increase in auricular pressure of the same magnitude.

Figure 21. The upper trace is a recording of the cephalic arterial sinus pressure response to reduced light in Haliotis corrugata #43. The trace directly below is the Doppler flowmeter output from the aortic bulb. The lower middle trace is a record of auricular pressure and the lowest trace is aortic pressure. ↑, room lights off.


1 min
15
CM H2O
0
6
CM H2O
0
16
CM H2O
0

(2) The aortic and cephalic arterial sinus pressures rise shortly after the light is turned off, but the heart output is slightly reduced with frequency also reduced.

(3) The aortic diastolic pressure falls to levels less than those recorded before the light was turned off.

A more quantitative analysis of the response of each individual animal to the reduction in room light will follow.

Figure 22 shows the results for animal 40. Unfortunately, the Doppler probe was not functioning properly for this experiment so no data on stroke velocity changes are shown. Notice that for this animal the aortic and auricular pressure peaks are nearly the same and that the heart stops beating for a period of 14 seconds following light reduction. Also, the pressures and heart rate recover in slightly over a minute. Animal 41 (Figure 23) responded quite differently to the light change. The auricular pressure remained fairly constant throughout the experiment but the anterior aortic pressure rose dramatically but then fell quickly. The heart rate responded to the stimulus by slowing briefly then finding a rate less than the pre-stimulus level. The stroke output (approximated by changes in aortic bulb velocity measured by the doppler) reduced after the stimulus and found a new lower level.

A third pressure catheter was added to animals 42 and 43 to record the pressures in the cephalic arterial sinus. Figure 24 details the light response of animal 42. Animal 42 responded to the light reduction in a manner very similar to animal 41, but more information was obtained. After approximately 30 seconds the pressures returned to pre-stimulus levels. The heart output and frequency reduced sharply immediately post-stimulus and stabilized at lower levels.

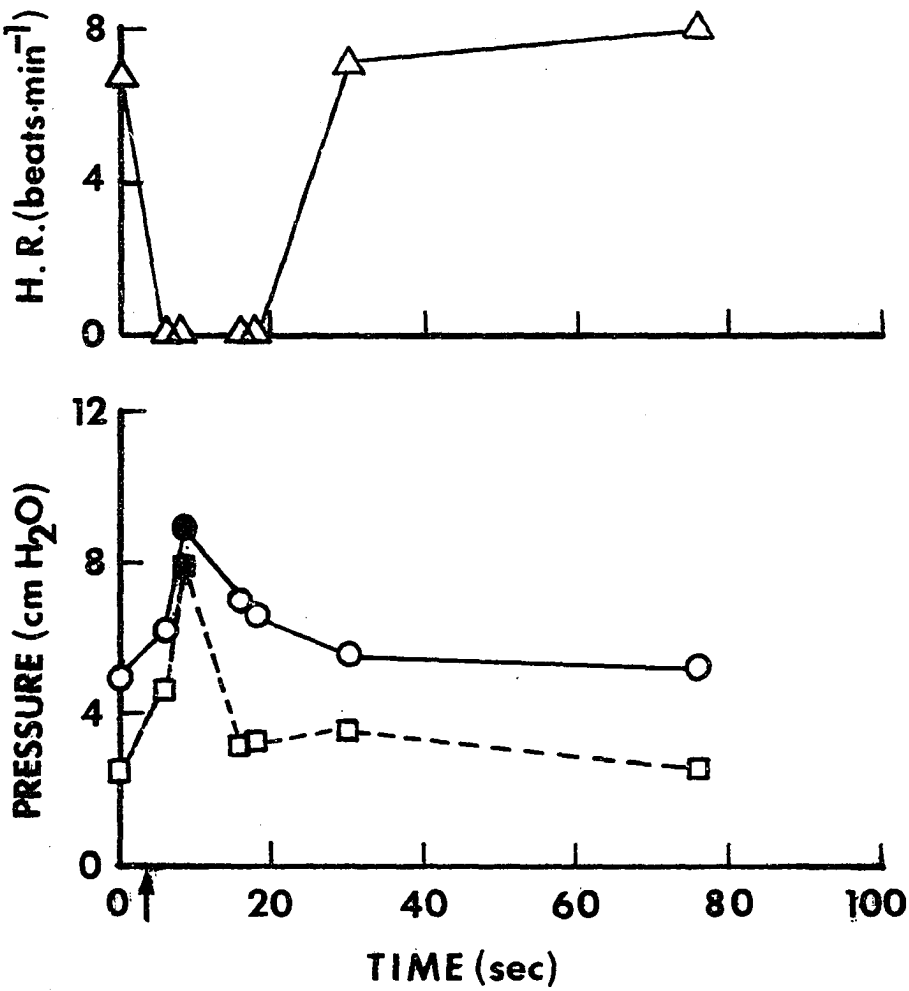


Figure 22. The response of circulatory pressures and heart rate to abrupt light reduction in Haliotis corrugata #40. ↑, room lights off; O——O, aortic pressure; □----□, right auricular pressure; Δ——Δ, heart rate; ●■, estimated records (record off scale).

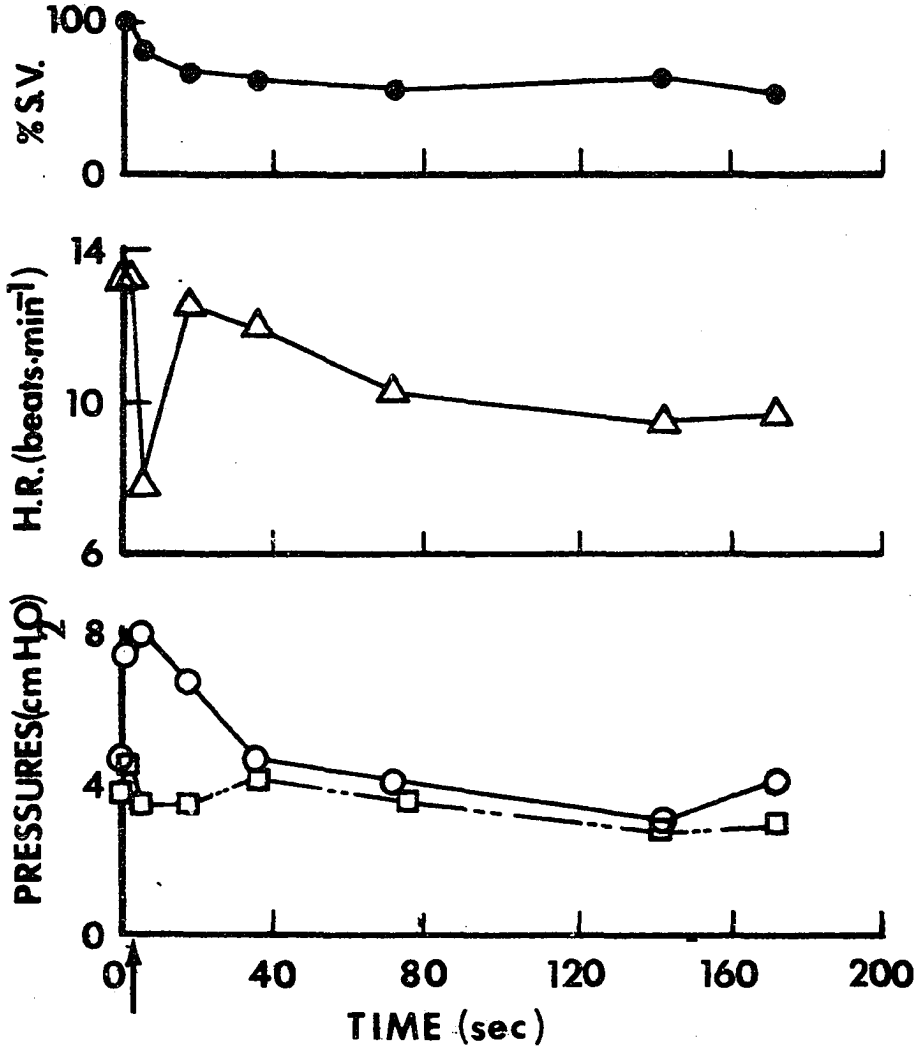


Figure 23. The response of circulatory pressures, heart rate, and aortic stroke velocity to abrupt light reduction in Haliotis corrugata #41. ↑, room lights off; O——O, aortic pressure; □----□, right auricular pressure; Δ——Δ, heart rate; ●——●, percent of pre-stimulus aortic stroke velocity.

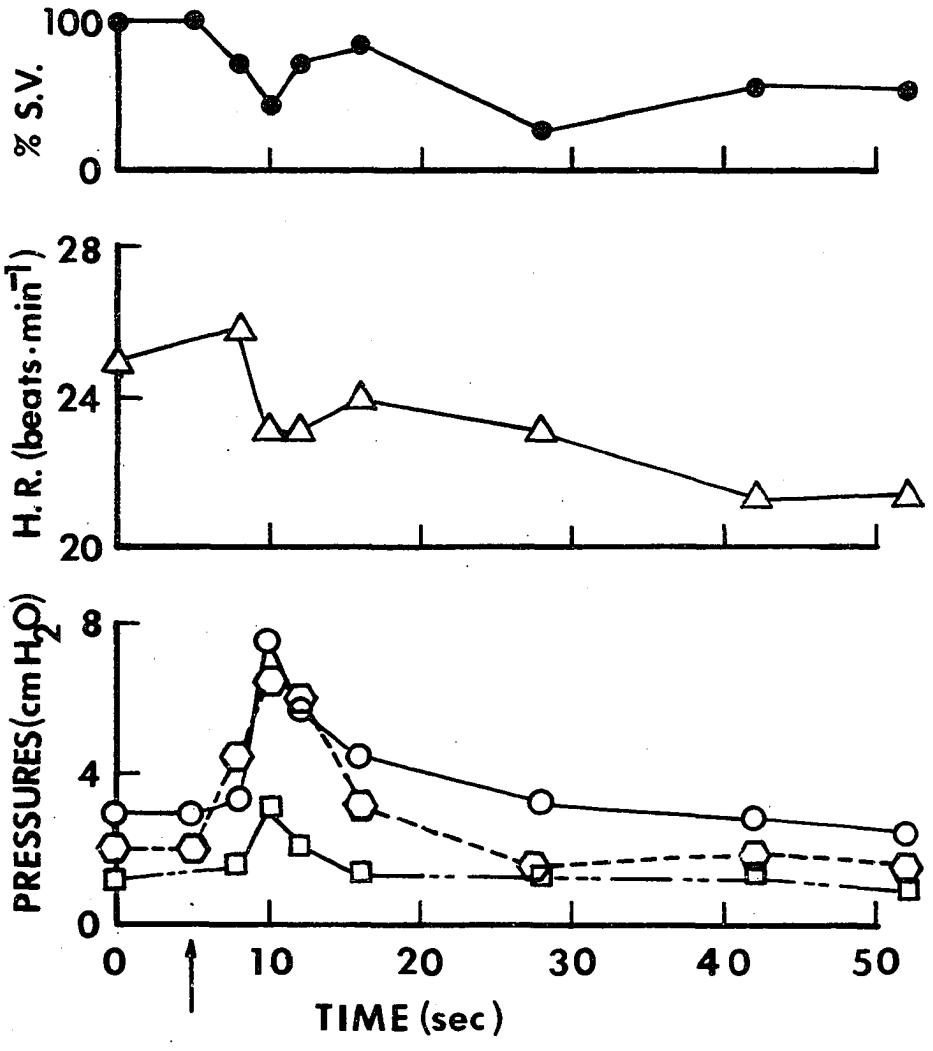


Figure 24. The response of circulatory pressures, heart rate, and aortic stroke velocity to abrupt light reduction in Haliotis corrugata #42. ↑, room lights off; O—O, aortic pressure; □---□, right auricular pressure, ⬡---⬡, cephalic arterial sinus pressures; Δ—Δ, heart rate; ●—●, percent of pre-stimulus aortic stroke velocity.

The results from animal 43 are summarized in Figure 25. This animal behaved somewhat differently from the preceding animals. The resting gradient between aortic to auricular pressure was large due to a high aortic diastolic pressure indicating this animal may have been in an excited state prior to light stimulus. The pressures responded in the now familiar pattern, but this time cephalic arterial sinus pressure did not exceed aortic pressure in the rising portion following the stimulus and there was very little gradient between the sinus and aortic pressures after 10 seconds into the experiment. Following the light change, the heart output and frequency dropped to zero even though blood pressures rose and the gradient from the aorta to the auricle increased greatly. At the 50 second mark the heart had returned to near normal levels of function.

Animal 44 had pressure taps inserted to measure cephalic arterial and cephalo-pedal venous sinus pressures. Thus, the difference between these pressures is the pressure gradient across the foot. The record of the responses of these sinuses to the light-off stimulus is shown in Figure 26.

Response to Increasing Temperature

The inducement of circulatory alterations by an environmental temperature stress was less predictable than for the light-off response. Very few trends could be seen from animal to animal. However, one definite trend can be reported: The pink abalone is not able to adapt to abrupt temperature changes greater than approximately 5°C. On all graphs

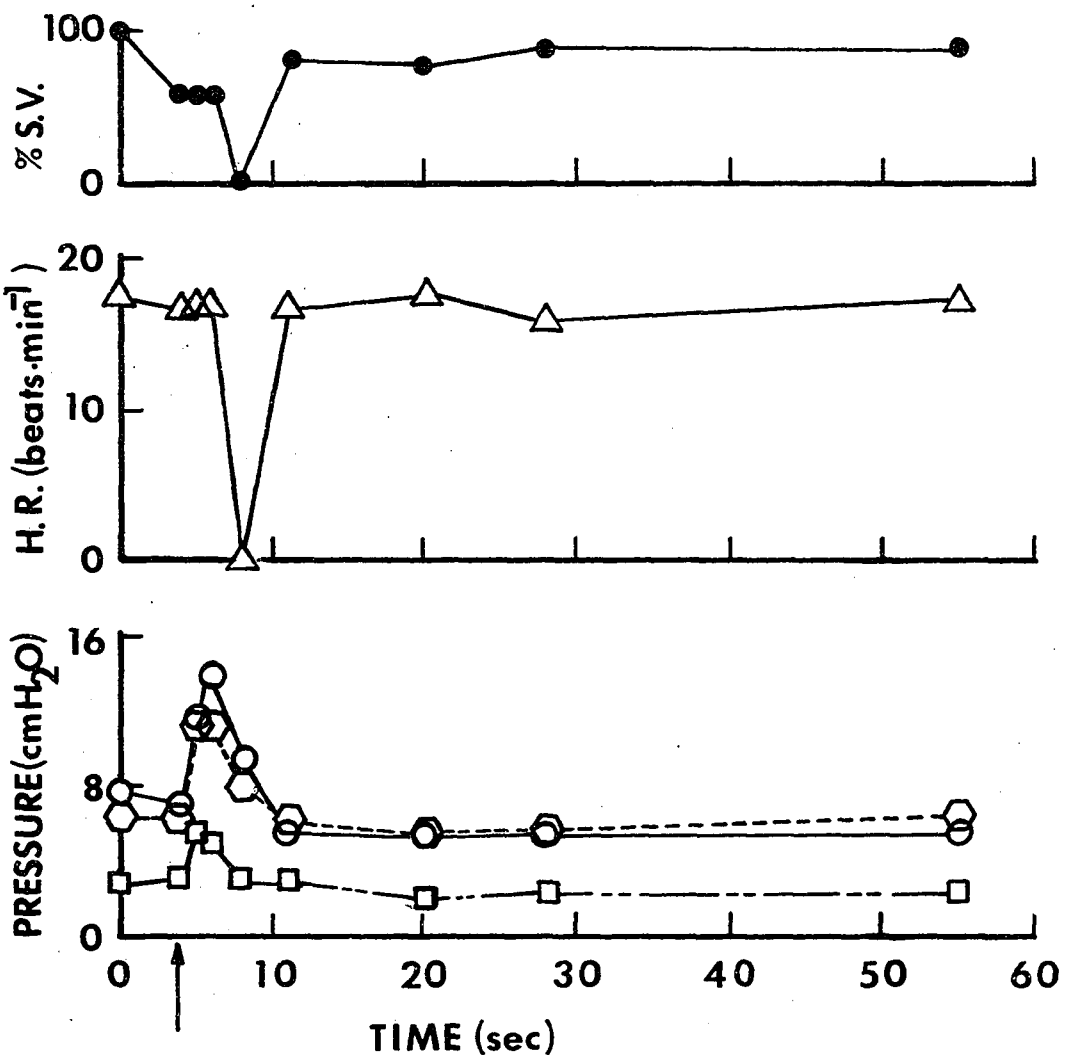


Figure 25. The response of circulatory pressures, heart rate, and aortic stroke velocity to abrupt light reduction in Haliotis corrugata #43. ↑ , room lights off; O—O, aortic pressure; □—□, right auricular pressure; ⬡----⬡, cephalic arterial sinus pressure; Δ—Δ, heart rate; ●—●, percent of pre-stimulus aortic stroke velocity.

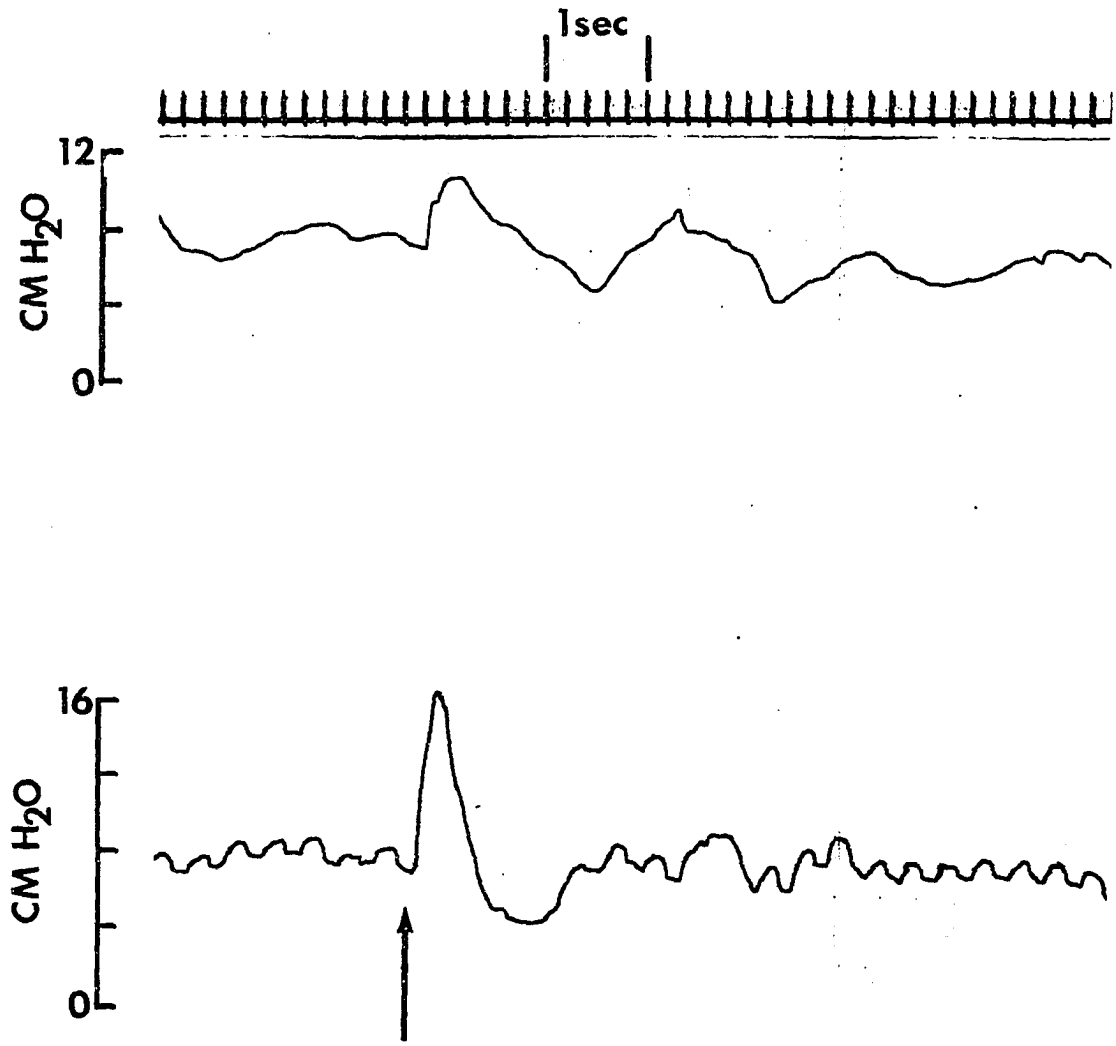


Figure 26. The upper trace is a record of cephalo-pedal venous sinus of Haliotis corrugata #44. The lower trace is cephalic arterial sinus pressure. ↑, room lights off.

the last points plotted represented the time beyond which the animal would not recover.

Figure 27 summarizes the results from animal 40. The water began to heat quickly at 600 seconds into the experiment and most of the changes occurred after that point. Both aortic and auricular pressures fell as the heart output remained reasonably steady. When the temperature reached 20°C the heart rate and aortic pressures climbed sharply. The animal was observed to move very little during the experiment. Animal 41 was quite different from animal 40 as can be seen in Figure 28. The temperature rose very quickly and the animal reacted violently to this change. The animal was observed to move away from the heating coil. The heart ceased functioning several times during the experiment; however, blood pressure was maintained.

Animal 42 is represented in Figure 29. The temperature was gradually increased to 20°C over a period of 40 minutes. The overall effect of the temperature changes was an erratic heart output and a reasonably stable group of body pressures. At 400 seconds for a short period, the auricular pressure had risen to a pressure greater than the aortic pressure creating a nonnormal pressure gradient across the inactive heart. Also, notice that as the heart resumed beating at 600, 1400, and 2400 seconds the aortic diastolic pressure remained constant or decreased slightly. Another possibly significant point to mention was that the auricular pressure was greater than the cephalic arterial sinus pressure for much of the temperature increase.

Figure 30 summarizes the changes in animal 43 as the water temperature is quickly raised to 18°C and then slowly increased to 20°C. As can be seen from this figure the temperature had very little effect on the

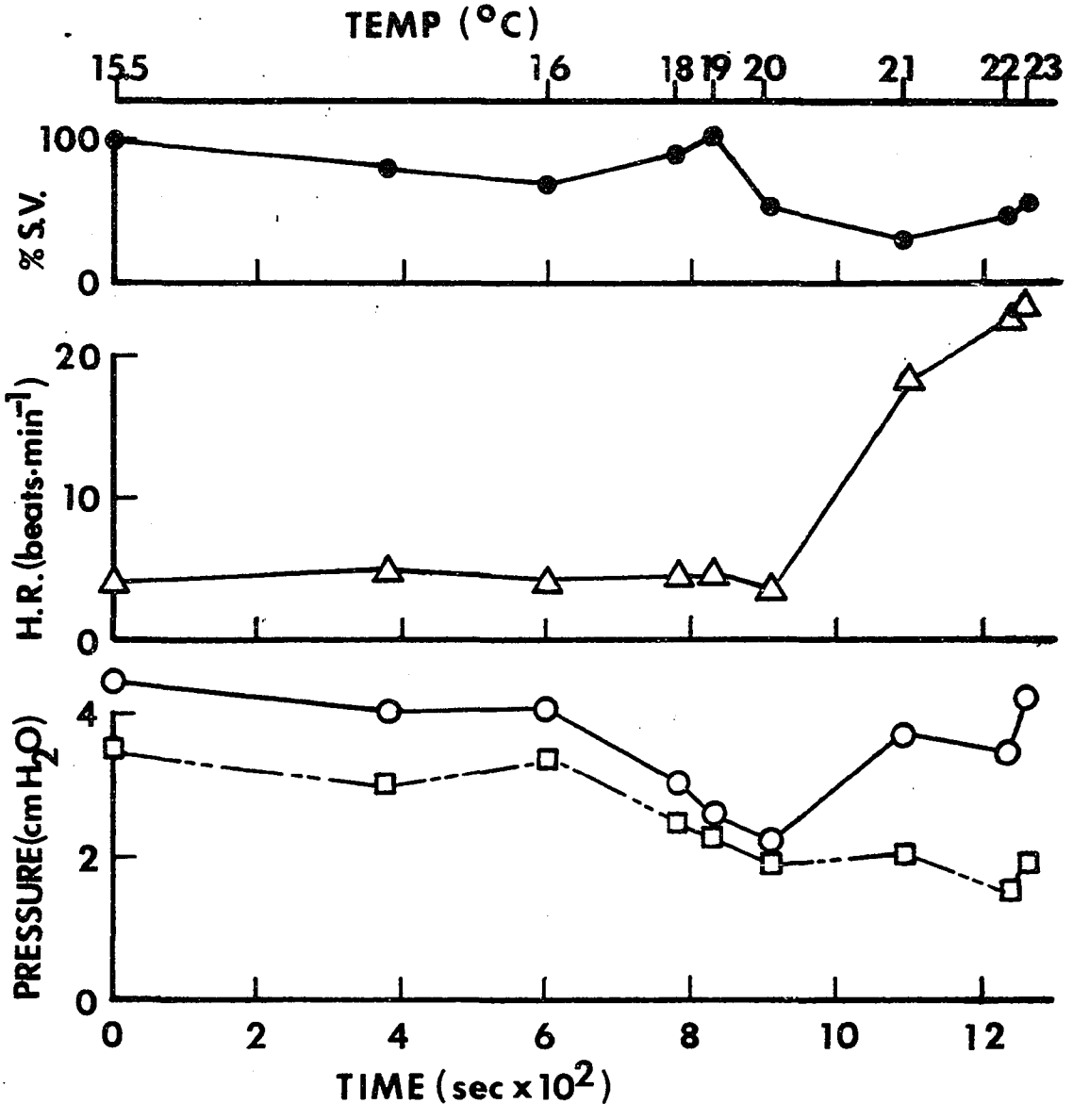


Figure 27. The response of circulatory pressures, heart rate, and aortic stroke velocity to increased environmental temperature in Haliotis corrugata #40. O—O, aortic pressure; □----□, right auricular pressure; Δ—Δ, heart rate; ●—● percent of pre-stimulus aortic stroke velocity.

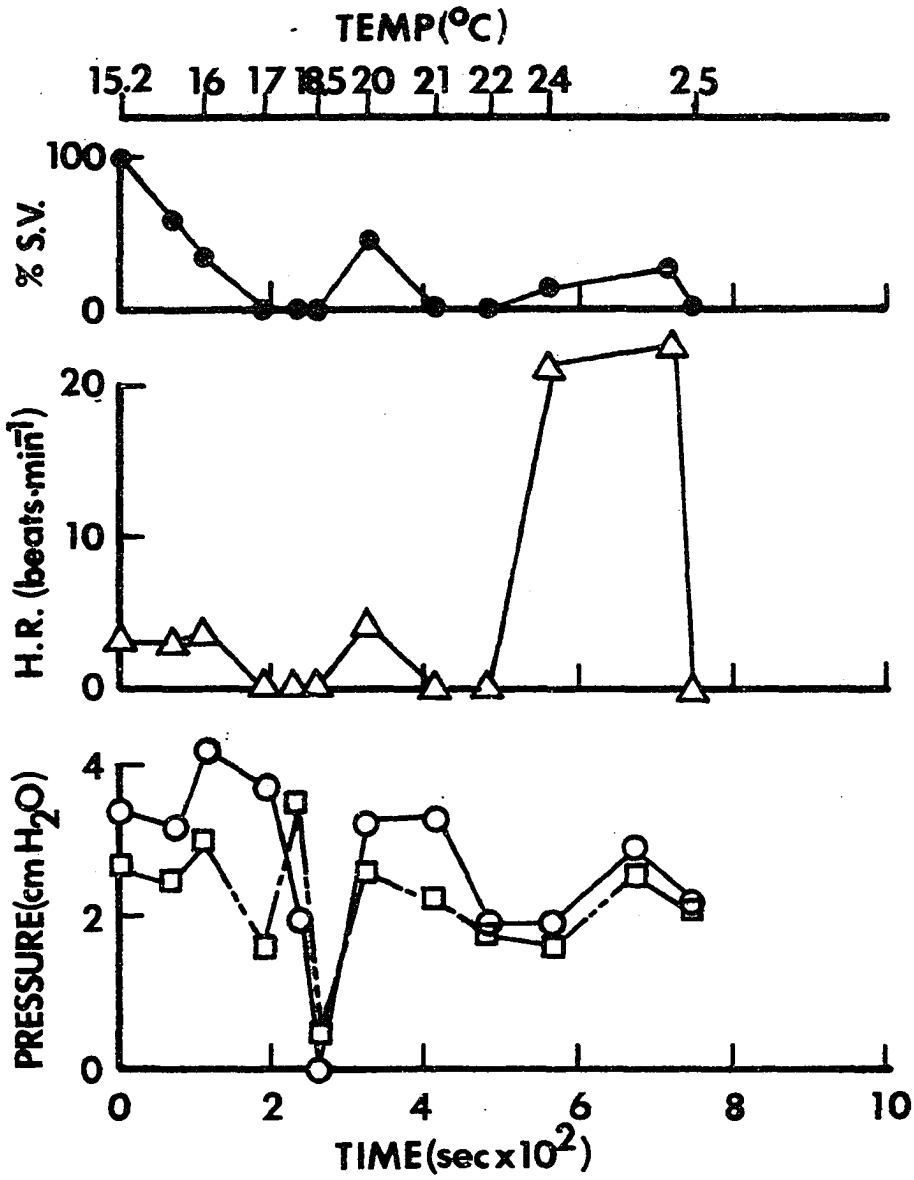


Figure 28. The response of circulatory pressures, heart rate, and aortic stroke velocity to increased environmental temperature in Haliotis corrugata #41. O—O, aortic pressure; □—·—□, right auricular pressure; Δ—Δ, heart rate; ●—● percent of pre-stimulus aortic stroke velocity.

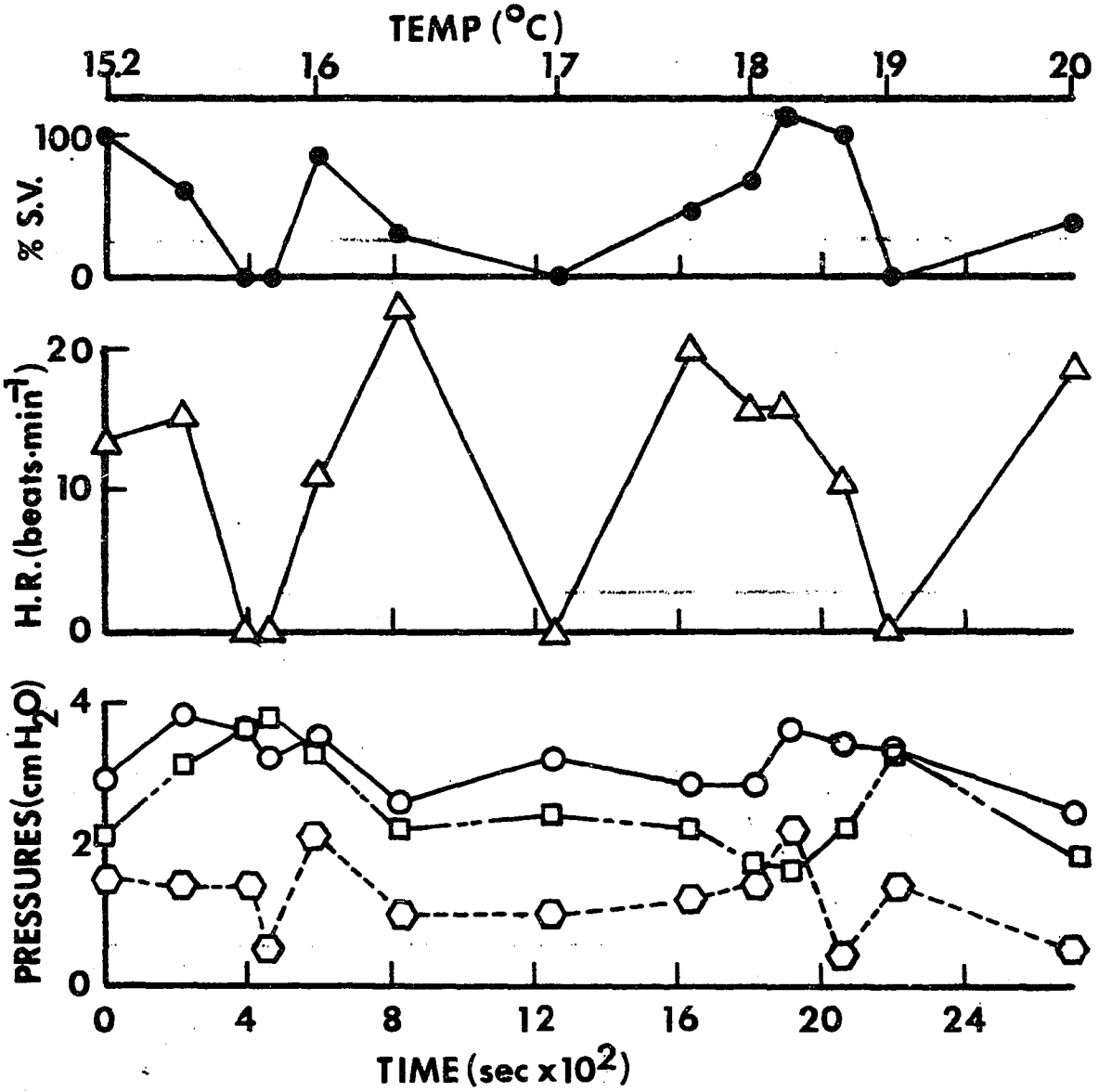


Figure 29. The response of circulatory pressures, heart rate, and aortic stroke velocity to increased environmental temperature in Haliotis corrugata #42. O—O, aortic pressure; □----□, right auricular pressure; ⬡----⬡, cephalic arterial sinus pressure; Δ—Δ, heart rate; ●—●, percent of pre-stimulus aortic stroke velocity.

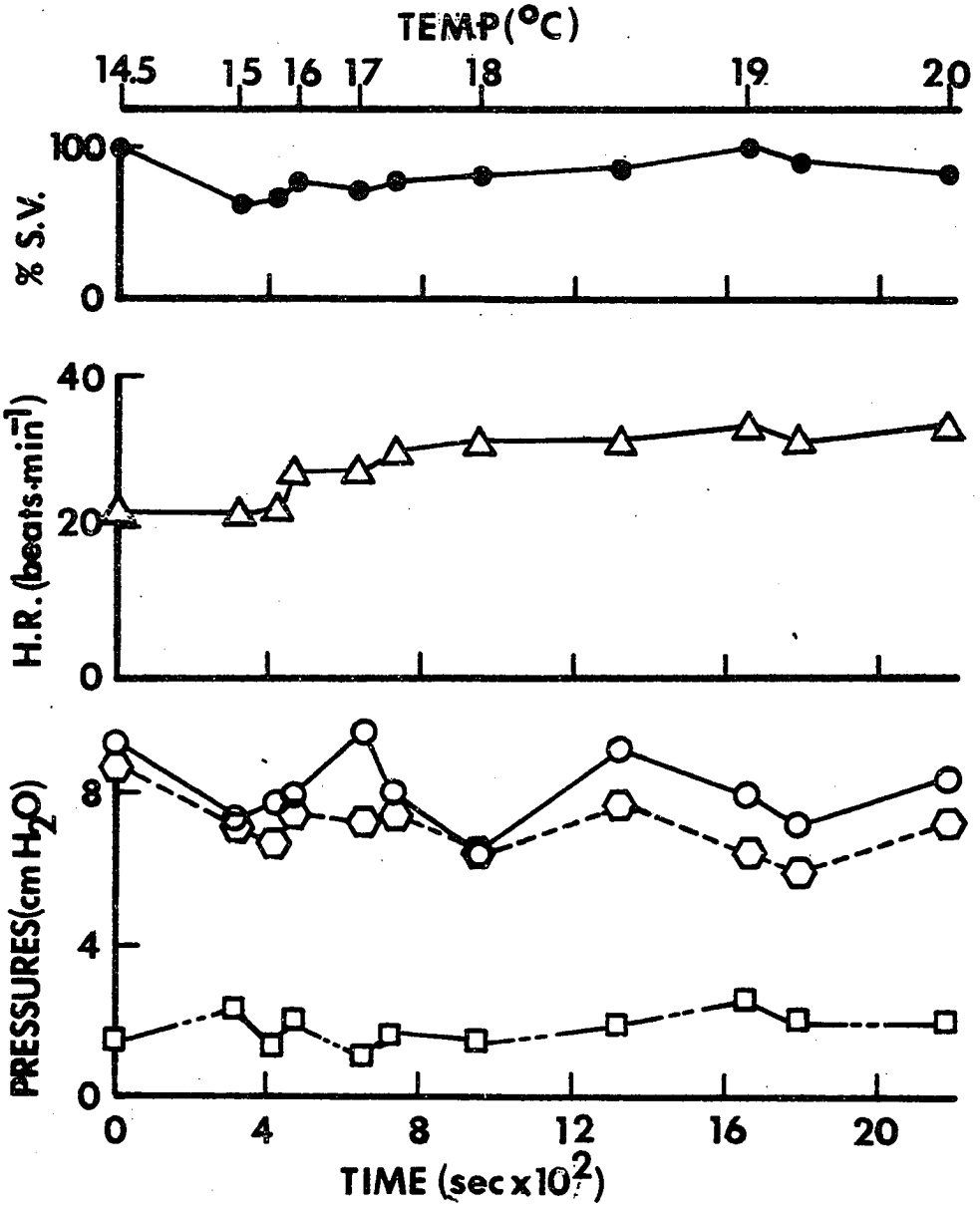


Figure 30. The response of circulatory pressures, heart rate, and aortic stroke velocity to increased environmental temperature in Haliotis corrugata #43. 0——0, aortic pressure; □——□, right auricular pressure; ⬡----⬡, cephalic arterial sinus pressure; Δ——Δ, heart rate; ●——●, percent of pre-stimulus aortic stroke velocity.

circulatory system other than the increase in heart rate. As was noted earlier, animal 43 had a very high resting aortic pressure (and large aortic to auricular pressure gradient) and this condition was maintained when the temperature was changed.

Low Frequency Pressure Oscillations

A phenomenon first reported by Bourne (1974) was observed in most of these animals. There were periodic pressure fluctuations of low frequency which were transmitted to all recorded pressures. These pressure fluctuations were most obvious in the cephalic arterial sinus and aorta, less predictable in the cephalo-pedal venous, and rarely seen in the right auricle and efferent ctenidial vein. An example of this fluctuation can be seen in Figure 21 before the light reaction.

DISCUSSION

The measured response of the abalone to stress conditions was only partially revealing of regulatory mechanisms. The biggest problem involved in measurement of pressures in nonrestrained animals is the uncertainty of the significance of measured pressure gradients if no local flow measurements are made between the points to confirm flow in the circuit. Therefore, once again the results asked more questions than were answered. However, several points of interest from the data should be discussed since some interesting trends have been discovered.

The light-off stimulus proved to be a very useful perturbation to measure the dynamics of circulatory adjustments. Among the most surprising recordings were the ones made following the light stimulus on a few of the animals where the pressure pulse in the aorta was greater than in the auricle, thus increasing the pressure gradient across the periphery (Figures 21, 23, 24, 25). This was not recorded by Bourne and Redmond (1977a) when the animal "clamped down" and indicates that there may be a selective muscular contraction that can create such a pressure gradient. Another point to mention is that the circulatory reaction to light-off is very fast, counteracting the statement that open systems are sluggish. Also, the heart output acts relatively independent of auricular pressure confirming the results of Part I and suggesting that the heart rate is controlled by quick changes in nervous or humoral factors. Unfortunately, the mechanism which varies stroke volume in these experiments is still a mystery with the hypothesis that was put forward in Part I neither confirmed nor refuted by the data.

Figures 24 and 25 show the cephalic arterial sinus pressure equalling aortic pressure several times during the maneuver. This could indicate a reduction or even a stoppage of flow in the aorta which might shunt flow to the visceral arteries; however, without an actual flow measurement this is only speculation. The pressure gradient across the foot during the light-off stimulus (as measured in the cephalic arterial sinus and cephalo-pedal venous sinus (Figure 26)) suggests flow through the foot into the right kidney and baso-branchial sinus from the venous sinus, indicating that blood is not always trapped in the foot during activity. However, once again, this was not confirmed by flow measurements.

The temperature reaction indicated that the abalone may be able to regulate to temperature for a short time before the heart rate begins to climb. It is significant that in the animal that was stressed by very quickly raising the temperature and that was able to move away from the heating coil, the heartbeat either stopped or was very erratic but blood perfusion pressures were maintained reasonably well (Figure 28). Also, once again it can be seen that the aortic diastolic pressure is as dependent on peripheral factors as on heart output. Several times the aortic pressure was seen to remain constant or even gradually rise while the heart was stopped (Figures 29 and 30).

As mentioned in the results section there were low frequency pressure pulses seen in most of the abalone. The significance of this periodic pulse is unknown although it is seen mostly in the arteries. It is possible that some muscle group (perhaps a radular muscle) may selectively compress the cephalic arterial sinus. However, the overall effect of this phenomenon is a mystery.

Model of the Circulation

Using the electrical analog of Bourne (1974), the input-output relationships of the heart and periphery (Part I and II), and the pressure-flow data from Part III; a hydraulic model has been developed which incorporates many of the characteristics that have been described. The main features of the model as shown in Figure 31 are:

(1) The heart with semi-rigid pericardium has regulatory mechanisms which are humoral, nervous, and possibly hydraulic.

(2) The capillary bed of the posterior and anterior viscera is supplied by the visceral artery branches from the aorta and branches from the cephalic arterial sinus which supplies the head region. Both of these circuits drain into the reservoir of the right urocoel (7).

(3) The cephalic arterial sinus which is surrounded by fluid and muscles and supplies the anterior viscera (2) and also supplies the foot through pedal and epipodial arteries.

(4) A one-way valve at the exit of the cephalic arterial sinus into the foot to prevent reverse flow from the foot into the sinus.

(5) A reservoir with a piston representing the foot haemocoel. Compression of the foot muscle will cause the pressure to rise in the foot.

(6) A collapsible tube apparatus which models the cephalo-pedal venous sinus and which may act as a vascular waterfall, with the foot muscle relaxed (piston in up position) the tube is opened and blood flows to the right urocoel governed by the pressure gradient in the two reservoirs. As the muscle compresses the tube it is collapsed and the pressure gradient now becomes the difference between foot and solid tissue pressure. When the foot clamps down hard the flow is cut off completely.

(7) Another piston controlled reservoir models the right urocoel and baso-branchial sinus which may be compressed by a different set of muscles than described in (6). In general, these blood reservoirs have low pressures and are assumed to have a large capacitance. If the animal is strongly clamped down, the reservoir would be compressed and pressures would rise in the auricle as seen in the aorta and arterial sinuses (Bourne and Redmond, 1977a and Figure 22).

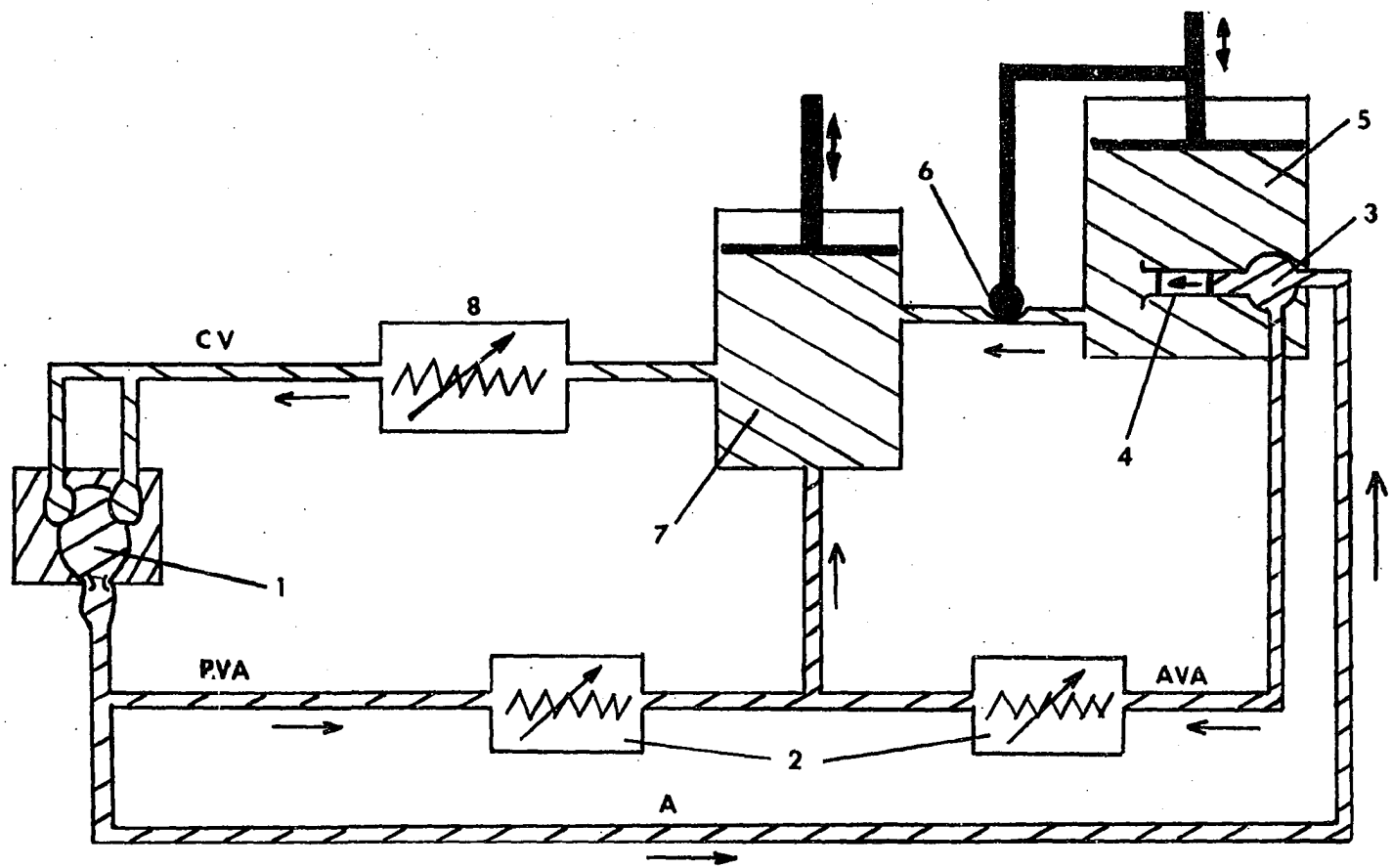


Figure 31. Hydraulic model of the circulatory system of Haliotis corrugata. PVA. posterior visceral arteries; A, anterior aorta; AVA, anterior visceral arteries; CV, efferent ctenidial vessels. (For an explanation of the numbered elements see text.)

(8) This large highly variable resistance represents the ctenidia which have contractile vessels.

Obviously, this model has some omissions and simplifies the physiology, but it may serve as a starting point for the complete elucidation of the important control mechanisms in the open circulatory system of Haliotis. It is now evident that the heart may be a main source of hydraulic motive energy at rest, but that during activity the body movement may contribute to the circulation of blood through the foot and the maintenance of perfusion pressure for the vital organs.

Further work is required to determine actual blood distribution patterns and relative flow rates in the peripheral circulatory system. Also, the exact mechanism of heart stroke volume regulation still needs to be determined.

GENERAL SUMMARY

1. The heart of the pink abalone, Haliotis corrugata, was cannulated in situ with the output pressure held constant and the input pressure to the auricle varied.
2. The heart required reperfusion of fluid in a "closed circuit" arrangement which recycled an apparent metabolite needed to maintain a steady (although lower than normal) heartbeat.
3. It was found that the stroke volume of the abalone heart remained relatively constant when changes in heart input pressure were applied. The stroke volume was greater than 0.3 ml.
4. The heart rate of the pink abalone in a "closed circuit" intact organ preparation is only slightly sensitive to changes in auricular pressure.
5. The product of stroke volume and heart rate (cardiac minute volume) was found to be nearly independent of changes in venous return pressures.
6. A hypothesis was formulated where the relatively constant volume of the pericardium limits the heart volume changes; the flow in the reno-pericardial canal may control the volume of the pericardium and thus indirectly regulates ventricular volume and cardiac output.
7. The resistance characteristics of the peripheral circulation of the pink abalone was studied by by-passing the heart, supplying blood to the cannulated aorta at a constant pressure, and measuring the flow to the cannulated efferent ctenidial veins when the venous return pressure was altered.

8. The mean total peripheral resistance level of 5 animals was 5.06×10^4 dynes•sec•cm^{-5} when perfused with instant ocean.
9. Several animals were compressed with weights placed on their shell to increase total tissue pressure in the foot muscle.
10. Two of the compressed animals exhibited nonlinear resistance curves suggesting the presence of a vascular waterfall in the peripheral system of the pink abalone.
11. It was pointed out that measurement of total peripheral resistance in molluscs by using the cardiac output and pressure gradients between aorta and auricles may be in error. The presence of a vascular waterfall or auxiliary heart would invalidate the standard technique.
12. The capacitance of the vascular system of the pink abalone is extremely large.
13. Pressures in the aorta, cephalic arterial sinus, cephalo-pedal venous sinus, and right auricle were measured simultaneously with flow in the aortic bulb while the animal was stressed by turning off the room lights and by abruptly increasing environmental temperature.
14. Under certain conditions the aortic pressures can be made to rise quickly by body movement where the auricular pressure does not increase an equal amount. This indicates that a pressure gradient for flow across parts of the circulation can be created by body movement.
15. Perfusion pressures can be maintained in the animal for long periods even when the heart has stopped. This occurred frequently when the animal was stressed.

16. A hydraulic model was proposed which simulates many of the circulatory mechanisms found in the abalone at rest and under environmental stress.

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