

NERVOUS CONTROL OF THE HEARTBEAT IN OCTOPUS

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SUMMARY

The circulatory system of cephalopods is based on a trio of hearts, with two pairs of associated ganglia linked to the CNS by a pair of visceral nerves. The beat of the hearts was recorded from free-moving octopuses before and after surgical removal or disconnexion of elements of the nervous system. Severing the visceral nerves does not stop the hearts, which continue to beat in a powerful well co-ordinated manner in isolation from the CNS. The nerves seem to be concerned in raising the cardiac output in exercise, and with stopping the hearts when mantle movements cease, but they are not necessary for the initiation or maintenance of the normal rhythm. Removal of the fusiform ganglia severs all nervous connexions between the two gill hearts, and deprives the systemic heart of its nerve supply. The trio of hearts continues to beat as strongly as before. Removal or disconnexion of a cardiac ganglion disrupts the beat of the corresponding gill heart which now tends to contract in an ill-coordinated and rather feeble manner, though at much the same frequency as before; with both cardiacs gone the systemic heart, which contracts only when it is filled, tends to drop in frequency and the mean aortic pressure falls. The system remains rhythmic, however, and the beat may recover, to the point where aortic pressures and frequencies approach those found in intact animals at rest; even octopuses with both fusiform and both cardiac ganglia removed can survive for many hours. From the performance of the isolated branchial heart, the existence of a pulsating vesicle in each cardiac ganglion, the effects of cardiac ganglion removal and the remarkable steadiness of heartbeat frequency shown by intact animals under a variety of conditions, it is argued that the heartbeat rhythm is normally controlled by pacemakers in the branchial heart/ cardiac ganglion complexes, and perhaps, in intact animals, from within the cardiac ganglia themselves.

The picture of the control of the heartbeat that emerges from the study of free moving essentially intact animals is quite different from that arising from *in vitro* and acute preparation studies. It suggests that the conventional wisdom about the control of the heartbeat in cephalopods (and perhaps by implication, in other molluscs) may need to be considerably revised.

INTRODUCTION

Cephalopods have a high pressure, fully enclosed blood system, with arteries, capillaries and veins, and some large-capacity venous sinuses. This complex system has a very elaborate innervation, and large parts of the central nervous system appear to be devoted to a detailed control of the peripheral circulation (references see Young, 1971; Wells, 1978).

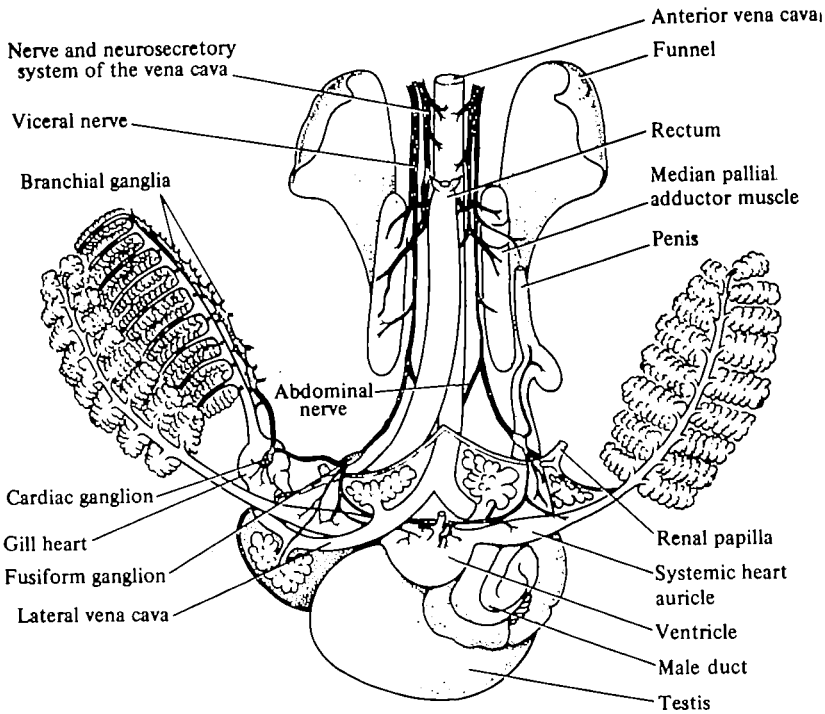


Fig. 1 The nerve supply to the hearts. Dissection from the ventral surface; the dorsal aorta running forward from the systemic heart is concealed in this view. Semi-diagrammatic, with the branchial and cardiac ganglia shown on the right side only (from Wells, 1978, after Young, 1967)

The principal pumps for the system (most of the veins show peristalsis and also help to move the blood) are the two branchial hearts, pressing the blood through the gill capillaries, and the single systemic heart into which the gills drain, supplying the rest of the body. The trio is innervated from the suboesophageal part of the brain through the two visceral nerves. The visceral nerve on each side passes into a fusiform ganglion (= first cardiac ganglion of some authors), and from there to a cardiac ganglion, attached to the branchial heart. A commissure links the two fusiform ganglia. In addition there is a chain of small ganglia along the length of each gill; these connect to the rest of the system through the cardiac ganglion on each side. An outline of the anatomy of the system is shown in Fig. 1, adapted from Young (1967). Detailed information about the nervous arrangements within the ganglia, and the innervation of the hearts is largely lacking for *Octopus*. Alexandrowicz (1913) gives some details and in a later paper (Alexandrowicz, 1963) describes the peculiar pulsating body to be found in the cardiac ganglion. But the most detailed information on the nervous arrangements in the ganglia associated with the hearts of cephalopods relates to *Sepia* (Alexandrowicz, 1960), which is unfortunate since decapods have no fusiform ganglia. The cardiac ganglia of decapods include elements that become separated into the fusiforms in the octopods.

Accounts of the function of the ganglia associated with the hearts have hitherto been limited to studies of the effect of cutting nervous connexions and/or stimulating

the visceral nerves in acute preparations. For most of these the animals were nailed to boards, upside down, with the mantle split or turned inside out. Hill & Welsh (1966) have summarized this work. The conclusions reached differ in several important ways from those arising from the experiments to be reported below, which were made with free-moving, essentially intact animals.

METHODS

Octopus vulgaris of between 500 and 1500 g were used. Most of the experiments were made in July and August, at Banyuls where the seawater in an open circulation runs at 21 ± 2 °C.

Two sorts of recording device were used. The first recorded pressure, through a T-piece tied into the dorsal aorta, about 5 cm downstream from the systemic heart. The second measured movement in terms of the changes in impedance between two fine wire electrodes, threaded into the heart or mantle musculature. Two such devices allowed simultaneous recordings to be made from the two branchial hearts. Details of equipment and its installation are given in Wells (1979).

Surgery was carried out under 2.5% ethanol anaesthesia.

RESULTS

(1) *The effects of cutting the visceral nerves (n = 13 animals)*

Both visceral nerves can be cut, between the point at which the abdominal nerves branch towards the guts and the fusiform ganglia, without stopping the heartbeat. The animals sometimes seem to take longer than usual to recover from the anaesthetic to the point where respiratory movements and the heartbeat become regular, but the effect is difficult to quantify since even controls are very variable.

This first result already contradicts convention which holds that respiratory movements cease after visceral nerves section (see Hill & Welsh, 1966). This belief appears to have originated with Ransom (1884). Ransom used acute preparations, partially dissected and pegged on their backs to a board, with the mantle slit open and folded aside. In this situation he showed (1) that cutting both visceral nerves stopped respiratory movements, (2) that the hearts nevertheless continued to beat, though rather irregularly and (3) that stimulation of the proximal cut ends of the visceral nerves induced mantle movements. Since he knew (from preliminary observations of the hearts through a hole cut in the mantle) that the heart beat rate often coincided with the respiratory rhythm, and was never slower than this, he concluded that mantle movements must be maintained in response to signals from the hearts. This conclusion appears to have been accepted by all subsequent workers (references see Hill & Welsh, 1966), who may or may not have repeated Ransom's experiments. But they were all using acute preparations. The present series of experiments shows that nervous connexions between heart and mantle are not a necessary condition for regular respiration, in unrestrained animals.

When the visceral nerves are cut, the branchial hearts continue to beat in phase with one another, preceding the systemic heartbeat by about two-thirds of a cycle, as in control octopuses (Fig. 2). Heartbeat frequencies, mean aortic pressures and the

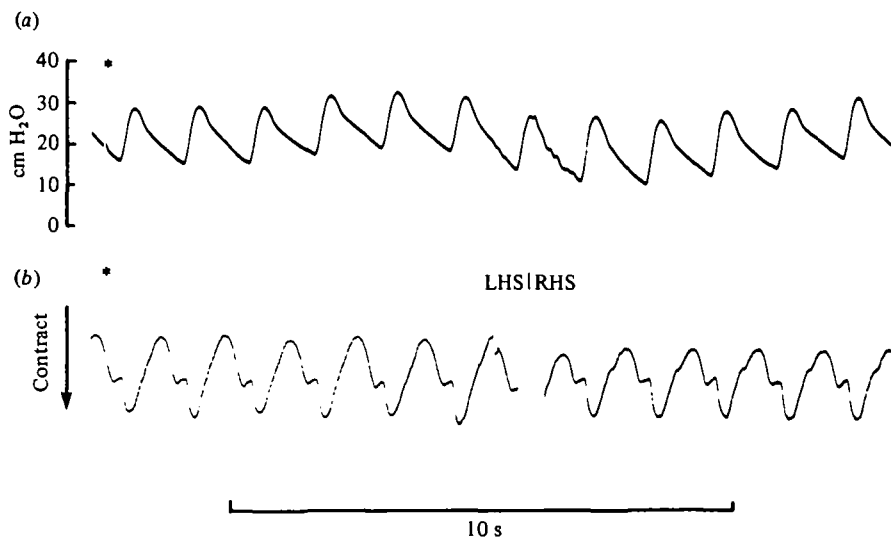


Fig. 2 The systemic heartbeat (a) continues and remains in phase with the branchial hearts (b) after section of the visceral nerves. (a) Is a pressure record from the dorsal aorta, from about 4 cm downstream of the systemic heart. (b) Is a record of the rate of change of impedance between two wires threaded into the wall of each branchial heart; halfway along this trace, recording is switched from the LHS to the RHS branchial heart. All records read from left to right. Deflections marked ** were created by a manual override operating through both recorders, to permit accurate time matching of the two traces (animal B34).

aortic pulses of resting animals have similar values before and after the operation (Table 1). High values, similar to those found in intact octopuses, are reached in, for example, recovery from anaesthetic or following the return of an octopus from O_2 deficient to O_2 saturated seawater.

The responses to acetylcholine, 5-hydroxytryptamine, adrenaline or tyramine injected into the hearts of freemoving octopuses were qualitatively and quantitatively similar in animals tested before and after visceral nerves section, as were the responses to extracts made from the neurosecretory systems discharging into the anterior vena cava or pharyngo-ophthalmic vein (Wells & Mangold, 1979). Again very high pressures, in excess of 100 cm of water, could be generated by the hearts in the absence of any nervous connexion with the CNS.

In intact octopuses the beat rate is closely related to temperature and oxygen tension, slowing progressively as either falls (Wells, 1979). Both responses continue, apparently unchanged, after section of the visceral nerves.

The fact that the steady rhythm of the hearts can continue and that the hearts can respond directly to a variety of stimuli in the absence of the visceral nerves does not necessarily mean that the nerves normally play no part in the control of the heartbeat. A considerable number of experiments has been made in the past, using an isolated systemic heart-visceral nerve preparation (references see Krijgsman & Divaris, 1955). In this situation stimulation of the nerves regularly induces inhibition followed by transient increases in the frequency and amplitude of the systemic heartbeat, effects that one might expect to find reflected in the performance of the heart *in vivo*, and absent in octopuses with the visceral nerves cut.

Table 1. *Typical systemic heartbeat frequencies and aortic pressures in resting animals before and after section of the visceral nerves*(S = Systolic pressure, D = Diastolic pressure (both in cm H₂O) and F = Frequency (beats per min).)

Animal	Before			After		
	S	D	F	S	D	F
601	40	20	58	25	10	55
	38	13	58	30	10	49
	38	10	54	25	10	54
B3	35	18	47	35	20	46
	35	22	48	40	25	46
	30	20	54	20	13	53
B26	30	20	45	28	22	45
	25	15	48	36	28	46
	28	18	42	35	25	44*
B34	22	17	35	24	16	44
	21	17	39	30	20	42
	22	15	45	30	18	43
B37	44	22	38	35	25	37
	50	30	30	36	24	39
	50	30	31	43	25	42
C14	48	28	42	70	50	50†
	50	25	44	90	50	50†
	33	20	43	Next day,		
C25	20	14	39	35	20	53†
	30	20	45	22	10	42
	30	17	51	20	14	42
C26				Next day,		
	32	25	40	25	15	43
	45	35	42	20	17	46
C60	32	25	41	27	22	48
	28	25	48	20	16	48
	30	25	48	30	25	43
C78	25	22	46	30	25	38
	30	20	48	27	20	39
	30	20	45	25	15	42
C130	22	18	46	24	15	41
	35	25	38	30	20	44
	37	28	35	45	30	37
	30	18	42	30	20	41
				28	18	41

Seawater temperatures were 25 °C in Naples (animal 601), 20–22 °C in Banyuls (the rest).

* Animal had both fusiform ganglia removed before these measurements were made.

† Animal had the cardiac to fusiform connectives cut as well as the visceral nerves; the reason for the very high pressures observed on the first day, which persisted for several hours after the operation, are not known.

The present series of experiments did not include any designed specifically to investigate inhibition of the heartbeat following visceral nerves section. But we know that cardiac arrest can occur *in vivo*, and there is evidence that this must at least sometimes be a direct nervous effect. The systemic heart will often miss one or more beats, if the animal is startled. Wells (1979) includes records of an octopus habituating to a

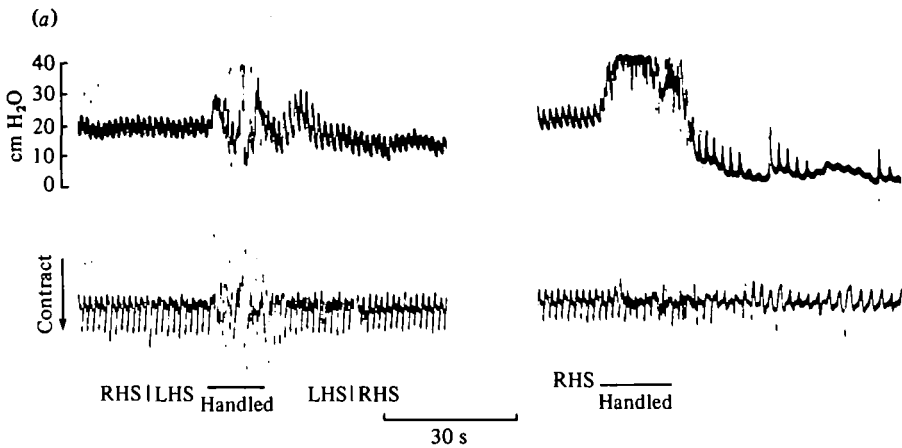


Fig. 3 Systemic and branchial heartbeats from an animal caused to move about by touching it and handling its arms. (a) Shows the response before and (b) after section of both visceral nerves (same animal, B34).

hand or a white rectangle shown at intervals, and of a male animal as it approaches and copulates with a female. It is true that the animal often tenses its muscles when startled. It may flatten out into the 'dymantic' response (see Wells, 1978) or grip tightly to the bottom of its tank. It often ceases to make respiratory movements. Any of these responses might restrict the venous return. But there would appear to be plenty of blood in the large vena cavae to supply at least two or three systemic heartbeats, and the effects observed (both the cessation of the beat and its return) often seem to be far too abrupt and complete to permit an explanation based on strangulation of the venous return, except just possibly by contraction of the gills (which in any case are innervated via the visceral nerves). It is far more likely that sudden cardiac arrest is a direct nervous effect, ordered by the CNS through the visceral nerves.

In the other direction, enhancement rather than inhibition of the heartbeat, there is more direct evidence of visceral nerve involvement. If an animal is handled, or otherwise obliged to take exercise, the normal response is to increase pulse amplitude and (to a lesser extent) heartbeat frequency; both increases persist for a while after the animal has come to rest (Fig. 3a). Animals with their visceral nerves cut, in contrast, often behave in the manner shown in Fig. 3b; the systemic heartbeat slows and may even stop, and the branchial heartbeat is disrupted. Table 2 summarizes the results obtained with 4 animals that were tested both before and after visceral nerves section. Reduction in beat frequency with concurrent falls in aortic pressure were seen to follow brief periods of exercise of 14 out of 18 occasions. In corresponding observations of the same animals before section of the visceral nerves short periods of exercise nearly always (in 15 out of 16 tests) produced an immediate rise in aortic pressure, and usually (in 10 out of 16 tests) a concurrent increase in beat frequency. Presumably it is undesirable for the animal to reduce, let alone to stop, the supply of blood to the arms during or after exercise and effects of the sort shown in Fig. 3b and in Table 2 represent a breakdown of a system that would determine an appropriate response to an increased oxygen demand in the intact animal.

Table 2. *Exercise and the response of the systemic heart before and after section of the visceral nerves*

(In each experiment the Systolic (S) and Diastolic (D) pressures and the beat frequency F were measured at rest immediately before and again immediately after a brief period of movement.)

	Before						
	S	D	F		S	D	F
601	40	20	58	→	75	40	63
	40	15	61	→	85	38	54
	38	12	60	→	80	40	42
B26	25	15	45	→	stops, then		
	28	18	40	→	30	20	40
B34	22	17	38	→	45	35	43
	22	15	45	→	26	18	48
	22	15	45	→	32	20	44
B37	44	22	38	→	60	35	40
	50	30	30	→	60	38	34
	50	30	31	→	60	30	35

(on 6 further occasions pressures and frequencies rose 4X, pressure dropped 2X, and frequency dropped once)

	After						
	S	D	F		S	D	F
601	25	5	49	→	heart stopped, then		
	26	10	54	→	58	15	51
B26	36	28	46	→	70	20	54
	40	28	44	→	almost stops, then		
					20	16	35
B34	24	16	44	→	10	0	28
	30	20	42	→	14	3	36
	30	20	48	→	22	15	43
(on two further occasions, pressures and frequency also fell)							
B37	35	25	37	→	27	20	35
	36	24	39	→	38	25	39
	43	25	42	→	30	18	39

(on 6 further occasions pressures and frequency fell every time)

(2) *Removal of the fusiform ganglia (n = 7)*

Removal of the fusiform ganglia cuts the visceral nerves, with effects upon the response to exercise that have already been noted above. Removing the ganglia had no additional effects upon systolic or diastolic pressures or upon the frequency of the systemic heartbeat; some typical examples, taken from animals tested both before and after the operation, are given in Table 3.

As well as being a pathway through which the visceral nerves might act, the fusiform ganglia link the two cardiac ganglia, and one might expect their removal to disrupt coordination of the branchial heartbeats. In the event, it was found that the two branchial hearts generally manage to remain in phase with each other and with the systemic heart after severance of the nervous connection between them (Fig. 4). They may, moreover, change their pattern of beat simultaneously and apparently spon-

Table 3. *Typical pressures and frequencies, and the effects of exercise on the systemic heart before and after removal of the fusiform ganglia.*

(In each experiment the Systolic (S) and Diastolic (D) pressures and the beat frequency (F) were measured at rest immediately before and again immediately after a brief period of movement.)

	Before						
	S	D	F		S	D	F
630	35	15	48	→	55	20	56
	30	15	50	→	60	25	57
	35	10	52	→	65	30	55
B22	30	20	46	No records of exercise effect before operation			
	35	22	54				
B26	28	18	40	→	45	35	43*
B37	50	30	31	→	60	30	35*
B40	40	25	34	→	42	30	36
	42	28	36	→	40	20	33
After							
	S	D	F		S	D	F
630	30	20	55	→	32	20	51
	25	22	58	→	45	25	57
	30	18	54	→	40	28	48
B22	50	30	48	→	30	20	40
	40	20	48	→	38	22	51
B26	35	25	44	→	24	15	43
B37	28	18	48	→	25	15	46
B40	25	12	44	→	25	10	44
	20	10	43	→	18	5	37
	23	15	34	→	18	7	30

* Ex Table 2. These animals had the visceral nerves cut, were retested, and then had the fusiform ganglia removed.

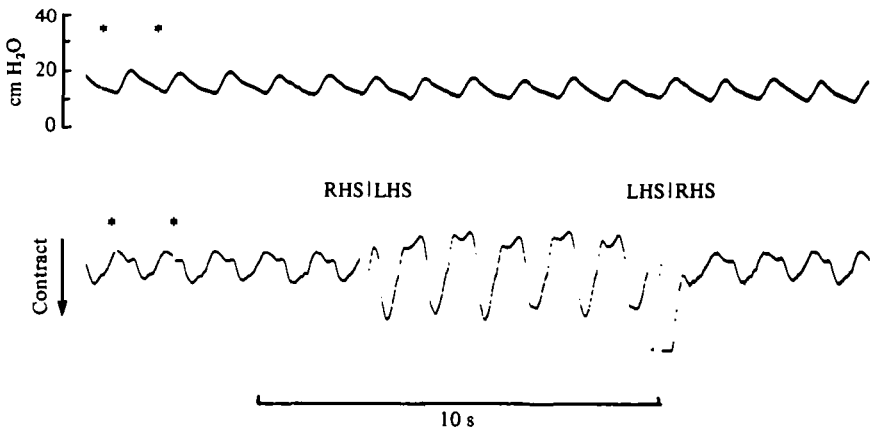


Fig. 4. The three hearts in phase after cutting both of the fusiform to cardiac ganglia connectives. As before, ** markers allow synchronization of the records (animal B32).

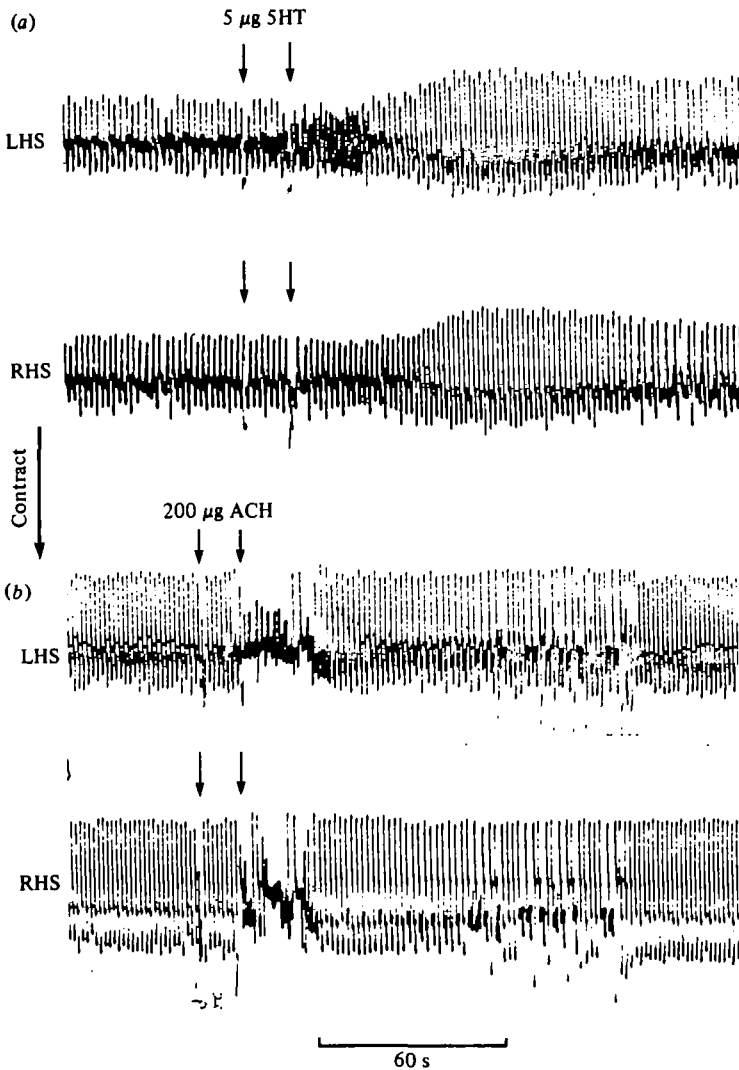


Fig. 5 Showing how the effects of drugs injected into one branchial heart are mirrored in the other. In each case $\downarrow\downarrow$ showed the period during which the injection was being made. Note that the increase in frequency induced by 5HT appears not to be relayed, while the increase in amplitude of contraction is. Impedance records (animal C41).

taneously. The branchial hearts share a common blood supply from the anterior vena cava and in such cases it seems possible that they are responding to pressure or hormonal stimuli arising upstream of them, a situation in which the nervous link might be redundant.

In an attempt to establish whether the activities of one branchial heart can influence the other as a result of stimuli arising downstream of the bifurcation of the anterior vena cava, drugs were injected into one branchial heart through a fine cannula, while recording the activities of both.

Fig. 5 shows a typical series of results. 5-hydroxytryptamine excites and acetyl-

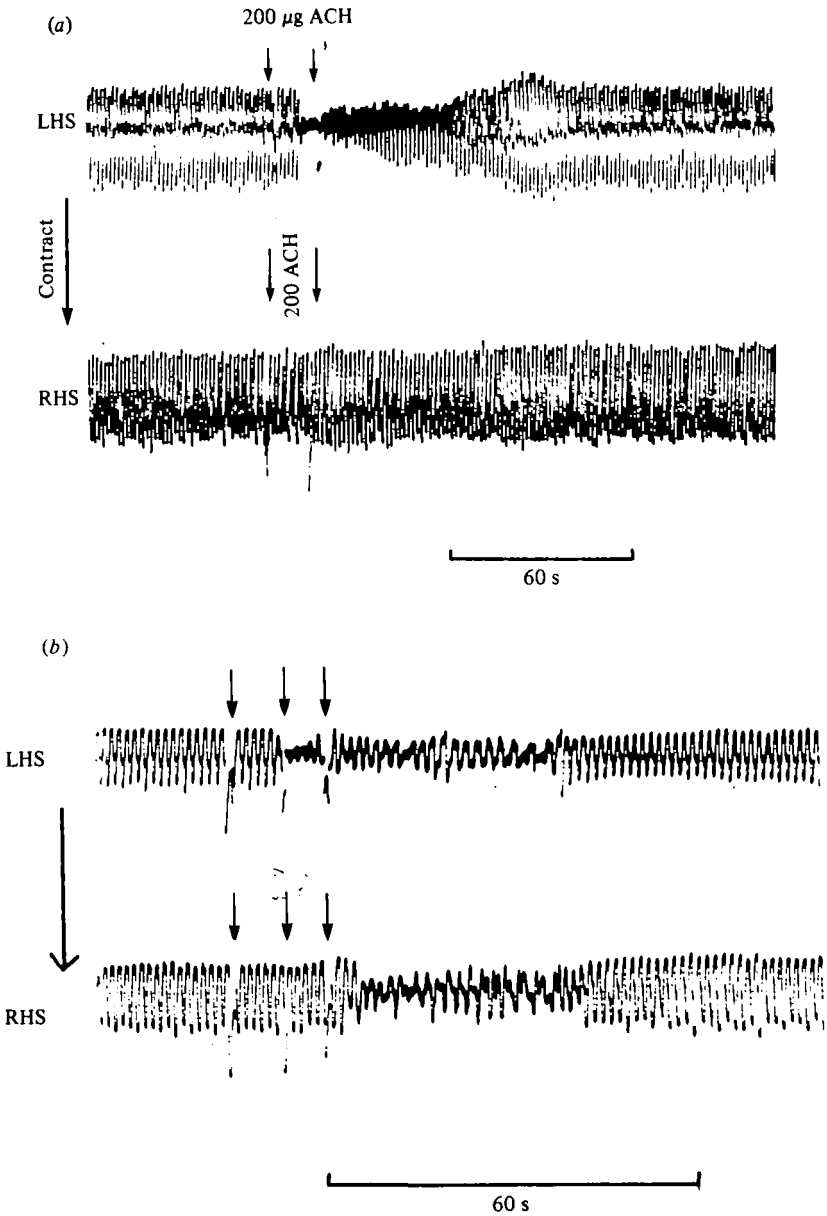


Fig. 6. (a) Following section of the fusiform to cardiac and gill to cardiac connectives on the RHS, the effect of an injection of 200 µg ACH into the LHS gill heart is no longer detectable on the RHS. Compare with Fig. 5 (b). (b) Later, following a further operation in which the connectives to the LHS ganglion were cut, the same dose of ACH inhibits both hearts; note, however, that there is a considerable delay in the response on the RHS side opposite to that injected.

In both recordings ↓↓ show the period during which the injection was being made. In 6 (b) there are three such markers; during the period between the first two the animal was injected with seawater, being the deadspace in the tube carrying the dose of ACH. (Impedance records, animal C46.)

choline inhibits the branchial heartbeat; the effect on the injected side is immediately mirrored in the activity of the 'control' side heart. Similar tests were made with adrenaline, tyramine, and neurosecretory materials derived from the anterior vena cava or the pharyngo-ophthalmic vein (Wells & Mangold, 1979). Ignoring instances where the dosage was subthreshold or the result uncertain, a total of 25 tests was made with 3 different octopuses. In 19 of these tests there was an immediate response by the 'control' side heart, closely paralleling the effect of the drug on the injected side.

Similar tests were made after cutting the fusiform to cardiac ganglion connectives on one or both sides in 3 octopuses, 2 of them from the trio already tested pre-operationally. In 15 out of 24 tests, the response now appeared only on the injected side (Fig. 6*a*). In 9 instances, there was a response on the 'control' side. In 6 (and perhaps 8) of these cases the control side response was noticeably delayed (Fig. 6*b*). Since there is a coronary circulation, fed by arteries that branch from the anterior aorta, downstream of the point of injection, it is likely that these delayed effects are blood-born rather than nervously relayed.

It seems reasonable to conclude that nervous signals from one branchial heart can reach the other only through the fusiform ganglia and that signals along this pathway could help to keep the branchial heartbeats in time in the intact octopus.

(3) *Severing the gill to cardiac ganglion connectives*
(*n* = 5, plus one unilateral operation)

Johansen and Martin (1962) have produced evidence for rhythmic contractions of the gills in *O. dofleini* which (assuming the same to occur in *O. vulgaris*) might contribute to the filling of the systemic heart. One might reasonably guess that severing the connexions between the gill ganglia and the hearts would produce some change in the output of the systemic heart. But no obvious consequent changes were seen in the present series of experiments.

(4) *Removal of the cardiac ganglia* (*n* = 7, plus 2 unilateral operations)

In contrast to the above, where very considerable damage scarcely affects the rhythm of the hearts, interference with the cardiac ganglia is seriously disruptive. If a cardiac ganglion is removed, the regular beat of the corresponding branchial heart generally becomes disorganised (Fig. 7*a*).

In one animal the nerves from the cardiac ganglia to the gill hearts were all cut, leaving the rest of the system intact. The effect of this operation was very similar to that of removing the cardiac ganglia. Fig. 8 shows the performance of the systemic and one branchial heart before and after the operation; the second branchial heart is presumably behaving in the same way. The consequences of malfunction by the pair of gill hearts show in the record of aortic pressure; the systemic heart beats irregularly and more slowly than usual. The form of the systemic beat, however, remains essentially normal, just as it does after removal of one or both of the cardiac ganglia.

In the course of these experiments it was often observed that while removal or disconnexion of a cardiac ganglion generally disrupted the beat of the corresponding gill heart, as in Figs. 7(*a*) and 8(*b*), the effect was by no means invariable. From time to time and sometimes for periods of several minutes (and perhaps for hours, though

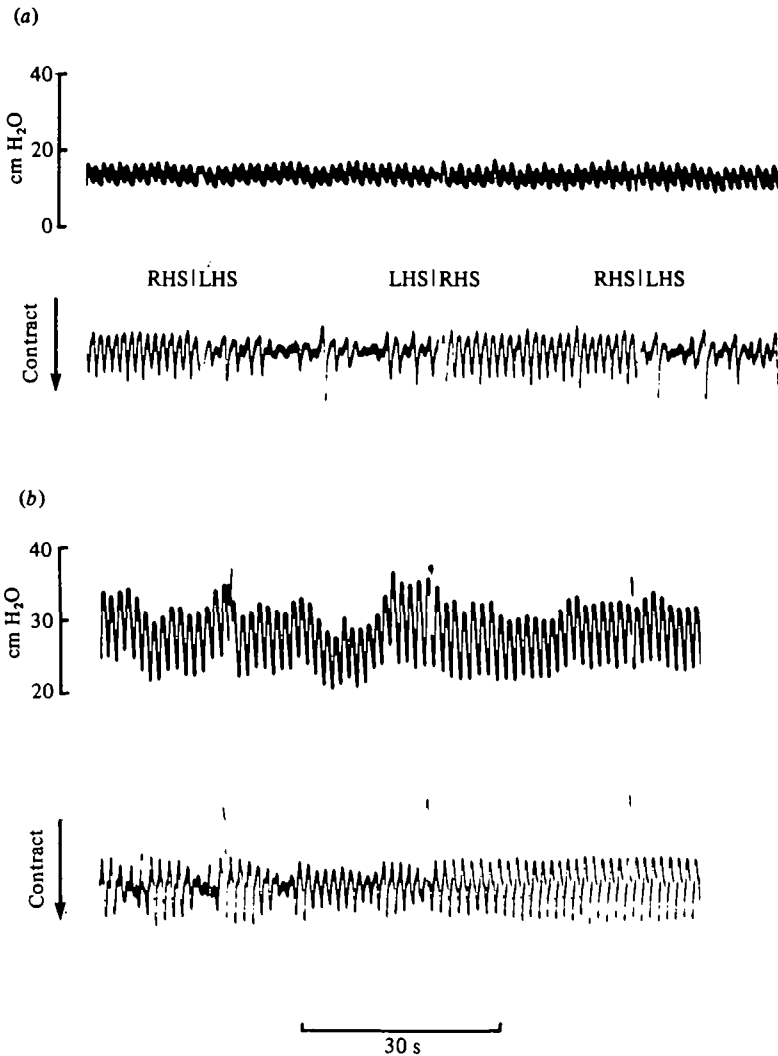


Fig. 7. The effect of removing a cardiac ganglion. In (a) the ganglion has been removed from the LHS. The ganglion on the other side is intact, but disconnected from the fusiform ganglion on that side. The LHS heart contracts irregularly, though with a frequency still recognizably the same as that of the RHS, while the RHS beats steadily and in phase with the systemic (animal B₃₂, as in Fig. 41. following a further operation). (b) Shows that gill hearts can sometimes beat in a well-coordinated manner even though deprived of their cardiac ganglia. This animal had both cardiacs removed. The record shows the beat of the systemic and the RHS gill heart, which at first cycles in beat amplitude before settling down to a steady beat; frequency remains constant and the same as that of the systemic (animal B₁₇).

continuous recordings were never made for so long) a gill heart without a ganglion would settle down to beat steadily, in time with its opposite number and in phase with the systemic. This could occur after removal of both cardiac ganglia (Fig. 7b) and even in animals from which the fusiform ganglia had been removed as well as the cardiacs. Four such animals survived (in some cases after several successive operations

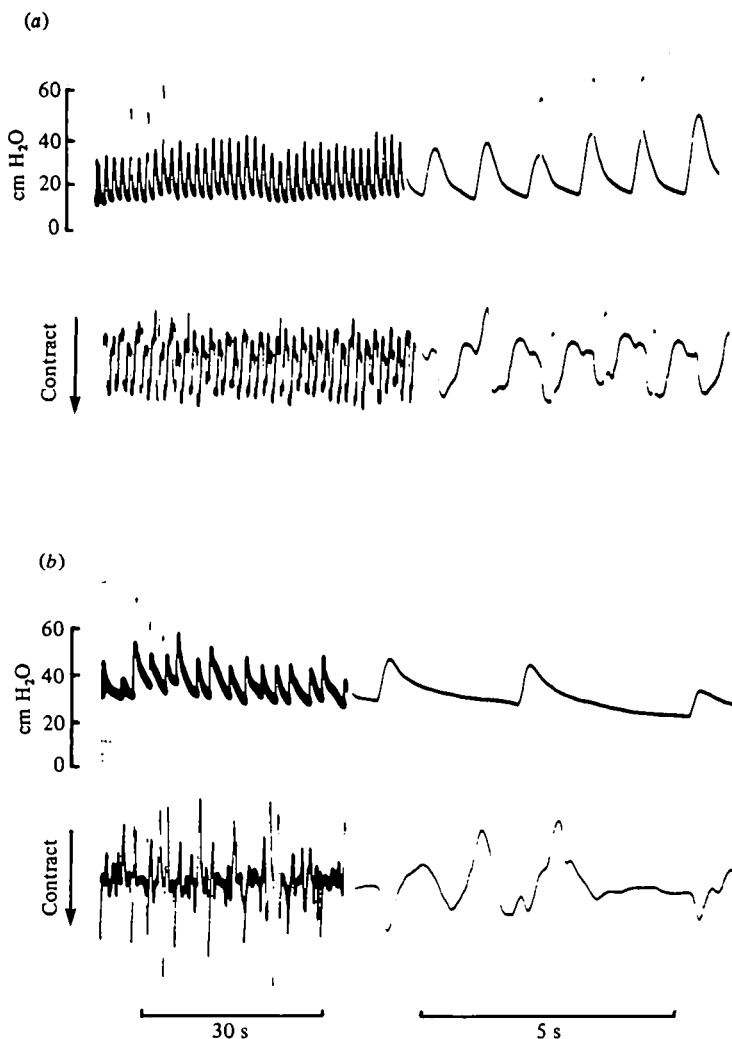


Fig. 8. The effect of severing the nerves from the cardiac ganglia to the branchial hearts; the rest of the system was left intact. (a) Shows a record made from the systemic and one branchial heart before and (b) after the operation. Erratic contractions by the denervated branchial hearts are reflected in the performance of the systemic (animal B29).

and many tests) overnight, so there is little doubt that the hearts system can function, at least sufficiently well to keep the resting animal alive, in the absence of all the heart ganglia. The maximum pressures and frequencies shown in these circumstances can approach the resting values seen in intact animals (Table 4). But there seems to be little or no capacity for a further rise; on the few occasions when attempts were made to induce such animals to move about there was either no change, or a sudden disorganization of the heartbeat, with aortic pressures and systemic frequencies collapsing towards zero. Fig. 11 includes the only instance in which there was any indication of a (small) sustained rise in pressure following a period of exercise.

Table 4. *Systemic heart pressures and frequencies after removal of both cardiac ganglia and (B22 onwards) both fusiform ganglia.*

As before, S = Systolic, D = diastolic and F = systemic heartbeat frequency, measured in the anterior aorta.

	S	D	F		S	D	F
B14	10	0	5	B26	35	25	40
B17	35	25	46	B38	38	30	34
B22	38	20	36	B39	30	20	41
B23	28	12	35				

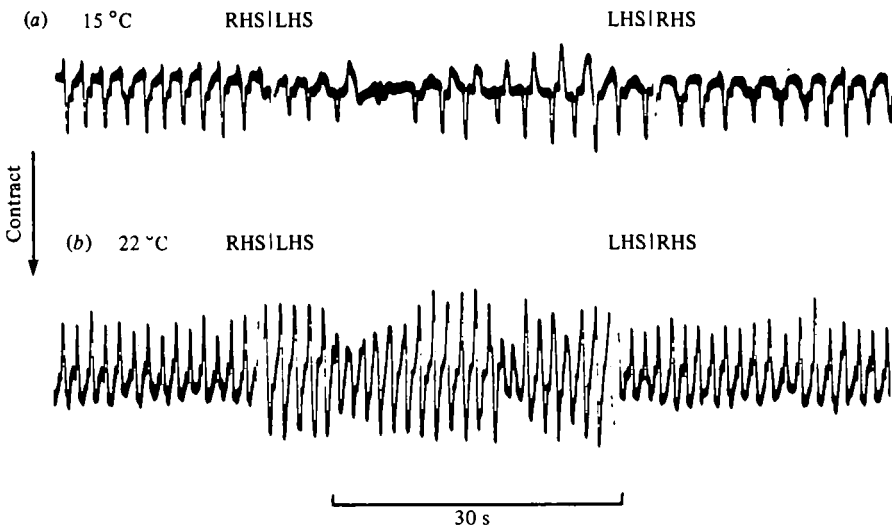


Fig. 9. Impedance records of the branchial heartbeats of an animal with the left hand side cardiac ganglion removed (a) at 15 °C and (b) at 22 °C. The heart lacking its ganglion is more irregular, but responds to temperature in the same way as that with the ganglion intact (animal B32).

(5) *Responses to Temperature and Oxygen Saturation after removal of the cardiac ganglia (n = 1 and n = 3)*

The heartbeat of an intact *Octopus vulgaris* is temperature sensitive, with a Q_{10} of about 3 over the range 7–27 °C. The hearts are also sensitive to the oxygen content of the seawater bathing them, slowing progressively as this declines; at about 2.5 ppm the beats become irregular, and may stop (Wells, 1979).

These responses continue after removal of the cardiac ganglia. Fig. 9 shows records of the branchial heartbeats of an animal with the cardiac ganglion removed from the LHS gill heart, in waters at 15 and 22 °C. The beat is notably slower at 15 °C and although it is a little irregular in the deganglionated heart, it is quite plain that the two are contracting at the same basic rate at each temperature.

Fig. 10 shows the effect of transferring an octopus from seawater at 6.8 ppm O_2 to seawater at 2.7 ppm O_2 and then returning it to the O_2 saturated water. The systemic heartbeat slows within a few seconds of transfer, and picks up again when returned to the oxygen-rich seawater. This animal had both cardiac ganglia removed.

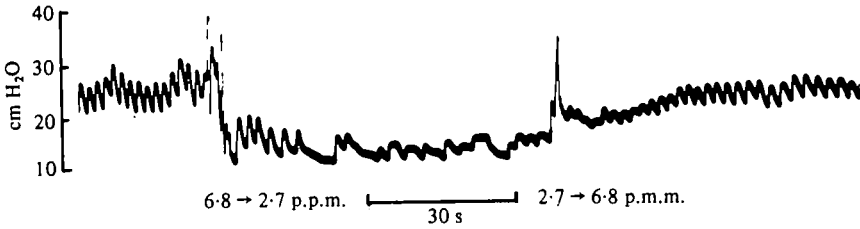


Fig. 10. The effect of a change in oxygen content on the heartbeat of an octopus with both of its cardiac ganglia removed. Water was boiled to reduce the O_2 content and refrigerated to the same temperature ($21.5^\circ C$) as the O_2 saturated water. The animal was transferred from one aquarium to another in this continuous record (animal B17).

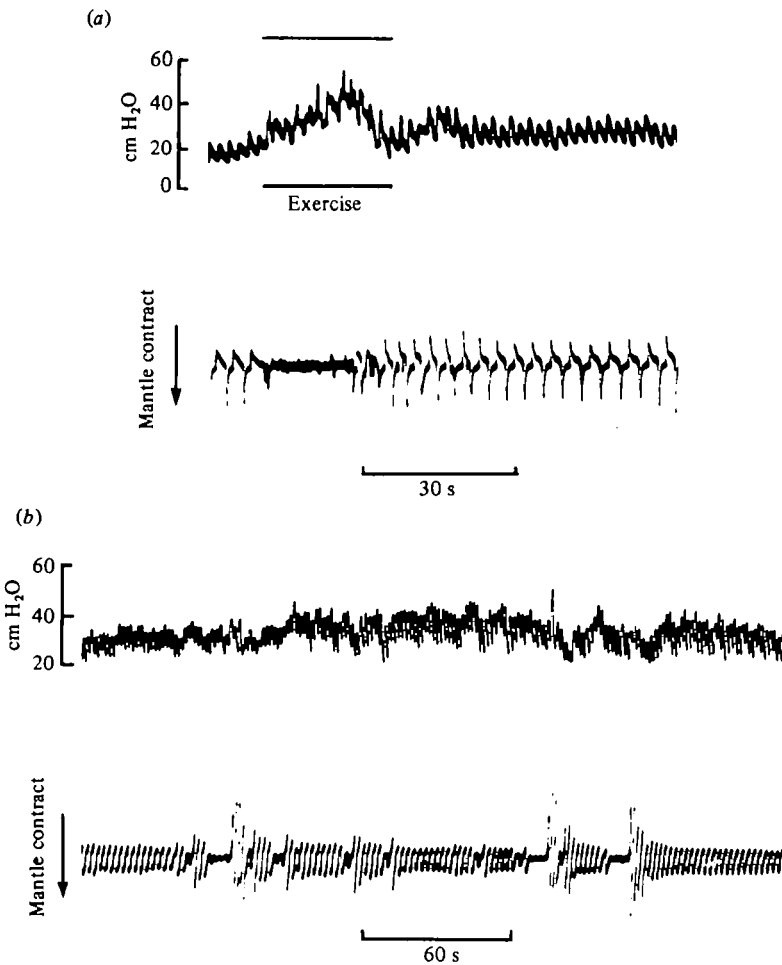


Fig. 11. Instances in which respiratory movements ceased without concurrent cardiac arrest. In each pair of records, systemic heartbeat is shown in the upper, and mantle movements in the lower trace. (a) Shows the effect of exercise on an animal (B22) which had both fusiform and both cardiac ganglia removed. (b) Illustrates a period of irregular respiration, including stops, from an intact animal (C6).

(6) *Respiratory movements and the heartbeat*

In recovery from anaesthesia respiratory movements generally return before there is any sign of a regular beat by the hearts. As the O₂ content falls so the hearts slow, to the point where they may finally stop while respiratory movements continue (Wells, 1979). The systemic heartbeat, at least, may stop 'spontaneously' for periods of a few seconds (*O. vulgaris*, Wells, 1979), or for many minutes (*O. dofleini*, Johansen & Martin 1962); in most cases respiratory movements continue regularly throughout the period of cardiac arrest.

It has been held that the reverse does not occur, that if the animal stops breathing, the heart stops (Johansen & Martin, 1962, *O. dofleini*). This was certainly found to be the case in most instances observed during the present study; cardiac arrest was typically abrupt, which would imply nervous inhibition of the heartbeat. It would plainly be economic for the animal to stop pumping blood through the gills while the water in the mantle remains unchanged.

The cardio-inhibitory reflex would be a further function for the visceral nerves and it should cease if these are cut. In experiments following visceral nerves section mantle movements were, unfortunately, rarely recorded and a sequence including a halt to these was observed only once, on an occasion when the animal was chased down its tank by the experimenter. This sequence is shown in Fig. 11(a); heartbeats continued throughout.

Fig. 11(b) shows a sequence in which both heartbeat and respiratory movements were irregular in an animal with its visceral nerves intact, apparently at rest in its tank. Respiratory movements ceased, or were at least very much reduced, on several occasions, without any obvious corresponding effect upon the systemic heartbeat. The matter plainly requires some further study.

DISCUSSION

When all the nervous ganglia associated with the hearts are removed or disconnected, the three hearts of *Octopus* will still beat. They normally remain in phase, within the two branchial hearts contracting together, followed by the beat of the systemic. Pressures and frequencies can approach the values found in intact resting octopuses. The beat frequency in such cases is temperature and oxygen dependent, falling with a decline in either, just as it does in animals having the full complement of nerve ganglia present and connected to the central nervous system.

Studies of the performance of the hearts of intact animals under a variety of conditions show that while the mean aortic pressure and pulse amplitude can vary enormously, heartbeat frequency is very stable (Wells, 1979; Wells & Mangold, 1979). This implies a system based on a pacemaker or pacemakers rather than one triggered reflexedly by the filling of the hearts, a conclusion that would eliminate the systemic ventricle as the source of the cardiac rhythm, since the systemic ventricle plainly varies in frequency, slowing down if its blood supply is limited. One must suppose either that the beat originates at the level of the branchial hearts, or that the pacemaker for the whole system lies outside the trio of hearts and their associated ganglia, perhaps within the walls of the vena cava.

Arguing against the latter possibility are two observations. One is that the isolated branchial heart continues to beat *in vitro* (and that, unlike the isolated systemic heart, no pressure-head of blood or seawater is required to cause it to do so; Ransom 1884). The second is Alexandrowicz's (1965) report that each cardiac ganglion contains a vesicle that pulsates with the same frequency as the branchial heart, and slightly in anticipation of the main muscular contraction; 'When the ganglion is cut out with its neighbouring organs its activity as a rule lasts longer than that of the branchial heart.' Taken together with the results of the present series of experiments which show the very disruptive effects of removal or disconnexion of the cardiac ganglia from the branchial hearts, these observations indicate the cardiac ganglion as the likely site of the main cardiac pacemaker on each side. The fact that the two branchial hearts regularly contract together, even after severing the nerves that connect the two cardiac ganglia through the fusiform ganglia, implies that the two branchial heart pacemakers are normally brought into phase by events upstream.

It has already been pointed out that the picture of the origin and control of cephalopod heartbeats that emerges from the present study differs radically from that to be found, for example, in the 'Physiology of Molluscs', where Hill & Welsh (1966) have summarized a literature based mainly on the behaviour of acute, partially dissected, preparations. From these, it has generally been accepted, following Ransom (1884), that the ventricle is the source of the beat, and that some connexion with the central nervous system is essential if a steady beat is to be maintained. In these acute preparations, moreover, it seems that the gill hearts cease to beat if the connexions between the fusiform and cardiac ganglia are severed, a result quite contrary to that now reported from free-moving and largely intact animals. The very unnatural conditions of much of the earlier experimentation make comparison of the two sets of results difficult. In particular it should be noted that restriction of mantle movements (for acute preparations it is split and pinned back, or folded inside out) may interfere with the heartbeat, through what appears to be an inhibitory reflex. This, and a number of other features (notably the means by which the two gill hearts tend to fall into phase after severance of all nervous connexions) of the hearts control system will require further investigation. What does seem quite plain even at this stage in the investigation is that much of the long-established 'conventional wisdom' about the control of cephalopod (and possibly other molluscan) heartbeats may be wrong and should now be re-examined under conditions more nearly resembling the state of affairs in the intact animals.

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