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THE PULSATILE ORGAN IN THE TIBIA OF *TRIATOMA PHYLLOSOMA PALLIDIPENNIS*

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The pulsatile organ, or "accessory heart," in the tibia of *Triatoma* consists of a membrane which divides the femur and tibia into a series of sinuses, a pulsatile muscle which propels the haemolymph through the sinuses, and a valvular membrane, which imposes unidirectional flow on the system. A possible innervation of the muscle is described, and simple experiments involving nerve stimulation demonstrate that the pulsatile muscle is under nervous control. Using partially isolated preparations of the pulsatile organ, it has been shown that the organ responds to dopamine and serotonin, but fails to respond to acetylcholine. The pulsatile muscle therefore resembles visceral muscle rather than skeletal muscle.

Introduction

The term pulsatile organ, or accessory heart, is one which has been applied to a wide variety of non-homologous structures. As their name implies, these accessory hearts are considered to assist in the circulation of haemolymph in areas where it might otherwise be languid. Structures in the legs, at the bases of the antennae, and at the bases of the wings have been assigned an accessory circulatory function in winged insects, and even the ventral diaphragm appears to play some role in circulation (Brocher 1920; Guthrie 1962). The present work concerns the pulsatile organ (PO) in the tibia of a heteropteran, *Triatoma phyllosoma pallidipennis* (Stål.).

Behn (1835) presented a detailed description of a PO in the tibia of *Nepa cinerea* Linn.; his observations were confirmed and extended by Verloren (1847), Mitchell (1859), Locy (1884), Richardson (1918), and Crozier and Stier (1927). The most comprehensive work has been that of Brocher, who looked at a variety of PO's in Hemiptera (1909), Lepidoptera (1919, 1920), and Diptera (1931).

From the work of these authors, it is possible to obtain at least a partial picture of the PO as it occurs in the tibia of various aquatic Hemiptera. The PO is situated chiefly in the upper portion of the tibia just below the femoro-tibial articulation. The muscular elements of the organ are attached to the cuticle of the tibia at one end, and at the other, to a membrane which extends throughout the tibia and partitions it into two sinuses. The current in one sinus is directed towards the tarsus, while the other current is directed towards the femur. Movement of the haemolymph results from alternating compression and aspiration of the two sinuses, which are confluent at the extremity of the tibia. The motive power is provided by muscular elements near the femoro-tibial articulation (Brocher 1909). The frequency of pulsing is more irregular and more rapid than that of the dorsal vessel, although the rate of pulsation in any one leg is relatively uniform over a long period of time. However, the frequency may vary among legs in the same individual. The fact that the PO continues to beat after amputation of the leg, albeit at a

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reduced frequency and amplitude, suggested to the earlier authors that there was no central control of the PO. No author has examined the possible innervation of the PO. The most complete histological description of the tibial PO is that of Debaisieux (1936), who examined the structure in *Notonecta*. He describes the blood sinuses, their limiting membranes, and the musculature, as well as the valvular membrane which ensures the unidirectional flow of haemolymph. The operation of this valve is not made clear.

The present work examines the structure and something of the functioning of the tibial PO of the very large tropical blood-sucking bug *Triatoma phyllosoma pallidipennis* (Stål.).

Materials and Methods

Morphology

Most of the information was obtained from serial sections, although living and permanent whole mounts were also useful. The main difficulty encountered was severe fragmentation of tissues due to the presence of thick cuticle. Attempts to overcome this difficulty were unsuccessful. The best series resulted when legs of freshly moulted third or fourth instars of *Triatoma* and *Rhodnius* were fixed in Duboscq-Brasil's modification of Bouin's fluid and embedded in tropical grade Ester Wax (1960 formula) (British Drug Houses) under vacuum (10 in Hg) for 30 minutes. Sections were cut at 7–10 μ with a heavy Jung rotary microtome. A variety of stains was employed the most useful of which was Hansen's trioxyhaematin (Pantin 1964). There were no important differences in morphology between the tibiae of *Triatoma* and *Rhodnius* or between larvae and adults.

Nerve Stimulation

Adult insects of both sexes were completely eviscerated to expose the fused ganglionic mass in the thorax and then pinned to a wax block. The cuticle overlying the PO was removed and the entire preparation kept moist with insect Ringer (9.82 g NaCl, 0.77 g KCl, 0.50 g $CaCl_2$ (anhydrous), 0.18 g $NaHCO_3$, 0.10 g NaH_2PO_4, 1 g glucose/l.). Silver-silver chloride electrodes were placed on the ganglion. The preparations were subjected to stimuli at frequencies of 1, 5, 10, 15, 20, 25, and 30/sec., from a simple stimulator. In each case, the PO was allowed to stabilize its rate under a given shock frequency before it was subjected to the succeeding frequency. The inferior flexor of the pretarsus (ifp) twitched synchronously with the electric pulses from 1/sec to 10/sec. Tetany of the ifp occurred at 15 to 20/sec and only rarely as high as 15/sec. The twitching of the ifp was regarded as indicating that the nerves were intact and electrodes in place. Only those animals which demonstrated this characteristic response from the ifp were tallied with the data of electro-stimulations.

The stimulus strength employed was usually 1 or 2 v. Higher voltages fatigued the preparations more quickly but did not otherwise alter the response elicited by the various stimulus frequencies. Consequently, the lowest strength at the frequency of 1/sec that resulted in twitching of the ifp was employed for each preparation.

After one or several series of experimental frequencies were tested and the final resting rate of the PO recorded, the pro-, meso-, and meta-thoracic nerves were severed; the series of experimental frequencies was then tested again. In all preparations after denervation, neither the ifp nor the pulsatile muscle responded to any electro-stimulation up to 20 v. In this way it was confirmed that the pulses

were effective via the nerves. All experiments were carried out at room temperature which varied from 21° to 24°C.

Pharmacological Studies

The effects of various drugs on the PO were studied by amputating legs from male and female adults, dissecting them to expose the PO, and suspending them in wax blocks in a simple muscle bath containing insect Ringer. The preparation was aerated continuously.

The bath was drained two or three times and the organ allowed to recover from surgical shock for at least 15 minutes. The preparation was observed for 5 minutes; if the organ was not beating, it was left undisturbed for a further 15 minutes or more. If the organ was still quiescent, it was discarded. Success with these preparations was not high: only 34 of the 176 organs observed were suitable for experimentation.

The drugs used were serotonin (13 preparations), dopamine (9 preparations), adrenalin (4 preparations), and acetylcholine (6 preparations). Further details of techniques used may be found in Kaufman (1967).

Results

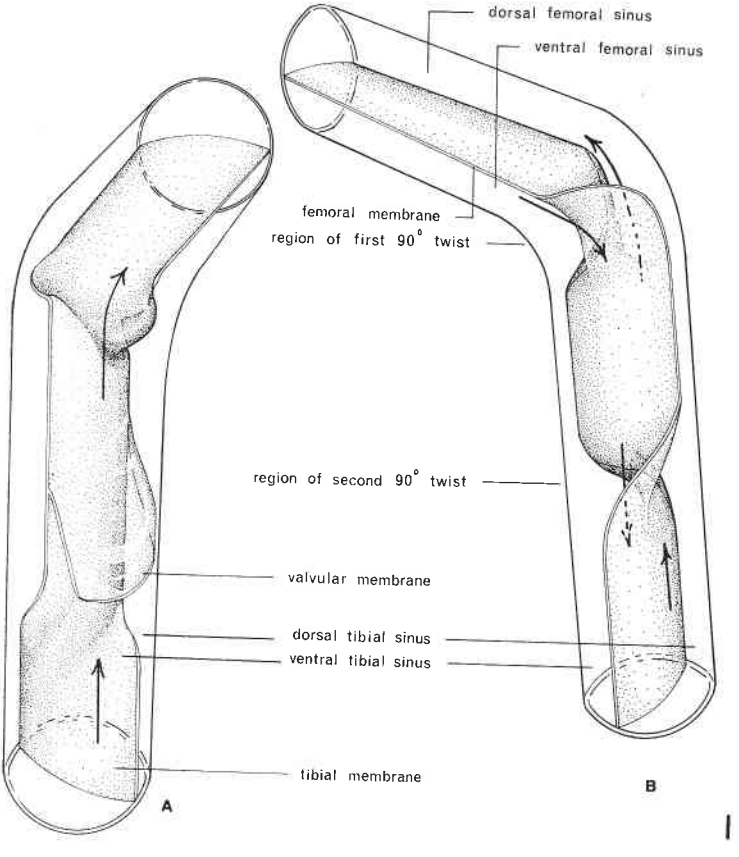
In the PO there are four major structures to consider: the *blood sinuses,* which are delineated by a series of *membranes,* the *muscles* which are responsible for pumping the blood, and the *nerves* which supply the muscles. Since the flow of blood is apparently unidirectional, a *valve* must also exist in the system.

The Membranes and Blood Sinuses

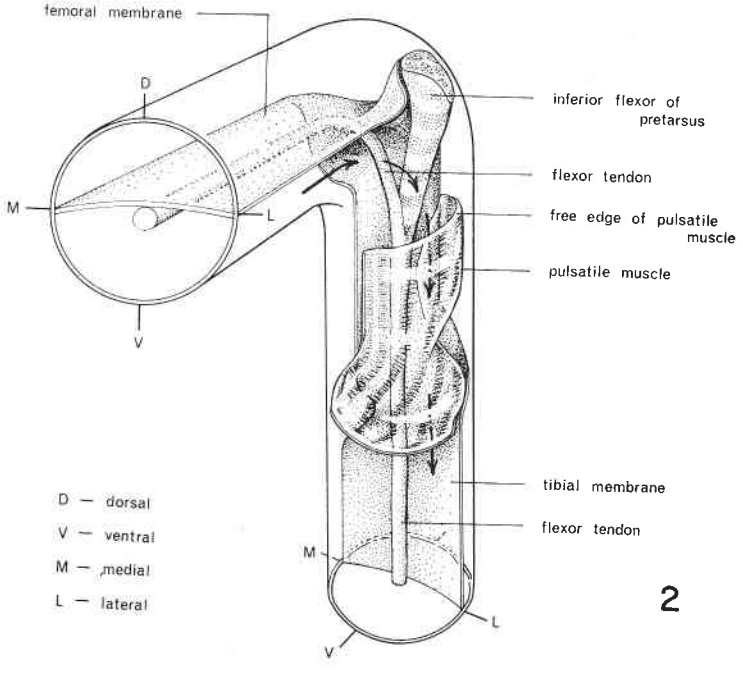
Figure 1 is a diagrammatic representation of the leg in the region of the femoro-tibial articulation. The femur and tibia are divided longitudinally by a membrane which twists through 90° in each of two separate regions. In the femur, the membrane is inserted in the medial and lateral cuticle of the leg and thus lies in a medio-lateral plane, dividing the femur into a dorsal and a ventral sinus. In the region of the femoro-tibial articulation, the lateral edge of the membrane veers towards the dorsal wall, and the medial edge veers towards the ventral wall resulting in the first twist through 90°. Lower down in the tibia, the membrane undergoes a second twist, returning to its original orientation. The upper part of the membrane, down to the region of the second twist, is here referred to as the *femoral membrane,* the *tibial membrane* being that part of the membrane below the second twist.

In the upper tibial region, where the insertions of the femoral membrane are dorsal and lateral, a flap diverges from the femoral membrane and inserts on the medial wall of the tibia: this is the *valvular membrane* (Fig. 1). Because we believe that this membrane functions as a one-way valve, its insertions are of some importance. It is clear from the sections that an insertion on to the medial and ventral walls of the tibia exists. The existence and extent of an insertion on the dorsal cuticle remains uncertain, but some degree of incompleteness in the attachments of this membrane is necessary to permit the observed flow of blood.

The sinuses of the femur are thus confluent with the sinuses of the tibia. Although they follow a somewhat tortuous course in the region of the two twists of the membrane, we have designated them the *dorsal and ventral sinuses of the femur and tibia.*


dorsal femoral sinus
ventral femoral sinus
femoral membrane
region of first 90° twist
region of second 90° twist
valvular membrane
dorsal tibial sinus
ventral tibial sinus
tibial membrane
A
B

1


femoral membrane
D
M
L
V
inferior flexor of pretarsus
flexor tendon
free edge of pulsatile muscle
pulsatile muscle
tibial membrane
flexor tendon
D — dorsal
V — ventral
M — medial
L — lateral

2

The Pulsatile Muscle

Debaisieux's (1936) description of the proximal musculature of the tibia in *Notonecta* corresponds with that in *Triatoma*. His *compressor muscle* is responsible for propelling the blood and is, therefore, the *pulsatile muscle* (Fig. 2). Debaisieux (1936) shows the muscle inserting on the flexor tendon. In *Triatoma*, however, the insertions of the pulsatile muscle are complex. Its lower end clearly inserts on the ventral wall of the tibia. The edges of the muscle have attachments which parallel those of the femoral membrane as it undergoes its second twist through 90°. Thus, at the upper end, these attachments are to the dorsal and ventral walls of the tibia, while at the lower end they have migrated to the lateral and medial walls of the tibia. For a part of its length, the dorsal edge of the muscle is attached to the femoral membrane. The upper end of the muscle has no attachments.

Innervation

The major nervous supply to the muscles of the tibia is derived from the two tibial branches of the large ventral femoral nerve. The latter enters the tibia along the ventral tibial wall and immediately divides into two nerves—the *posterior tibial nerve* (ptn) and the *median tibial nerve* (mtn).

Neither of these nerves has been seen to send fibres to the pulsatile muscle. The ptn supplies the ifp, and with the mtn, passes into the tarsus. Both the ptn and the mtn receive numerous branches from the hypodermal region; these are likely sensory axones from sense organs on the cuticle. Further details concerning the course of these nerves may be found in Kaufman (1967).

Apart from the neurons abundant below the hypodermis and associated with the sensory cuticular hairs, there exist at least three larger accumulations of neurons deeper in the leg: their functions are unknown. The first appears in the femur as part of the dorsal femoral nerve (Fig. 3). The second is located in the ventral tibial sinus just below the dorsal insertion of the femoral membrane (Fig. 4). It is most often composed of two neurons, the more proximal of which rests against the femoral membrane and a fibre of the pulsatile muscle; the posterior axones lead to the distal end of the ventral sinus where they approach the median tibial nerve. Whether or not they join that nerve is uncertain: they definitely do not reach the distal end of the tibia as independent axones. They must, therefore, either terminate in the tibia or continue through to the tarsus incorporated with the median tibial nerve. While this ganglion may be the one responsible for transmitting the sensory impulses from the *scolopidial organs* (described in greater detail by Debaisieux (1935), it is here referred to as the *pulsatile ganglion*, because of its close association with the pulsatile muscle. In some specimens, this ganglion is spindle-shaped, consists of 3 or 4 cells, and is not in direct contact with the tibial membrane (Fig. 5). In such cases, it produces a nerve proximally toward the pulsatile muscle although no nerve endings of the conventional type were seen. The

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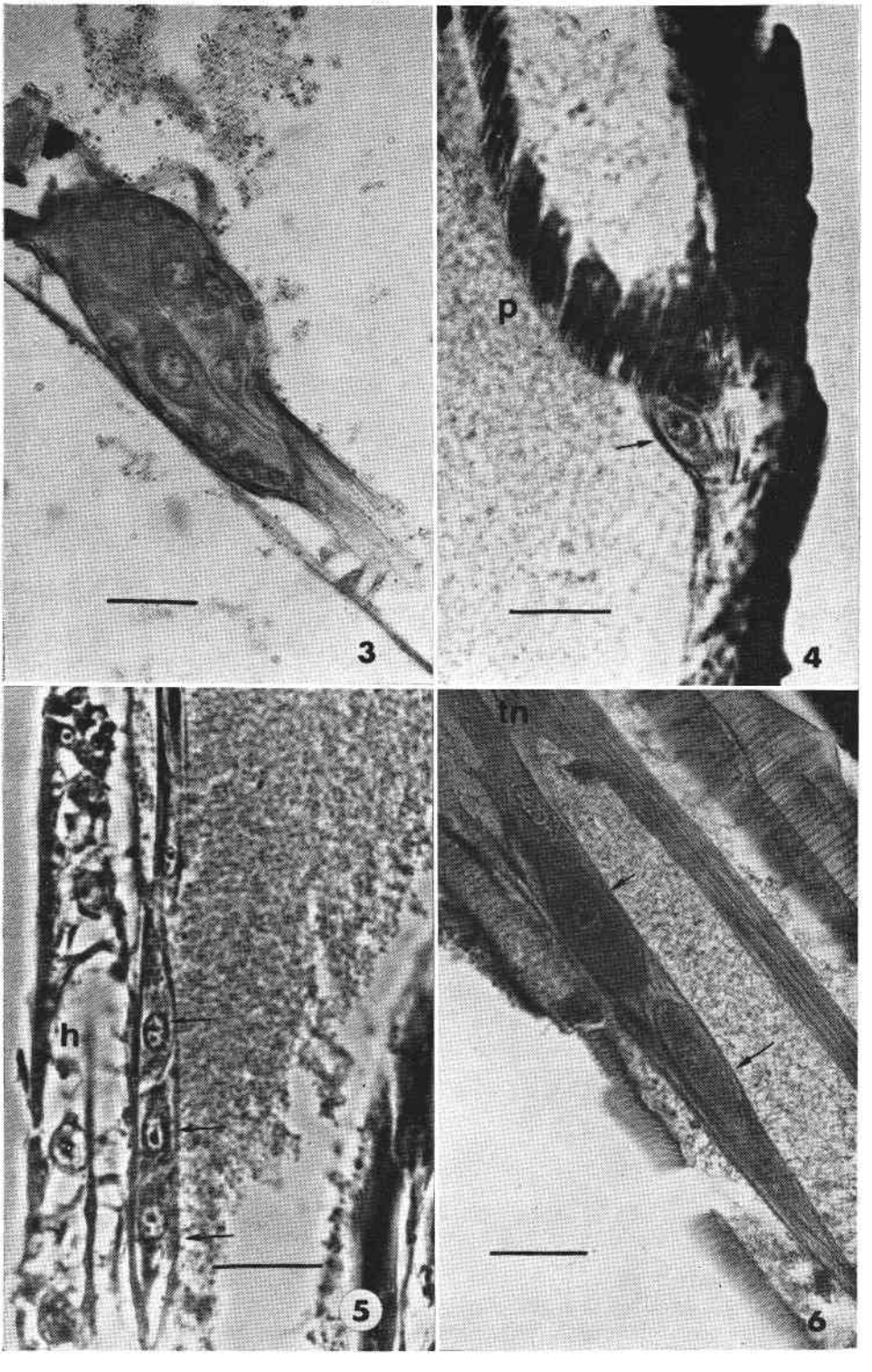
FIG. 1. Diagram of a right leg of *Triatoma* in the region of the femoro-tibial articulation to demonstrate the membranes and sinuses. A. Medio-frontal aspect. B. Latero-frontal aspect. The arrows indicate the direction of flow of the haemolymph.

FIG. 2. Diagram of a right leg of *Triatoma* showing the association between the membranes, the ifp, and the pulsatile muscle. The arrows indicate the direction of the flow of the haemolymph.


3
p
4
h
5
tn
6

nerve apparently terminates on the surface of the muscle, but we have been unable to trace with certainty the proximal connections of this ganglion.

The third group of neurons (Fig. 6) is associated with a branch of the posterior tibial nerve and thus connects with the fused ganglion in the thorax.

Nerve Stimulation

The pulsatile organ *in vitro* does not beat continuously but experiences periods of activity, alternating in an irregular way with periods of inactivity. Thus, it is possible to stimulate a resting organ and an active organ. When 10 resting organs were stimulated at 1 cycle/sec they were excited into activity. When 27 active organs were stimulated at the same frequency, there was a net inhibition when the effects were averaged. This is to be expected. A resting organ can, after all, only respond by starting to beat. A beating organ on the other hand, can increase or decrease its rate; it so happens that the average effect of stimulation at this frequency is inhibitory. After the first stimulation, when all of the organs are excited, subsequent stimulation has the same result. Figure 7 shows the results of stimulation at various frequencies upon the rate of beating of the organ. At stimulation frequencies up to 10 cycles/sec, there is a marked inhibition of beating. Beyond 10 cycles, there may be a gradual increase in beating, but the responses are variable.

Other less systematic observations are also of interest. Amputation of a leg stops the pulsatile organ. Although the organ may resume beating, it does not attain as rapid a rate as it had in the intact leg. Severing the nerves from the thoracic ganglion similarly stops the beating, even when the operation is performed on legs that had been prepared for nerve stimulation, and thus had recovered from any surgical shock.

Furthermore, there appears to be a relationship between the skeletal muscles of the leg and the pulsatile organ, such that there is a tendency for the two muscles not to contract at the same time. In *Rhodnius,* where it is possible to observe the PO through the cuticle in an intact specimen, the PO is normally active in resting animals, but ceases when the insect struggles. Furthermore, if the ifp is contracting in time with a volley of applied shocks at 10–15 cycles/sec, it may be inhibited momentarily while the PO beats a few times.

Pharmacology

All four PO's exposed to adrenalin were blocked at concentrations between 10^{-5} M and 10^{-4} M. However, lower concentrations either had no effect (two organs) or an inhibiting effect (two organs). Acetylcholine at concentrations from 10^{-9} M to 10^{-3} M had no consistent effect (six organs). On the other hand, dopamine applied to five preparations had a marked and consistent stimulating effect, with a threshold of 10^{-8} M or less. Blockade occurred at about 10^{-5} M.

FIG. 3. Ganglion in dorsal femoral sinus of *Triatoma* along the course of the dorsal femoral nerve. Stain: Hansen's trioxyhaematin. The horizontal line indicates 25 μ.

FIG. 4. Neurone (arrow) of the pulsatile ganglion situated in the ventral tibial sinus. Stain: Azo-carmine. p = pulsatile muscle. The horizontal line indicates 25 μ.

FIG. 5. Neurones (arrows) of the pulsatile ganglion in *Triatoma.* Stain: Azo-carmine. h = hypodermis. The horizontal line indicates 25 μ.

FIG. 6. Sagittal section of tibia of *Triatoma* showing neurones (arrows) of the ganglion associated with the posterior tibial nerve (tn). Stain: Azo-carmine. The horizontal line indicates 25 μ.

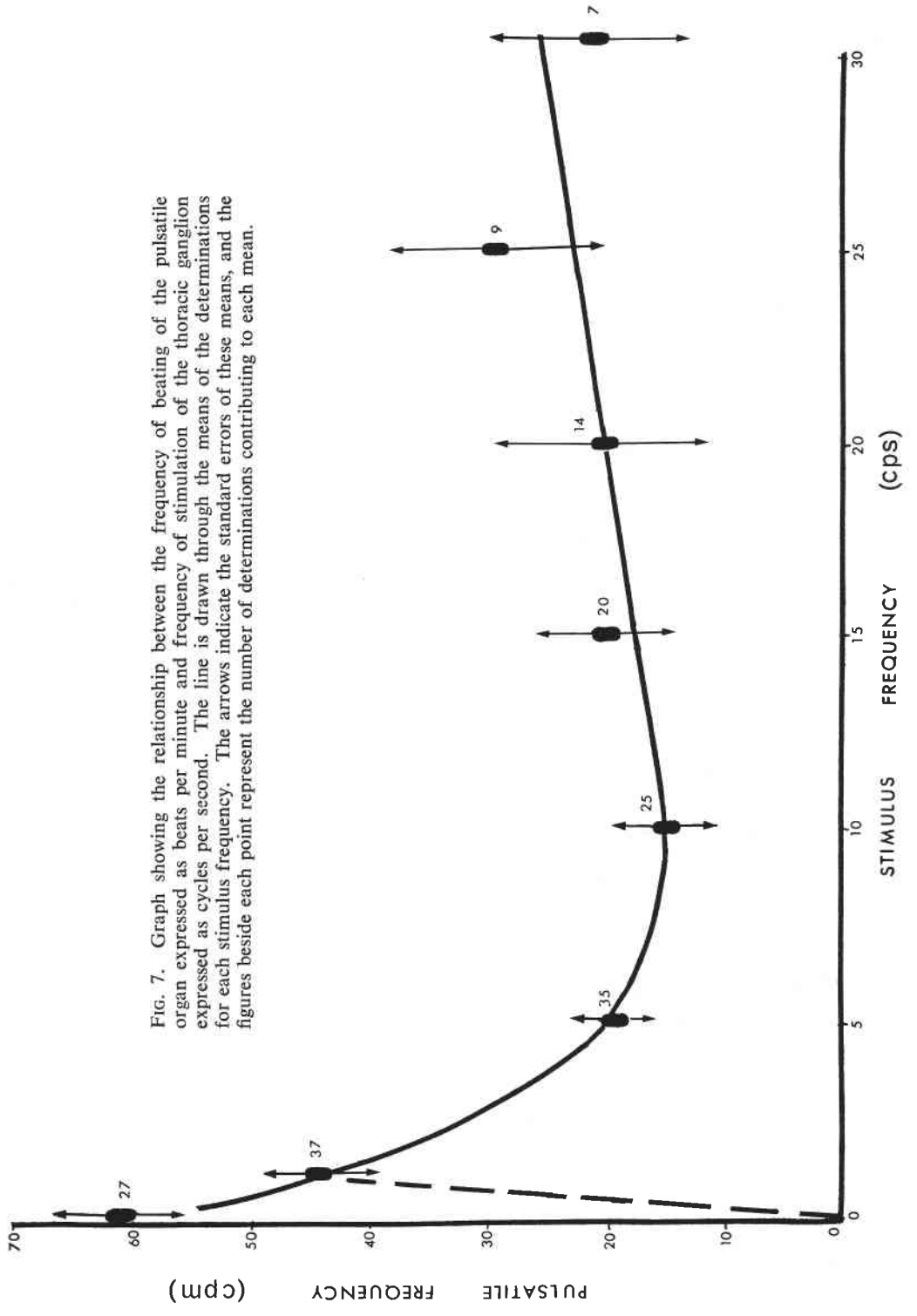


FIG. 7. Graph showing the relationship between the frequency of beating of the pulsatile organ expressed as beats per minute and frequency of stimulation of the thoracic ganglion expressed as cycles per second. The line is drawn through the means of the determinations for each stimulus frequency. The arrows indicate the standard errors of these means, and the figures beside each point represent the number of determinations contributing to each mean.

Serotonin was tested on 13 organs. In eight preparations it stimulated the rate at concentrations between 10^{-11} M and 10^{-6} M. One preparation was inhibited by concentrations of 10^{-6} M and greater. One failed to respond to concentrations from 10^{-9} M to 10^{-5} M. The remaining three organs responded erratically.

Discussion

The morphology of the tibial PO in *Triatoma* is similar to that in other Heteroptera. The organ in *Triatoma* differs only in detail from that in *Notonecta* as described by Debaisieux (1936). Basically, the organ consists of a series of membranes defining a number of blood sinuses. The blood is propelled through these sinuses by the action of the pulsatile muscle.

The Pulsatile Valve and Direction of Flow

The evidence from the literature is overwhelmingly in favour of the hypothesis involving unidirectional circulation in the insect limb (Behn 1835; Verloren 1847; Brocher 1916, 1920, 1931; and Debaisieux 1936). Consequently, although the presence of a valve associated with the pulsatile apparatus has not been proved, such a valve, which hinders the reflux of haemolymph, must be postulated. Debaisieux (1936) mentions a valve in the pulsatile organ of *Notonecta,* but unfortunately he does not treat it adequately in his paper. It was observed in the tibia of *Rhodnius* that although the net movement of blood may be in one direction, flow is spasmodic; the valve is evidently not entirely efficient.

The hypothetical mechanism described below accounts for the direction of flow as observed in this study and by the previously mentioned authors. The system requires an orifice associated with the valvular membrane (Fig. 1). Although we have been unable to observe this orifice, it most likely occurs as an incomplete attachment of the valvular membrane to the dorsal cuticle of the tibia. When the pulsatile muscle contracts, haemolymph is forced distally in the ventral tibial sinus (heavy arrows, Fig. 2), and into the tarsus where the ventral tibial sinus becomes confluent with the dorsal tibial sinus. The haemolymph is forced proximally in the dorsal tibial sinus and reaches the dorsal femoral sinus by passing through the postulated orifice in the valvular membrane (Fig. 1). When the pulsatile muscle relaxes, distal or return flow of the blood in the dorsal femoral sinus is inhibited, presumably by the collapse of the hypothetical orifice resulting from back-pressure on the valvular membrane.

The functional significance of such a circulation is obvious. The Heteroptera in general do not have a particularly conspicuous dorsal aorta, and in any case, the circulation induced by the aorta would be largely confined to the main body cavity. Circulation in the tibia would otherwise have to rely on the stirring which is induced by locomotor activity. In this connection, it is significant that the PO functions only when the limb is not moving.

While the information from experiments on nerve stimulation and the effects of pharmacological agents must be regarded as preliminary, some tentative conclusions are possible. In spite of its location among skeletal muscles, the pulsatile muscle does not resemble skeletal muscles in its functioning. When it contracts, it does so in a more or less rhythmic fashion. The frequency of contraction can be increased by dopamine or serotonin at relatively low concentrations. In this respect it resembles the visceral muscles such as heart (Davey 1964). Skeletal muscles, on the other hand, appear to be unaffected by biogenic amines at low concentrations, while very high concentrations lead to neuro-muscular block (Hill and Usherwood 1961), It is possible, of course, that the drugs were acting through some local

nervous system. If this is the case, however, the failure of acetylcholine to affect the system is puzzling.

The role of the nervous system in the control of the PO is not clear. Certainly the organ continues to beat when its connections with the central nervous system are severed, but stimulation of the fused ganglion in the thorax leads to inhibition of the PO. Since this stimulation also brings about contractions of the skeletal muscles, the notion that the PO functions in the absence of locomotor activity is given further support. It is not clear whether the experimental inhibition of the PO is a direct result of nervous stimulation or is a consequence of mechanical disturbances caused by contractions of the skeletal muscles. More sophisticated experiments are clearly desirable, but the isolated preparations have not been sufficiently reliable.

Acknowledgments

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