Apart from one paper (Nadort, 1943) dealing with some somatic reflexes in *Anodonta cygnea* and *Unio pictorum*, the only references to the behaviour of *Anodonta* seem to relate to isolated observations made by workers interested in other aspects of the physiology of these animals.

Pawlow (1885), in a paper demonstrating the existence of a double innervation of the adductor muscles, mentions both reflex and spontaneous adduction of the valves, and adds the remark that occasionally the spontaneous movements assume a regular periodicity. In 1906, Marceau showed that rhythmical activity of the adductor muscles was exhibited by many lamellibranchs of widely differing structure and mode of life. This rhythmical activity comprised alternate rapid adductions and slow abductions of the valves, the amplitude of the movements tending to decrease until the valves came to rest, in some genera, in the closed position, and, in other genera, in a widely gaping position. *Anodonta* was one of the former group. In a later paper (1909), he reproduced some kymograph records which demonstrated that in *Unio* (and, less obviously, in *Anodonta*) this rhythmical activity was merely part of a much slower rhythm, composed of alternate periods of activity and quiescence. He did not mention this, however, in the text of his paper. Marceau did not investigate the mechanism controlling these rhythms, nor did he offer any explanation of the function of the muscular contractions other than the rather strange teleological one: 'Ces conditions de distension constante, avec contractions et relâchements intermittents, sont nécessaires à la vie des muscles adducteurs, et le grand principe de physiologie générale “tout organe qui ne travaille pas s’atrophie” trouve là une éclatante confirmation.'

Koch & Hers (1943) have studied the movements of the siphons in *Anodonta*, and have shown that, during the animal’s period of activity, the apertures are alternately open and closed, thus causing intermittent respiratory currents. When the animal first opens its shell valves after a period of quiescence it has incurred an oxygen debt, but as this debt is paid off the periods of flow of water through the siphons become progressively shorter and less frequent. The movements of the siphons may, therefore, be regarded as subject to a rhythm which is modified according to the respiratory needs of the animal. As far as one can tell from their kymograph recordings, there is no correlation between this rhythm and the adductor rhythm.

In his paper of 1943, Nadort first reviews the literature on the nervous systems of lamellibranchs, and then goes on to describe the method of locomotion and also
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several reflexes found in *Anodonta*. As a result of experiments involving the extirpation of ganglia, he draws conclusions as to which of the ganglia are responsible for controlling these various activities.

The behaviour of *Anodonta* may be considered as composed largely of (a) activity of the adductor muscles, producing movements of the shell valves, (b) activity of the mantle margins, and of the siphons in particular, and (c) activity of the foot, resulting in locomotion. In so far as these activities are under nervous control, they could be induced either by stimulation of receptors (i.e. reflexly) or by 'spontaneous' functioning of the nervous system, or by both factors. The purpose of this paper is to describe experimental work on (a) which points to the importance of both factors in the neurophysiological basis of behaviour in *Anodonta*. A preliminary account of some of this work has already been published (Barnes, 1952).

**MATERIAL AND METHODS**

Most of the experiments described in this paper were carried out on large specimens of *A. cygnea* L., but in a few experiments where thick-shelled animals were desirable *A. anatina* L. was used instead. The latter experiments were, however, repeated with *A. cygnea* to ensure that any conclusions drawn from the experiments were equally applicable to this species.

The animals were kept in running water in laboratory sinks until required, and, although the specimens used for experiment were collected fresh every few weeks, some specimens were kept in an apparently healthy condition in this way for many months; their behaviour after nearly 4 months captivity was, as far as could be seen, identical with that when they were freshly collected.

For the purpose of recording the movements of the shell valves, the animal was placed on its left side with its valve embedded in low melting-point paraffin wax in a dissecting dish, leaving its right valve free to move about the axis of the hinge. A piece of thread fastened by means of sealing wax at some suitable point on the surface of the right valve was attached to a frog heart lever which wrote on the smoked surface of a kymograph drum. The animal could then be covered with still, running, or aerated water. The contractions of the two adductor muscles could be recorded separately by cutting across the right valve from the mid-point of its margin to the mid-point of the hinge, and attaching the two portions to two separate levers. Operations on the nervous system were performed by inserting instruments through small windows cut at appropriate places in the right valve. When it was desirable to exclude water after the operation, the windows were 'glazed' by means of cover-slips fixed in place with stiff petroleum jelly. The actual recordings were made on one or other of (a) a standard physiological kymograph, giving a range of speeds from 1 rev./sec. to 1 rev./hr. approximately, (b) an improvised kymograph, driven through gearing by a government-surplus electric motor, and controlled by a variable resistance to give a continuous range of speeds from 1 rev./4 hr. to 1 rev./day, and (c) a Casella barograph drum, giving 1 rev./day or 1 rev./week.
During the course of this work, the movements of the valves of sixteen intact specimens of *A. cygnea* have been recorded, each for a period of several days, so that the recordings cover more than 200 specimen-days. All of these recordings demonstrate the same type of behaviour. The animals spend alternate periods in activity and quiescence.

![Graph showing normal behavior](image1)

**Fig. 1.** Normal behaviour. Part of one period of activity. Time marker, 2 min. In all figures, recordings run from left to right, with contractions (adductions) downwards.

![Graph showing normal behavior](image2)

**Fig. 2.** Normal behaviour. The whole of one period of activity. Total time, 3 hr.

During the period of activity the shell is typically open, with the ventral margins of the valves separated by about 2–6 mm., but from time to time there occurs a rapid adduction of the valves followed almost immediately by a slow separation. These movements are repeated at intervals usually sufficiently regular to leave no doubt that one is witnessing truly rhythmical activity (Fig. 1). At its commencement the rhythm has (at room temperature) a frequency of approximately 12–20 adductions/hr., but towards the end of a period of activity the rhythm slows down. The adductions are not strong enough to lead to complete closure of the shell, but they are rapid enough to produce a vigorous jet of water from the exhalant siphon. The end of the period of activity is marked by progressively less complete abductions and stronger adductions, until the valves meet along their margins (Fig. 2).
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Throughout the period of quiescence the shell is kept closed, with its valve margins held firmly together, but, if the thread to the recording lever is attached over the insertion of the posterior adductor muscle in a thin-shelled specimen, small rhythmical kinks in the recording indicate that the rhythmical activity of that adductor muscle has not ceased with the complete closure of the valves. In most instances the retardation of the rhythm, already noticeable during the active period, leads sooner or later to a cessation of these kinks during the quiescent period. In a few instances, however, the rhythmical kinks have persisted until the commencement of another active phase, when the frequency has again increased (Fig. 4A). The new active phase is initiated by increasing relaxation of the adductor muscles, causing the valves to gape, and allowing the rhythmical adductions to take place.

The alternate periods of activity and quiescence themselves conform to a rhythmical pattern, which, however, is not so regular as the more rapid rhythm (Fig. 3). The periods of activity are usually shorter than the intervening periods, and they recur with frequencies ranging from 3 to 30 per week in the specimens investigated. Some of the irregularities in the rhythm are attributable to internal, and some to external, factors, e.g. (a) the occasional protrusion of the foot (particularly in well-aerated and agitated water), causing a great extension of the active phase possibly for a day or two; (b) considerable variations in the temperature of the laboratory, which are likely to influence the frequency significantly (Pawlow (1885) observed a marked change in the rate of activity of the animal over a temperature change of 3–4°C); and (c) unavoidable vibrations, which have been observed to induce reflexly the commencement of an active period (see p. 171). But despite the irregularities the behaviour of the specimens investigated conforms to such a consistent pattern as to leave no doubt that it is rhythmical.

For the sake of brevity the two rhythms described above will hereafter be termed the ‘rapid’ and the ‘slow’ rhythms respectively.

THE NEUROMUSCULAR MECHANISM OF THE RHYTHMS

If the valve which is free to move is cut across from the mid-point of its ventral margin to the mid-point of the hinge, and simultaneous recordings are made of the movements of the two halves, it is found that both adductor muscles participate in the rapid rhythm. In order to demonstrate this satisfactorily, however, it is usually...
necessary to weight the anterior lever to abduct the valves between contractions, since the elastic ligament of the hinge operates only on the posterior part of the shell. If these recordings are continued it is found that both portions of the cut valve are after a few hours fully adducted, and that they remain fully adducted until the animal becomes moribund after a week or two. It is therefore impossible to ascertain by this means whether both adductor muscles are concerned in the slow rhythm as well as the rapid rhythm. But this experiment does indicate that both muscles are, at least, capable of maintaining a strong contraction over a long period of time. Whether they both in fact do so during the period of quiescence has to be determined by other experiments. The most satisfactory method is the following.

An animal with a thin and therefore pliable shell was selected and a window of about 1/2 in. in diameter was cut into the right valve in the region of the shell's greatest bulge, so that when the specimen was fixed in the dissecting dish in the usual manner the window was at the highest point of the shell. Water was then poured into the dish until it rose to a level just below the window. The animal continued to show its normal type of behaviour. Then during a period of quiescence a fine scalpel was introduced through the window and the posterior adductor muscle was cut from its insertion on the upper valve. Immediately the valves separated by about 3 mm. at their posterior end, while at the anterior end they remained in close contact. This result indicated that before it was cut the posterior adductor muscle was responsible for keeping the valves together by its contraction. Another experiment was then set up to test the effect of cutting the anterior adductor muscle in the same way, but, as the elastic ligament operates only on the posterior end of the shell and would be counterbalanced by the contracted posterior adductor muscle, it was necessary to employ a means of abducting the anterior end of the valves so that the effect of cutting the anterior muscle could be ascertained. For this purpose a thread attached to the upper shell above the insertion of the anterior adductor muscle was tied at its other end to the hook of a spring balance, which was suspended vertically from a movable clamp. The clamp was adjusted so that a tension of 100 g.-wt. (a small tension compared with that exerted by the elastic ligament) was recorded by the spring balance when the valves were completely adducted. This did not interfere with the normal rhythmical activity. When the anterior adductor muscle was cut during a period of quiescence the anterior end of the shell gaped by about 2 mm., the spring in the balance contracting, of course, by the same amount. Thus the anterior adductor muscle must also be in a contracted condition during the quiescent phase of the slow rhythm. During the period of the animal's activity it is obvious that both adductor muscles are relaxed except for the periodic contractions of the rapid rhythm. So it may be concluded that both anterior and posterior adductor muscles exhibit the same type of activity, both participating in the rapid and the slow rhythms.

Marceau (1909) showed that the adductor muscles of *Anodonta*, like those of many other bivalves, are composed of two portions, one consisting of unstriated fibres...
The behaviour of A. cygnea L., and its neurophysiological basis capable of maintaining a tonus for long periods of time, and the other consisting of spirally striated fibres capable of rapid contractions but incapable of maintaining a tonus. It seems probable then that the movements of the rapid rhythm are brought about by the phasic fibres, while the slow rhythm is explicable in terms of increased and decreased tonus of the tonic fibres.

This is confirmed by the following observations. Injection into the foot of an intact specimen of 5–7 ml. of 2% morphine hydrochloride (which prevents or abolishes the state of tonus in the tonic muscle—see later) allows the rapid rhythm to continue but abolishes the slow rhythm, i.e. the active phase of rhythmical activity persists indefinitely (Fig. 4 A, B). Cutting the tonic portions of the adductor muscles has the same effect on the rhythms. These results demonstrate that the slow rhythm is, in fact, a function of the tonic portion, and that the rapid rhythm is exhibited by the phasic portions, but they do not rule out the possibility that the tonic portions may also take part in the rapid rhythm. The only way to settle this point would be to separate the two portions of the muscles and remove the phasic portions completely (merely cutting the phasic fibres from their attachment to the shell is inadequate since rhythmical tensions produced by the continued contractions
of these fibres are transmitted to the valves via the tonic fibres to which they are intimately bound by connective tissue), but this is found to be impracticable since the nerves to the tonic portions pass through the phasic portions of the muscles, and they would be severed when the muscle portions were separated. So, for the time being, this point will have to remain unsettled.

If the cerebropleural ganglia are excised, or if the cerebrovisceral connectives are cut, the posterior adductor muscle goes into a state of increased tonus, drawing the two valves together, and often so vigorously that the movable valve is snapped in two. (It is for this reason that *A. anatina* was sometimes substituted for *A. cygnea*, the former having thicker valves less likely to break). This state of tonus is maintained indefinitely, until the animal becomes moribund after a week or two. During the whole of this time, the phasic portion of the muscle is continuing its rapid rhythmic activity (Fig. 5). The same result is produced if a specimen is completely eviscerated, leaving only the posterior adductor muscle and the visceral ganglia. There are two conceivable explanations of this commencement of tonus: it could be due to the arrest of impulses normally travelling along the connectives, or it could be due to stimulation of the connectives by cutting them or by removing the cerebropleural ganglia. The second explanation is unlikely since electrical stimulation of the connectives has not been observed to induce tonus in the muscle; it has, on the contrary, been repeatedly observed to inhibit a state of tonus. So it may be concluded that the state of tonus is due to the interruption of inhibitory impulses passing along the connectives. Pawlow (1885) demonstrated the presence of inhibitory fibres running from the cerebropleural ganglia to the posterior adductor

Fig. 5. The effect of 'decerebration' on the activity of the posterior adductor muscle. The first part of the recording is of the activity of an animal intact except for a small window cut in the shell in the region of the cerebropleural ganglia. At X the two cerebropleural ganglia were excised, and the anterior adductor muscle was cut (in order to record only posterior adductor activity). Total time, 3 days.
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muscle, and his conclusions had been confirmed before his paper came to hand. But mere removal of an inhibitory stimulus is not a complete explanation of the assumption by the muscle of a state of tonus; there must be some activating stimulus present in addition. The activating factor might be intrinsic in the muscle fibres themselves, or it might reside in the visceral ganglia. That the visceral ganglia stimulate the muscle to go into a state of tonus is shown by the following experiment. A 'muscle-ganglion' preparation was made, i.e. a preparation consisting of the posterior adductor muscle, the visceral ganglia, an inch or so of the two cerebrovisceral connectives still attached to the visceral ganglia, and sufficient of the movable valve to obtain a recording. The connectives were then subjected to faradic stimulation until the state of tonus in the muscle was inhibited, and the preparation was then left undisturbed. Within 15–20 min. the tonus reappeared, and the process could be repeated. If, however, the ganglia were extirpated while the muscle was in the relaxed condition the tonus did not return. The same observation was made if the ganglia were extirpated in an intact animal during the period of activity when the tonic muscle is naturally relaxed. It therefore follows that as far as the posterior adductor muscle is concerned the slow rhythm is explained by the activity of the visceral ganglia which tend continually to maintain the state of tonus, and of the cerebropleural ganglia which intermittently inhibit that tonus.

The rapid rhythm of the posterior adductor muscle is, on the other hand, independent of the cerebropleural ganglia; as has been said, it is exhibited by a muscle-ganglion preparation. There could be three explanations of this fact: (1) the rhythm could be intrinsic in the striated muscle, as it is, for example, in vertebrate cardiac muscle, or (2) the rhythm might result from proprioceptive reflexes between the muscle and the visceral ganglia, or (3) the rhythm could be intrinsic in the ganglia. Which of these explanations is the correct one has not been satisfactorily settled.

When the visceral ganglia are extirpated from such a preparation the rhythmical activity of the muscle normally stops immediately, but in one specimen of \textit{A. anatina} the rhythm was found to persist for several hours after all visible (under low-power binocular microscope) traces of the ganglia had been removed. (Note: two specimens of \textit{A. cygnea} showed similar continued activity of the anterior adductor muscle after both cerebropleural ganglia had been excised.) Pawlow (1885) recorded a similar observation, but suggested that the continued activity of the muscle was due either to incomplete removal of the ganglia or to the presence of nerve cells in the substance of the muscle. These are probably the most likely reasons for continued rhythmical activity of the muscle, but, nevertheless, the possibility cannot be ruled out that there may be an intrinsic muscular rhythm which normally requires nervous activation for its continuance.

If proprioceptive reflexes are responsible, one would expect that stretching the muscle might modify the frequency of the rhythm, but experiments involving loading the muscle with various weights have yielded inconclusive results. Inducing extra contractions by direct electrical stimulation of the muscle does not
interfere with the rhythm: subsequent spontaneous contractions occur at the approximate times at which they would be expected if no artificially induced contractions had taken place. Furthermore, when the rapid rhythm is of relatively low frequency, recordings often indicate that the muscle is in a state of complete relaxation for several minutes before a spontaneous contraction occurs. These facts suggest that proprioceptive reflexes are not involved in the maintenance of the rapid rhythm.

This leaves the third of the above explanations, namely that the rhythm is intrinsic in the ganglia. Now it has been found that extra contractions of the muscle reflexly induced by electrical or mechanical stimulation of the mantle edge do have the effect of delaying subsequent spontaneous contractions. Since, then, stimulation of the nervous system does affect the rhythm, while direct stimulation of the muscle does not, it seems highly probable that the rhythm is intrinsic in the visceral ganglia. This conclusion must, however, be regarded as only tentative. Probably the only way of confirming it with any certainty is to record the electrical activity of the ganglia before and after their excision, and it is hoped to try this approach to the problem in the near future.

The nervous control of the rhythms in the anterior adductor muscle has not been investigated in the same detail, but the experiments which have been performed suggest a mechanism analogous to that found at the posterior end but entirely under the control of the cerebropleural ganglia.

That these ganglia control the rapid rhythm of the muscle can be demonstrated by injecting a specimen with morphine hydrochloride to cause relaxation of the tonic fibres, cutting across the movable valve so as to record the activity of the anterior muscle only, and then cutting the cerebrovisceral and the cerebropedal connectives and the pallial nerves, leaving only the cerebropleural ganglia connected with the muscle. Such a preparation continues to exhibit the rapid rhythm. If one or other of the cerebropleural ganglia is then extirpated the rhythm still persists, but if both are removed it normally ceases immediately (but see note on p. 165).

The cerebropleural ganglia can be shown also to control the slow rhythm in the anterior muscle. The mere excision of the visceral ganglia is sufficient to stimulate the posterior adductor muscle to go into a state of tonus, from which it may not recover for several days. During this period any continuance of the slow rhythm by the anterior adductor muscle will, of course, be masked. This may be overcome by inserting a fine knife between the shell valves in the position of the exhalant siphon and cutting the tonic fibres of the posterior adductor muscle. It then becomes possible to demonstrate that the slow rhythm can be maintained by the anterior adductor muscle under the control of the cerebropleural ganglia. Even if the cerebropleural ganglion on one side is excised, and the cerebropedal connective and the pallial nerve on the other side are cut, leaving only one cerebropleural ganglion connected to the muscle, the slow rhythm still persists for a few days until the animal dies. If both cerebropleural ganglia are removed all rhythmical activity of the anterior adductor muscle normally stops (Fig. 6).
The behaviour of A. cygnea L., and its neurophysiological basis

The conclusions to which these experiments lead can be summarized as follows: each adductor muscle consists of two portions, a phasic and a tonic portion; the phasic portions of the two muscles exhibit rapid rhythmical contractions under the control of the nearest ganglia, while the tonic portions exhibit rhythmically alternating periods of contraction and relaxation under the ultimate control of the cerebropleural ganglia. This control is exercised by the rhythmical inhibition of the contraction produced in each muscle by continuous stimulation from its nearest ganglia.

The question then arises whether the slow rhythm is intrinsic in the neuromuscular system or whether it depends upon some peripheral stimulation or other. It is a simple matter to show that the rapid rhythm is intrinsic because a muscle-ganglion preparation can be made at the posterior end of the animal. The anterior ganglia and muscle, owing to their anatomical relations, present a much greater problem, and it has so far proved impossible to get a suitable muscle-ganglion preparation. Nevertheless, a tentative answer to the question can be given.

It has been shown that the phase of the slow rhythm which is actively controlled is the period of relaxation of the adductor muscles, and therefore of gaping of the shell valves. If, in fact, peripheral stimulation of the cerebropleural ganglia is responsible, one can guess at the most likely stimuli operating at the end of a period of closure of the valves. They are: (i) lack of oxygen, (ii) excess of carbon dioxide, (iii) accumulation of faeces in the rectum or exhalant siphon, (iv) accumulation of pseudofaeces at some point on the mantle edge, (v) lack of food at some point in the alimentary mechanism, and (vi) accumulation of excreta in the mantle cavity. Experiments have been performed as follows to test whether any of these stimuli are essential for the maintenance of the slow rhythm.

Fig. 6. Activity of anterior adductor muscle under control of the cerebropleural ganglia (visceral ganglia excised, and tonic fibres of posterior adductor cut). At X both cerebropleural ganglia were removed.
(a) Recordings have been made of the activity of an intact specimen in an environment devoid of oxygen. This was achieved by covering the animal with water that had been well boiled and allowed to cool to room temperature in an atmosphere of air that had been passed slowly through alkaline pyrogallol solution. To prevent further oxygen going into solution during the experiment the whole recording apparatus was placed in a sealed glass tank containing a pot of alkaline pyrogallol, and communicating with the outside air only through a wash-bottle containing yet more pyrogallol. The only oxygen then available was that contained within the animal at the beginning of the experiment. These conditions were maintained for 5 days, during which the animal continued to exhibit the usual rhythmical activity.

(b) Another specimen was immersed in water through which was passed a continuous stream of carbon dioxide from a Kipp's apparatus. Although the periods of activity were very much reduced in length, the slow rhythm nevertheless persisted for nearly a week. It became somewhat irregular, but this irregularity was shown for a week after fresh aerated water had replaced the CO$_2$-saturated water, so it must be interpreted as representing permanent damage to the rhythmical mechanism rather than as the result of interference with the normal stimuli.

(c) During several different experiments animals were kept under observation for many weeks while immersed in running tap water, so that no food was available from the beginning of the experiments, and no faeces were passed out of the exhalant siphon after the first few days. The regular rhythmical activity nevertheless persisted unaltered.

(d) A small notch was cut into the ventral edge of one shell, without damage to the mantle, and a fine glass nozzle was inserted into the mantle cavity, through one of the gills, and thus into the epibranchial space. The nozzle conducted a continuous stream of tap water which washed out the mantle cavity and then escaped via the same notch when the shell was closed and via the exhalant siphon when the shell was open. A flow of 1-1.5 l of water per hour was maintained during the experiment. This, one would imagine, is more than adequate to wash away excreta discharged into the mantle cavity. Normal rhythmical recordings were, nevertheless, obtained.

These experiments between them rule out the possibility that any of the likely stimuli mentioned above are essential factors in maintaining the slow rhythm. Thus experiment (a) rules out factor (i), experiment (b) factor (ii), experiment (c) factors (iii), (iv) and (v), and experiment (d) factor (vi). It seems highly probable then that the slow rhythm as well as the rapid rhythm is spontaneous.

**THE EFFECT OF MORPHINE HYDROCHLORIDE**

Pawlow (1885) had difficulty in performing his experiments on the innervation of the adductor muscles because of the prolonged tonic contractions induced during the dissection of his animals. He therefore decided to narcotize the specimens before dissecting them, and for this purpose he used a 2% morphine hydrochloride solution, which he injected into the foot in sufficient quantity to bring about relaxation of the adductor muscles.
When therefore an anaesthetic was required for a similar purpose in this present work it was decided to use the same drug as the appropriate dosage was already known. But before any operations were performed under the influence of morphine, it was felt desirable to ascertain (a) how the drug affected the behaviour of an intact animal, (b) how long its effects lasted, and (c) whether the normal behaviour returned completely at the end.

For this purpose, 5 or 6 ml. (according to size) of 2% morphine hydrochloride solution were injected into four animals which had been exhibiting normal behaviour for at least a week, and recordings were continued thereafter for 2 or 3 weeks. It was then observed that the injection of the drug had the effect of abolishing the slow rhythm while allowing the rapid one to continue, i.e. the periods of tonic contraction were done away with. This suggested that treatment with morphine might be a useful tool in the investigation of the rhythms, quite apart from its possible use as a narcotic. If it could be discovered at which point in the neuromuscular system it was acting it would indicate a physiological mechanism which is essential in the slow rhythm but inessential in the rapid. This was therefore investigated. The effect upon the posterior adductor muscle of injecting morphine hydrochloride was observed in several specimens which had had various parts of their nervous systems cut or extirpated. In every case the effect was the same—the tonus was abolished. This suggested that the drug influenced the muscle directly and not through the nervous system. This was confirmed by the observation that the injection of 0.5 ml. of the solution into a completely isolated adductor muscle in a state of tonus also led to its relaxation. As Marceau (1909) showed, the tonus is maintained by the unstriated portion only of the adductor muscle, so the site of action of the morphine must be this unstriated portion.

The morphine hydrochloride acts not only upon the adductor muscles. If 5-6 ml. of the 2% solution be injected into an intact animal, it is found that the foot is invariably extended to its fullest degree. This must involve the relaxation of the retractor muscles of the foot as well.

In some experiments it was found that this dose of morphine was sufficient to abolish the tonus of the adductor and retractor muscles, but did not prevent reflex contractions of the adductors and other muscles when various parts of the animals were touched. Slightly increased doses in the same animals, however, did abolish the reflexes, although the rapid rhythmical contractions of the adductor muscles still persisted. Hence, morphine in certain doses does have a narcotic effect upon the nervous system of Anodonta; but the observations that Pawlow was making were due not to the narcotic effect, as he supposed, but to the inhibitory effect on the muscle tonus. As a result, some of his conclusions are, in fact, erroneous. Thus he failed to realize that the muscle fibres which he was stimulating reflexly in his morphine-treated specimens are not the ones responsible for keeping the valves adducted during the period of shell closure, and he therefore wrongly identified the tonus of the unstriated fibres with tetanus of the striated ones.
A REFLEX WHICH INHIBITS TONUS OF THE ADDUCTOR MUSCLES

Nadort (1943) described and investigated a number of reflexes exhibited by *A. cygnea*, but, although he searched for one, he failed to find any that led to the opening of the shell by inhibiting the tonus of the adductor muscles.

It seemed to me, however, that in all probability one does exist, since whenever a specimen of *Anodonta* was taken from the storage sink, cleaned, dried, fixed in a dish, and covered with water for the purpose of recording, it invariably commenced to open its shell within a few minutes. The effective stimulus might be one of several: *(a)* the temporary removal from water, *(b)* the agitation of the water as it is run into the dissecting dish, *(c)* the increase in temperature as the specimen is removed from the storage sink and fixed in molten wax in the dissecting dish, *(d)* the decrease in temperature as the new water is run into the dish, or *(e)* the inadvertent shaking and/or rotation of the specimen during its handling.

The following experiment was therefore performed. An animal that exhibited very regular rhythmical behaviour was chosen, and set up intact for recording in the normal way, except that a heavy (and therefore very stable) dissecting dish was used and this was immersed in water in a glass tank sufficiently large to allow the dish to be rotated while still under water. For 9 days a continuous recording was made, and during that time the periods of shell closure were very regular and of approximately 24 hr. duration. It seemed therefore reasonable to assume that the next period of quiescence would be of similar length if the animal were undisturbed. Five hours after it commenced, however, the water surrounding the animal was violently agitated for 2 min., and then left undisturbed for approximately 45 min. No relaxation of the adductor muscles occurred during this time. Then all the water was gently siphoned out of the tank into another container, the specimen was left exposed to the air for half an hour, and the same water, still at room temperature, was once more gently siphoned back. Again no response was observed during the next 1 hr. 40 min. Then the effect of changing the temperature was tried. A lump of ice was suspended in the tank, and the water gently stirred, with the result that a thermometer placed near the specimen indicated a fall of temperature from 15 to 10° C. in 70 sec. The ice was then removed. During the following 3/4 hr. the shell remained closed. By this time the temperature had risen to 13° C. Some warm water was next poured in, with stirring, and the temperature rose in 10 sec. from 13 to 18° C. The increase in temperature likewise produced no response during the next half an hour. Lastly the animal was disconnected from the recording lever and was, with its dissecting dish all under water, gently rotated 10 times about its longitudinal axis, and then reconnected. The whole process took less than half a minute. Within 5 min. the shell was widely gaping and the rapid rhythm had commenced. This was less than 10 hr. after the last period of activity and therefore 12–14 hr. before the next was otherwise expected. The result of this experiment pointed to the rotation of the animal as being the effective stimulus.

At the time when this conclusion was reached there were seventeen live speci-
mens of *A. cygnea* or *A. anatina* in the storage sink. These were all rotated 10 times without being removed from their water. Before they were rotated only six had gaping shells; 10 min. later all seventeen were gaping.

An attempt was made to analyse the stimulus still further to ascertain whether vibration without rotation was an adequate stimulus or whether the rotation was a necessary part of the stimulating movement. This was done very crudely merely by shaking a number of specimens up and down 10 times in the water of the storage sink without this time rotating them. Whereas seven out of fifteen specimens were gaping before shaking, twelve were gaping ten minutes afterwards. This result and subsequent observations made at various times during the course of other experiments indicate that vibration without rotation is an effective stimulus, but that specimens vary enormously in their sensitivity to the stimulus. Some require vigorous shaking in the hand, while others respond to vibrations set up by moving a heavy object on the laboratory bench upon which the animal in its dissecting dish is lying (Fig. 7). This latter fact proved to be an occasional source of irregularity in the recordings made of the slow rhythm (see p. 161).

The fact that a much greater proportion of animals responded to vibration with rotation than to vibration alone suggests that rotation is an additional stimulus for the shell-opening reflex. No attempt has been made to identify the receptors for the two stimuli, but it is conceivable that rotation stimulates the statocyst, while vibration causes intermittent pressure of the foot against the mantle lobes. Woortmann (1926) found that the latter was an effective stimulus for shell opening in *Mytilus*, but Nadort (1943) failed to demonstrate this in *Anodonta*; he may, however, have been unfortunate in using specimens of low sensitivity.
Lowy (1953) investigated the contraction and relaxation of the adductor muscles of *Mytilus*, and obtained experimental results which he considered favour the 'tetanus' hypothesis of prolonged contraction, although he had difficulty in reconciling certain of his results with this view. The main evidence in support of this hypothesis is that prolonged contraction of the posterior adductor is accompanied by electrical activity of the muscle. Two facts, however, do not accord well with this view, but favour rather the 'catch mechanism' hypothesis of von Uexküll (1929): they are (a) the continued state of contraction for about 20 hr. after denervation of the muscle, and (b) the occurrence of bursts of muscle potentials during the spontaneous relaxation of the muscle.

If therefore a theory could be propounded which incorporates the 'catch mechanism' explanation of adductor tonus and at the same time offers an explanation of the electrical activity associated with it, it might prove to be the solution to the problem in *Mytilus*. Such a theory is suggested by the foregoing conclusions regarding *Anodonta*.

In *Anodonta* there must be a treble innervation of the adductor muscles, one group of motor nerve fibres supplying the striated muscle fibres and producing phasic contractions (which may summate to produce a tetanus), another group of activating fibres supplying the unstriated muscle fibres and producing increased tonus, and yet a third group of inhibitory fibres supplying the same muscle fibres and producing decreased tonus.

*Mytilus*, like *Anodonta*, is capable of both phasic and tonic contractions of its adductor, but there is no obvious differentiation of the muscle into two parts, so it must be accepted either that the muscle fibres are all capable of exhibiting both types of contraction or that there are two types of fibres present but completely interspersed. But in either case, the nervous mechanism controlling the adductor activity may be the same as in *Anodonta*. This postulate offers an explanation of apparently all the experimental data in Lowy's paper.

Thus the prolonged tonic contractions such as occur when the mussel is out of water could be regarded as being of the 'catch mechanism' type, and therefore requiring a volley of nerve impulses to bring it to an end. The shorter periods of contraction such as occur when the animal is showing spontaneous activity in sea water could be regarded as periods of tetanus, which would cease when electrical activity ceased. This type of electrical activity would tend to occur in bursts or volleys corresponding to the onset of the contractions, and would be equivalent to the impulses of the rapid rhythm in *Anodonta*. But it has been stated that the rapid rhythm often persists long after a period of tonic contraction has started. If the same thing happens in *Mytilus*, the continued electrical activity during tonic contraction is explained (Lowy has pointed out that this electrical activity is exactly the same as that accompanying spontaneous phasic contractions), as is also its cessation when the visceral ganglia are removed. This hypothesis explains also the continued tonic contraction of the 'deafferentated' posterior muscle-ganglion...
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preparation, since, if *Mytilus* is like *Anodonta* in this respect, the inhibitory nerves to the posterior adductor muscle arise in the cerebropleural ganglia and merely pass through the visceral ganglia.

Other facts in Lowy’s paper could be interpreted in terms of this hypothesis, but enough has been said to show that this hypothesis is worth investigating as a possibly more satisfactory explanation of the neuromuscular physiology of *Mytilus*.

**SUMMARY**

1. *Anodonta cygnea* L. in captivity exhibits rhythmical behaviour as follows:

   (a) Periods of activity and quiescence alternate, with frequencies varying with different specimens from 3 to 30 per week. During the periods of quiescence the shell valves are completely adducted. During active periods the valves normally gape. (The ‘slow’ rhythm.)

   (b) During the active periods the adductor muscles show frequent rapid contractions followed by slow relaxations. This activity is also rhythmical, and has a frequency of up to 20 per hour. (The ‘rapid’ rhythm.)

2. The neuromuscular mechanism of these rhythms has been investigated, and it is shown that: (a) both adductor muscles participate in the rhythms; (b) the slow rhythm is a function of the unstriated portions of the muscles; (c) the rapid rhythm is a function of the striated portions of the muscles; (d) the rapid rhythm in each adductor muscle is controlled by its nearest ganglia; (e) the slow rhythm in each adductor muscle is controlled by the combined effect of the nearest ganglia, which tend always to produce a tonus, and the cerebropleural ganglia, which at intervals inhibit that tonus; (f) the control of the rhythms appears to be intrinsic in the nerve ganglia, and independent of peripheral stimulation.

3. The effect of morphine hydrochloride on *Anodonta* has been investigated, and it is shown that, in addition to a possible narcotic effect, it abolishes the tonus of the unstriated portions of the adductor muscles.

4. A reflex is described which leads to the relaxation of the tonus in the adductor muscles, and the commencement of a new period of activity. The stimulus is vibration and/or rotation.

5. On the basis of the above conclusions regarding *Anodonta* a hypothesis is put forward to account for certain facts about the contraction and relaxation of the adductor muscles of *Mytilus*.

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