

THE MOVEMENTS OF THE PROBOSCIS IN *GLYCERA DIBRANCHIATA* EHLERS

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(With Five Text-figures)

IN an earlier paper (Wells, 1937), the writer studied experimentally the movements of the proboscis in *Arenicola marina*, a worm which lives in a burrow and feeds by taking in the surrounding mud, and in which the brain and sense organs are very much reduced. It was shown that the intact worm, observed in a glass tube, generally exhibits periodic outbursts, either of rhythmic extrusion and withdrawal of the proboscis or, in less vigorous worms, of movements of similar type which do not result in actual extrusion, the activity outbursts alternating in either case with periods of rest. This probably means that the worms, under natural conditions, have a "feeding rhythm", ingesting mud for a few minutes, then resting, and so on. Moreover, by considering a series of dissected preparations, it was shown that the pacemaker determining this rhythm was situated in the oesophageal wall. Periodic outbursts of excitation originate in the oesophagus and are transmitted forwards along the wall of the proboscis to the central nervous system, calling forth correlated movements of the musculatures of the proboscis itself and of the body wall.

In certain circumstances, as is well known, *Arenicola* can be made to exhibit continuous burrowing activity for a considerable time. This occurs, for instance, if it is dug out of its burrow and placed on the surface of the sand. Here again, it is at least possible that the excitations responsible for the movements of the proboscis and body wall originate in the oesophagus and not in the central nervous system. If one injects adrenaline into an intact *Arenicola*, the animal burrows for half or three-quarters of an hour; and in the dissected preparations it was shown (*a*) that adrenaline induces continuous activity of the proboscis and body wall, and (*b*) that this is the result of a specific action of the drug on the oesophagus, which now discharges its excitations forwards with a continuous rhythm instead of the usual intermittent rhythm. The following picture of the mechanism of proboscis activity in *Arenicola* was therefore suggested: the immediate cause of extrusion is an impulse (or volley of impulses) originating in the oesophagus; normally these excitations arise in periodic outbursts, producing the "feeding rhythm"; in certain circumstances ("flight reaction") the oesophageal rhythm becomes continuous, perhaps as a result of adrenaline secreted into the blood or by means of adrenergic fibres running to the oesophagus from the central nervous system. Whether any mechanism exists for inhibiting the oesophageal pacemaker is not known.

Arenicola is of course highly specialized for its very atypical mode of life. In most errant polychaetes the proboscis is used for seizing suitable food masses (e.g. living prey) when they present themselves, and one would therefore expect it to be under the immediate control of the brain. *Arenicola* does not actively seek its food. It simply lies with its head at the blind end of its burrow and eats away at its environment. As fast as the mud is ingested, fresh supplies slide down from above to replace it (Thamdrup, 1935). It is therefore not surprising that the rate of ingestion is controlled by rhythmic processes of visceral origin. With errant polychaetes, on the other hand, food is only occasionally available, so the feeding act may be presumed to be a reflex elicited by chemical, mechanical or visual stimuli.

Various observations of reflex activity of the proboscis in errant polychaetes have been made. In *Glycera unicornis*, for instance, Stolte (1928, 1932) describes ejection of the proboscis in response to chemical or mechanical stimulation. Either single or repeated ejections can be elicited by a single mechanical stimulus of brief duration. If ejection occurs in response to disturbance of the water, the proboscis may be thrown accurately in the direction from which the disturbance comes: "ja sogar beim Blasen auf die Wasseroberfläche erhebt das Tier sein Prostomium und den Kopfteil und schleudert zielsicher seinen Rüssel aus." The receptors for this reflex are situated on the prostomium, and Stolte's description, quoted above, very strongly suggests that the proboscis, at least for the purposes of this reflex, is immediately controlled by the brain.

A similar reflex control of proboscis activity is indicated by the observations of Copeland & Wieman (1924) on *Nereis virens*. If suitable food (fish, crab or mollusc meat) is dropped near the burrow, the worm creeps out in the direction of the food and seizes it with its jaws. However, proboscis activity may also occur spontaneously in this species, in the apparent absence of chemical stimulation. Dr Manton Copeland (private communication), while watching the behaviour of *N. virens* kept in glass tubes for considerable periods of time, on several occasions saw a worm everting its proboscis gently but regularly within the tube for a while—an observation which recalls the periodic outbursts shown by *Arenicola*, although in *Nereis* the outbursts did not occur at regular intervals.

In view of these facts, information is to be desired on the relative roles of the circumoesophageal commissures and brain on the one hand, and the stomatogastric ganglia on the other, in determining movements of the proboscis in errant polychaetes. Investigators who have studied the anatomy of the polychaete stomatogastric system have frequently commented on its size and elaboration and have suggested that it has important motor functions. Thus Gravier (1898), after describing in detail the structure of the stomatogastric system in *Glycera convoluta*, writes: "Si l'on tient compte de l'importance du développement des ganglions qui appartiennent en propre à la trompe, dont le volume est notablement supérieur à celui des centres nerveux prostomiaux, on est amené à considérer le système nerveux proboscidien, non comme une dépendance du cerveau moyen, auquel il ne se rattache, du reste, que par deux nerfs, mais comme un ensemble ayant une

véritable autonomie, superposé au système nerveux du reste du corps." However, no physiological investigations have yet been published which enable us to determine how far the stomatogastric system is a "dépendance" of the central nervous system and how far it enjoys a "véritable autonomie". The present paper is a contribution towards the bridging of this gap.

During August and early September, 1936, at the Marine Biological Laboratory, Wood's Hole, I made some experiments on errant polychaetes, working along the lines of my previous work on *Arenicola*, to investigate the relation between the stomatogastric and central nervous systems. The first experiments were made on *Nereis virens*, which has an elaborate stomatogastric system described by Turnbull (1876). I found that the excised pharynx, with a short length of oesophagus attached but free of brain or circumoesophageal commissures, generally showed vigorous rhythmic activity when suspended in sea water. In some of the preparations, grouped beats appeared which were not unlike the rhythmic outbursts of the isolated extrovert of *Arenicola*. However, the type of behaviour obtained varied greatly, not only from preparation to preparation but even from time to time in the same preparation; thus a pharynx which has been relatively inactive may suddenly begin, and continue, a vigorous contractile rhythm. Evidently, the stomatogastric centres have a considerable degree of potential autonomy, and equally evidently, the factors determining their actual activity are complex. Partly because of the great variability of behaviour exhibited by the preparations, and partly because the proboscis of *Nereis* is too compact to be conveniently dissected for simultaneous kymographic recording of the movements of the various parts, I gave up the work on *Nereis* and turned to *Glycera dibranchiata*, a species which proved to be admirably suited for experiments of this kind.

(1) THE ANATOMY OF THE EXTROVERT

The anatomy of the front part of the gut in *Glycera* has been described by Ehlers (1868) and in great detail by Gravier (1898). In what follows Gravier's nomenclature of the parts is not followed. The names here adopted follow the general English practice, as exemplified, for instance, by Benham (1901), according to which the proboscis of an errant polychaete is regarded as consisting of an eversible buccal portion followed by a pharynx, which is not turned inside out during eversion and contains the jaws and associated glands.

The relevant parts of the gut in *Glycera* are (see Fig. 1, I):

(1) The *buccal tube* ("gaine pharyngienne" of Gravier, "Rüsselröhre" of Ehlers), a long, reddish, muscular tube running back from the mouth. Generally it traverses about twenty body segments, but it can contract down to a small fraction of its relaxed length. This is the portion of the proboscis which is turned inside out during extrusion. In this respect, it corresponds to two distinct regions of the *Arenicola* extrovert—the "buccal mass" and "pharynx" (terminology of Ashworth, as adopted in my earlier paper)—taken together.

(2) The *pharynx* ("trompe pharyngienne" of Gravier, "Kieferträger" of Ehlers), a shorter region, reddish and very muscular. The front part of the pharynx

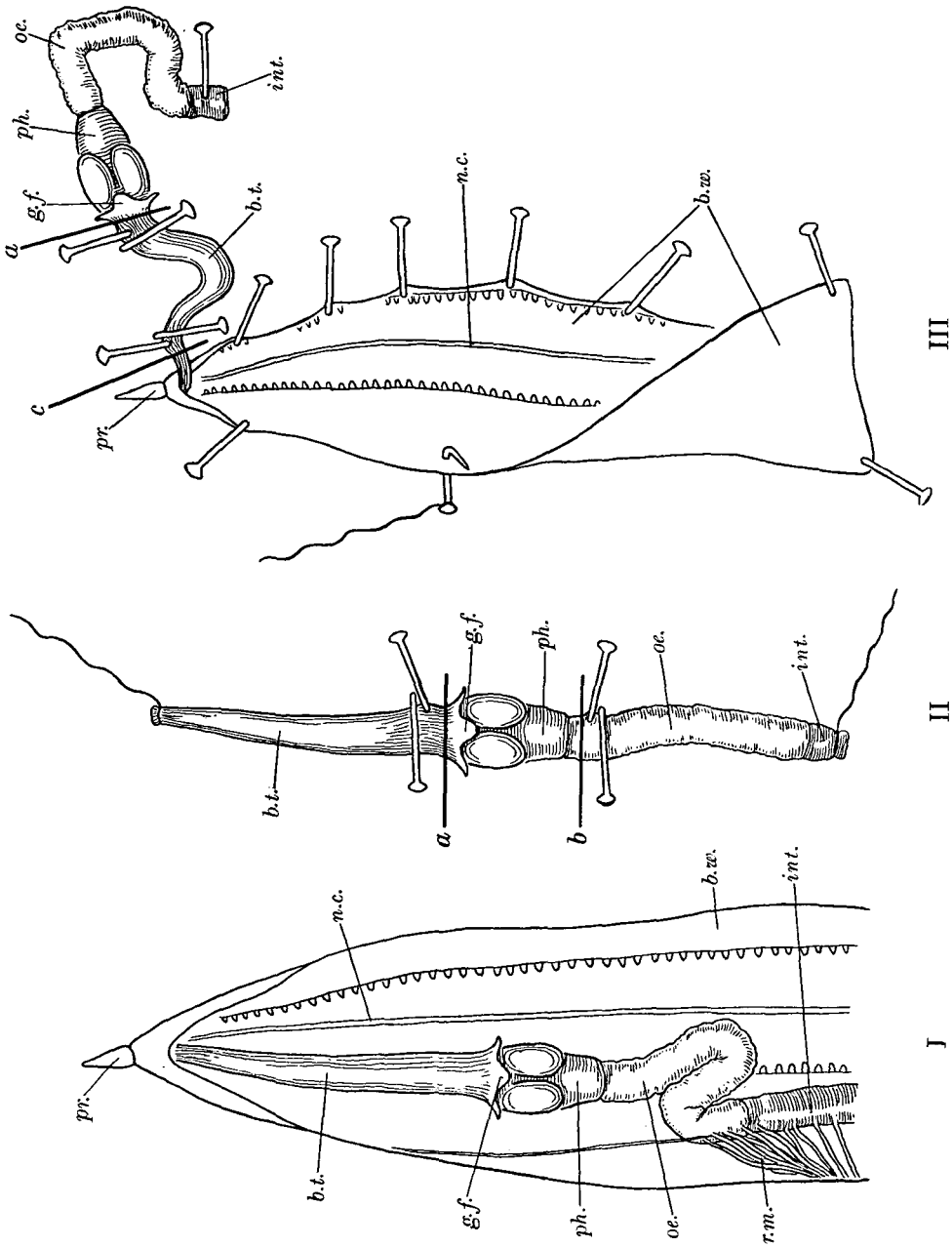


Fig. 1. I. Anterior end of *Glyceria*, opened along the dorsal side. II. "Isolated extrovert" preparation. III. "Extrovert-body wall" preparation. *b.t.* buccal tube; *b.w.* body wall; *g.f.* ganglionic fringe; *int.* intestine; *n.c.* ventral nerve cord; *ph.* pharynx; *pr.* prostomium; *oe.* oesophagus; *r.m.* retractor muscle. *a, b, c*, indicate positions in which cuts were made during the experiments.

has four cushion-like swellings on its walls containing the jaws with their associated muscles and glands. The anterior boundary of the pharynx, where it meets the buccal tube, is marked by a delicate four-lobed fringe ("membrane quadrilobée" of Gravier) which projects from the coelomic surface of the pharynx. As Ehlers showed, this fringe is ganglionic in nature. *Arenicola* has no jaws, and no pharynx of this type. Probably the pharynx of *Glycera* corresponds to that part of the extrovert in *Arenicola* which, in my earlier paper, I termed the "post-pharyngeal ring".

(3) The *oesophagus* ("ventricule" of Gravier), a white tube, normally long and thrown into an S-curve when the proboscis is withdrawn, but capable, like the buccal tube, of contracting down to a small fraction of its relaxed length. The boundary between pharynx and oesophagus is clearly indicated (*a*) by a constriction, and (*b*) by the change from the pink colour of the pharynx to the white of the oesophagus.

(4) The *intestine*, a thin-walled, yellowish tube, running through the remainder of the body, and in which digestion occurs.

Operative procedures on *Glycera* are greatly facilitated by the fact that the buccal tube, pharynx and oesophagus lie free in the body cavity. There are no transverse septa, and the only structures suspending the gut are as follows: (1) A series of short muscles runs from the mid-dorsal line of the body wall to that of the intestine, and serves to anchor the latter. The anterior members of this series, inserted into the gut in the region where oesophagus and intestine join, are longer and thicker than the rest and act as retractors of the proboscis. (2) Two exceedingly fine muscles, one dorsal and one ventral, run forwards from the junction of oesophagus and intestine to the body wall.

The stomatogastric nervous system is minutely described in Gravier's memoir. For our purposes, it is only necessary to note the following facts. The system arises as a single pair of nerves from the sides of the oesophageal connectives. Almost at once, these nerves give rise to a ring nerve running round the buccal tube close to the mouth. From this ring, eighteen longitudinal nerves, symmetrically disposed round the buccal tube, run back along it to its hinder end. Here they communicate with a complex of ganglia, lying in the front end of the pharynx and including, among other structures, the delicate ganglionic fringe already noted. From this complex, whose detailed anatomy need not be considered here, four nerves run back along the pharynx and oesophagus. These nerves lie, one in the plane of symmetry of each jaw. They have ganglionic swellings ("ganglions de renforcement" of Gravier) about half-way down the pharynx, and again in the extreme front end of the oesophagus, immediately after its junction with the pharynx.

In what follows, the ganglia at the anterior end of the pharynx and the "ganglions de renforcement" have been treated as a single unit, and will be termed collectively the *pharyngeal ganglia*. No attempt has been made to study separately the functions of these various ganglia.

(2) AUTONOMOUS ACTIVITY OF THE PHARYNGEAL GANGLIA

The activity of the isolated proboscis was studied by cutting the gut across immediately behind the mouth, and again a few millimetres posterior to the junction of oesophagus and intestine. The part thus isolated was pinned out on a disk of cork glued to the bottom of a finger bowl. The method of pinning is shown in Fig. 1, II. A pin is inserted through one side of the buccal tube, as near as possible to its edge, and a couple of millimetres oral to the ganglionic fringe of the pharynx; a second pin slopes across the buccal tube, without perforating it, to prevent it from sliding up the first. A similar pair of pins holds the oesophagus, a couple of millimetres aboral to its junction with the pharynx. Threads are attached to the free ends of the buccal tube and oesophagus, and pass to light isotonic levers, so that their movements are separately recorded. The pharynx also contracts actively, but its movements were not recorded. In this preparation, owing to the radial symmetry of the stomatogastric nervous system, there is little interference with the conduction paths from region to region of the gut. During an experiment, the various regions can be isolated from each other by cutting at *a* or *b*, without interrupting the continuity of the record.

Sea water was used as bathing medium throughout this investigation.

Both halves of a preparation of this kind show spontaneous activity, but they differ from each other in the type of contraction exhibited (Fig. 2). The oesophagus gives somewhat irregular tone-waves, occasionally with more rapid and frequent contractions superposed upon them. The buccal tube, on the other hand, gives contractions of wide amplitude and brief duration, which do not follow each other in a regular rhythm but occur in irregular bursts. Occasionally, the buccal tube may show a tone-wave resembling those seen in the oesophageal tracing.

Another important difference between the oesophagus and buccal tube emerges when the preparation is cut at *a* or *b* (Fig. 1). After cutting at *a*, the buccal tube generally becomes absolutely relaxed (Fig. 2). This cut severs its connexion with the pharyngeal ganglia. In a few preparations only, the buccal tube shows very small, slow contractions after isolation from pharyngeal influences. These are, however, quite different from the vigorous, rapid contractions seen before the cut was made, and it seems clear that the buccal tube itself has only a slight degree of autonomy, the powerful contractions seen in the intact preparation being due to impulses coming forwards from the pharyngeal ganglia. The activity of the oesophagus, on the other hand, is not abolished by section of the preparation at *b* (Fig. 2). Tone-waves of wide amplitude, sometimes with more rapid contractions superposed, still persist. Evidently, the oesophagus has considerable powers of autonomous movement, and does not depend, as the buccal tube mainly does, on impulses from the pharyngeal ganglia.

In a few cases (but not in most), synchronous tone-waves occur in the two ends of the preparation. An instance of this is seen in the first part of Fig. 2. Unhappily I have not been able to find out whether these correlated contractions are initiated by the oesophagus or by the pharyngeal ganglia. They appeared only in a minority

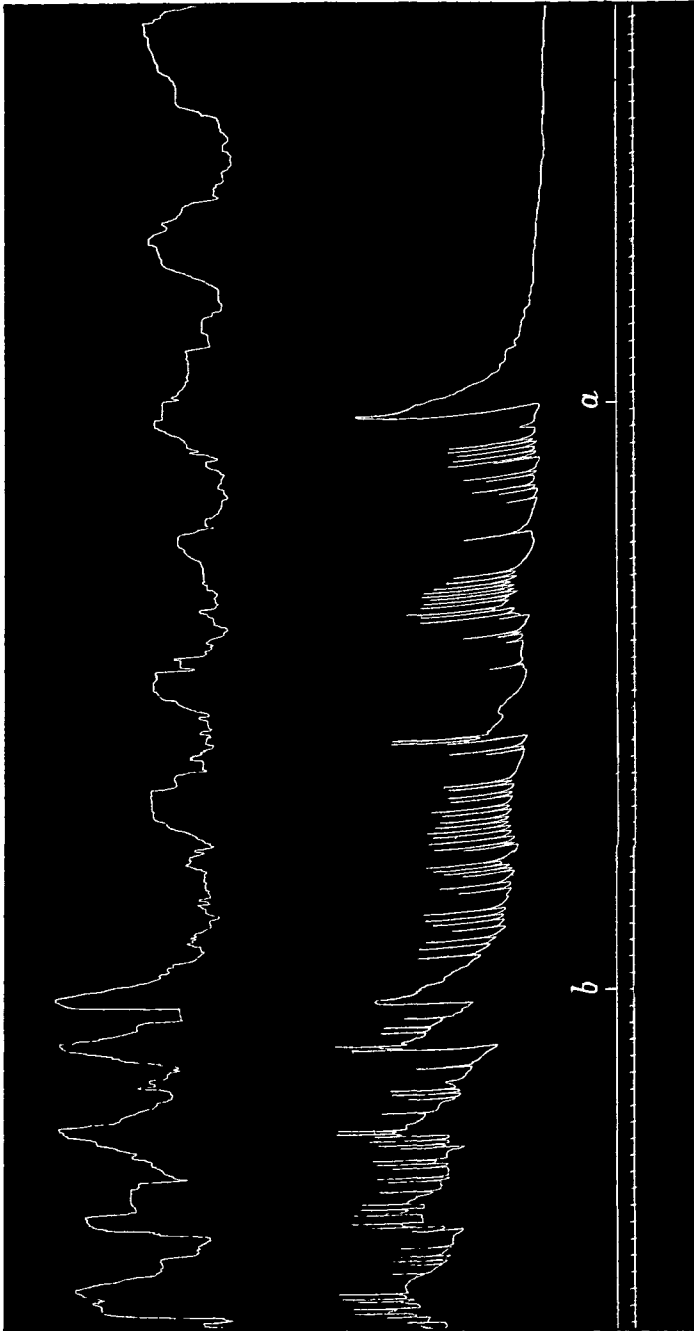


Fig. 2. "Isolated extrovert" preparation. Oesophagus writes above, buccal tube below. Preparation transected at *b* at first signal, and at *a* at second (see Fig. 1, II).
In all records: read from left to right; upstroke of lever means contraction of preparation; time signal marks once a minute.

of preparations, and after cutting the gut at *b* the behaviour pattern of both parts is usually somewhat modified, as in Fig. 2, so that one cannot say with certainty that the waves continue after the cut in one part while ceasing in the other.

A further point was noticed in some, but not all, of the preparations. On cutting at *b*, the buccal tube may begin to contract with a strikingly regular rhythm, the effect gradually passing off until, in half an hour or so, it has ceased altogether. This effect is seen in the experiment of Fig. 3, but not in that of Fig. 2. If, during the period of regular contraction resulting from a cut at *b*, the preparation is cut again at *a*, the buccal tube immediately becomes quiescent. The effect is therefore due to impulses discharged into the buccal tube by the pharyngeal ganglia. Probably, these have been excited by the section of afferent fibres from the oesophagus.

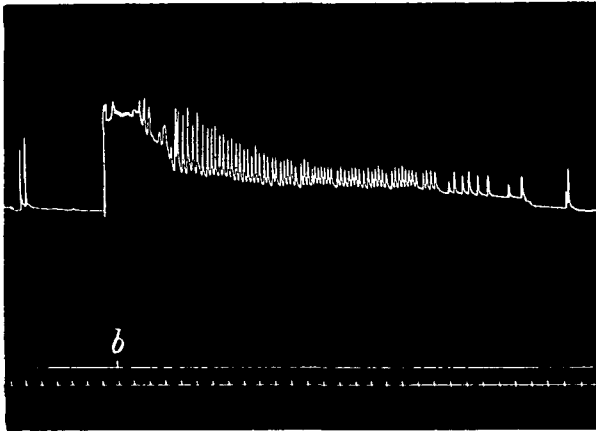


Fig. 3. "Isolated extrovert" preparation. Movements of buccal tube only shown. At signal, preparation was transected at *b* (Fig. 1, II).

If these results are compared with those reported in my previous communication on the *Arenicola* extrovert, a certain degree of parallelism emerges. In both cases, that part of the gut which is turned inside out during extrusion (buccal tube in *Glycera*, buccal mass plus pharynx in *Arenicola*) has only a limited degree of autonomy and is subject to invasion by impulses coming from the hinder part of the extrovert. The hinder part is however more complex, both anatomically and physiologically, in *Glycera*, where it includes the oesophagus and pharynx, than in *Arenicola*, where it consists of the oesophagus and perhaps the post-pharyngeal ring.

(3) INHIBITION OF THE PHARYNGEAL GANGLIA BY THE CENTRAL NERVOUS SYSTEM

In *Arenicola*, excitations originating in the oesophagus spread, via the proboscis, to the central nervous system and thence to the muscles of the body wall, determining extrusions of the proboscis. I have therefore set up dissections of *Glycera* in which the movements of the buccal tube and body wall were separately recorded

while the normal conduction paths between them were still intact, to find out whether a similar invasion of the central nervous system by impulses of stomato-gastric origin could be detected.

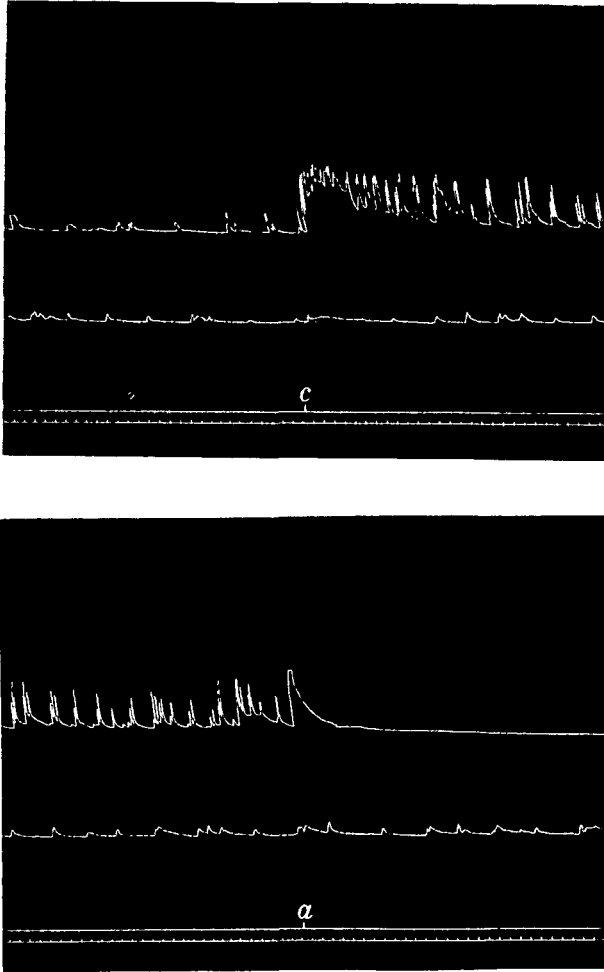


Fig. 4. "Extrovert-body wall" preparation. Buccal tube writes above, body wall below. Preparation transected at *c* at first signal, and at *a* at second (see Fig. 1, III). Cut *a* was made 1 hour 38 min. after cut *c*.

The preparation is illustrated in Fig. 1, III. A *Glycera* is opened by a cut along one of the lines of parapodia; the extrovert is freed by dividing the intestine shortly behind its junction with the oesophagus and cutting through the retractor muscles; and the parts are pinned out as shown. A hook through one of the body-wall flaps bears a thread running to a lever. Movements of the buccal tube are recorded by passing a second hook (not shown in the diagram) underneath the buccal tube, without perforating it. The weight of a light lever, connected by a thread to this

second hook, is enough to pull up the buccal tube into a loop; when the tube contracts the hook is depressed and the writing point rises.

The most striking feature of most of these preparations is their extreme quiescence. The body wall generally shows slight movements but the buccal tube, in contrast to its behaviour in the isolated preparation described in the last section, is very still. If, however, one cuts through the gut at *c* (Figs. 1 and 4), the buccal tube becomes active, and usually continues to contract with an irregular rhythm until a second cut is made at *a*, when it again becomes passive.

This latter result, of cutting at *a*, indicates, as already pointed out in the last section, that the contractions of the buccal tube are due to impulses coming forwards from the pharynx. The former result—that the buccal tube is quiet until cut at *c*, when it becomes active—might be due to either of two causes: (1) the cut at *c* excites the pharyngeal ganglia, much as a cut at *b* sometimes does (Fig. 3), or (2) the pharyngeal ganglia are inhibited by the central nervous system while the preparation is intact, and cutting the conduction paths at *c* releases the pharynx from this inhibitory influence. The latter view seems more probable than the former. Such exciting effects in the pharynx as are certainly due to cuts pass off comparatively rapidly, but the activity which appears after cut *c* usually continues, without any sign of diminishing, for hours. In one experiment, for instance, the buccal tube of the intact preparation was quiet for 2 hours 12 min. Cut *c* was then made, and the buccal tube at once became vigorously active, its activity continuing without obvious diminution for 2 hours 10 min. At the end of this time cut *a* was made and the buccal tube again became quiet. It is unlikely that section of the nerves could produce an exciting effect of such long duration.

(4) CORRELATED ACTIVITY OF THE PROBOSCIS AND BODY WALL

Most of the preparations, as already stated, showed little or no mechanical activity in the proboscis, and only little in the body wall, before cuts were made. In a few, however, associated outbursts of contraction appeared in the body wall and buccal tube (Fig. 5). These outbursts recall the similar outbursts, involving both extrovert and body wall, in *Arenicola*, but whereas in the latter worm they are due to excitations originating in the oesophagus, in *Glycera* this is not the case. If the preparation is transected at *a*, the outbursts continue as before (Fig. 5). Evidently, they do not originate in the pharynx or oesophagus. If it is cut at *c* instead of *a*, the outbursts continue unaltered in the body wall. The outbursts are therefore presumably due to excitations originating in the central nervous system.

It is possible, at least, that these outbursts correspond to extrusion movements of the intact worm. As Gravier (1898) pointed out, the actual extrusion act is caused by a contraction of the body wall muscles, which raises the pressure of the coelomic fluid and thus drives out the proboscis through the mouth. If, however, one watches carefully an intact worm, the appearance strongly suggests that this "squeeze" action of the body wall is immediately preceded by a vigorous contraction of the longitudinal muscles of the buccal tube. The pharynx can often be

plainly seen through the semi-transparent body wall to slide swiftly forwards towards the mouth before its ejection, and a moment or two may elapse between the end of this movement and the beginning of the actual eversion of the buccal tube. It is during the latter movement that the body wall can be seen to contract. This makes it pretty plain that, in *Glycera* as in *Arenicola*, extrusion involves co-ordinated movements of the body wall and the proboscis itself. The only co-ordinated movements of the two structures seen in my dissected preparations were those described in the last paragraph. If this argument be admitted, and the

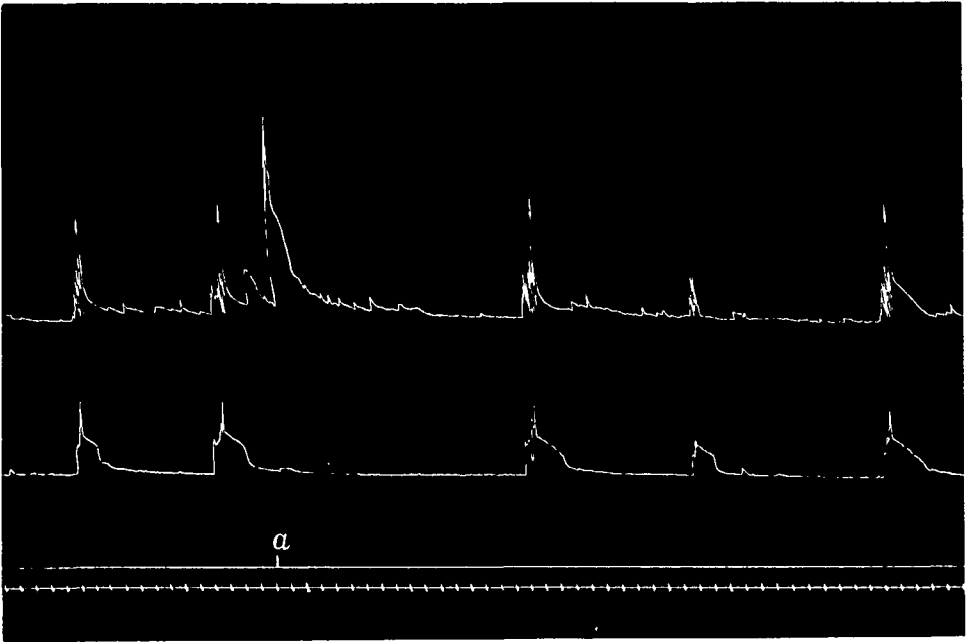


Fig. 5. "Extrovert-body wall" preparation. Buccal tube writes above, body wall below. Preparation transected at *a* at signal (see Fig. 1, III).

outbursts in question be regarded as outbursts of extrusion, then it follows that the extrusion act in *Glycera* is brought about by impulses coming directly from the central nervous system.

In any event, even if the somewhat speculative argument of the last paragraph be set aside, it is clear that the dissected preparations of *Glycera* differ markedly in behaviour from those of *Arenicola*. In *Glycera*, the buccal tube serves two masters. It contracts in response to impulses coming from the pharynx (Fig. 2) or from the central nervous system (Fig. 5). In *Arenicola*, impulses of stomatogastric origin invade the central nervous system and the former system seems therefore to play a dominant role. In *Glycera*, on the other hand, the tables are turned; the stomatogastric ganglia are apparently inhibited by the central nervous system, which may also invade the proboscis with excitation outbursts of its own.

SUMMARY

1. The gut of *Glycera* consists of (a) the buccal tube, (b) the pharynx, containing the jaws with their associated muscles and glands and the principal stomatogastric ganglia, (c) the oesophagus, leading from the pharynx to (d) the intestine, in which digestion occurs.

2. An "isolated extrovert" preparation is described, consisting of the buccal tube, pharynx and oesophagus. The movements of the buccal tube and oesophagus are recorded separately. The preparation has the following properties:

(a) The buccal tube shows vigorous, rapid contractions with a somewhat irregular rhythm. These contractions are due to impulses coming forwards from the pharynx, the buccal tube itself having little power of spontaneous movement.

(b) The oesophagus shows tone-waves, on which more rapid contractions of small amplitude may be superposed. These contractions and tone-waves are due to impulses originating in the wall of the oesophagus itself.

(c) In a few preparations only, synchronous movements of buccal tube and oesophagus were seen. The site of origin of this synchronous activity was not determined.

3. An "extrovert-body wall" preparation is described, in which the movements of the body wall and buccal tube are separately recorded while the normal nervous conduction paths between them remain intact. The preparation has the following properties:

(a) In most cases the body wall shows slight movements only, and the buccal tube moves little or not at all. If, however, the buccal tube be cut across close to the mouth, it begins an irregular rhythm of vigorous contractions, due to impulses originating in the pharynx, which usually continues without diminution for hours. The quiescence of the buccal tube before this cut is made indicates that the central nervous system normally exerts an inhibitory influence on the pharynx.

(b) In a few preparations, correlated outbursts of contraction in the body wall and buccal tube were seen. These outbursts, which possibly correspond to extrusion movements of the intact worm, are due to impulses originating in the central nervous system.

4. The results are compared with those previously obtained on *Arenicola marina*, and reported in an earlier paper.

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