FUNCTION OF THE PENIAL APPARATUS OF *HELIX ASPERSA* MÜLLER

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[*Manuscript received June* 9, 1961]

Summary

The distribution of the penial nerve in *Helix aspersa* Müller is described.

If the penis sheath is removed from the animal with the brain and penial nerve attached, it shows rhythmic activity which is at a maximum in autumn and again in spring–early summer; at other seasons activity is minimal or non-existent. In the absence of the brain similar activity can be induced by adrenaline.

There is reason to think that the activity of the penis sheath is due to an "injury discharge" from the central nervous system, caused by the damage sustained at dissection. There is some evidence also for the view that a similar discharge is caused by the calcareous dart at copulation. On the strength of this evidence it is suggested that the dart serves to induce penial activity at copulation.

Extracts of liver or gonad or of both are capable of affecting the rhythmic activity of the penis sheath–brain preparation; the significance of this finding is obscure. Extracts of certain other tissues and solutions of acetylcholine and histamine did not affect the preparation at the concentrations tested.

I. INTRODUCTION

There now exists a modest amount of information on the functions of the various parts of the reproductive tract of pulmonate molluscs (Duncan 1958; Goddard 1960*b*). However, almost nothing is known of how these functions are controlled and coordinated. Analogy with vertebrates and deductions from anatomical evidence suggest that certain functions (e.g. penial activity, use of the dart) are under nervous control, whilst others (the glandular activities of the reproductive tract) are under endocrine control. It is the purpose of this work to investigate certain aspects of the control of penial function in *Helix aspersa* Müller.

The penis is located in a penis sheath which opens into the genital atrium. At copulation it is extruded from the sheath "comme le doigt d'un gant" (Moquin-Tandon 1855). Details of the nerve supply to the penial apparatus are given in Section III.

An accessory reproductive structure, the calcareous dart, will feature in this work, and it seems advisable to review the literature on this remarkable organ.

Darts are "nearly confined to the genus *Helix*", though not all species of the genus possess darts (Ashford 1883), and in some the darts appear to be non-functional (Collinge 1890).

The dart is produced by, and is carried in, the dart sac which opens into the vagina. In *H. aspersa* it is a tubular structure some 8–10 mm long, tapering distally to a fine transparent point. The sides are raised at four points around the circumfer-

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ence to form longitudinal ridges which serve to strengthen the shaft and act as cutting blades. The base of the dart is shaped like an inverted cup; it sits upon, and is lightly attached to, the apex of the "dart tubercle". The tubercle is located in the fundus of the dart sac and is thought by some workers to be responsible for secretion of the dart. (Others hold that the walls of the sac are at least partly concerned in its formation.)

It is generally agreed that the mineral component of the dart is calcium carbonate. When this substance is removed with acid, there remains a matrix of organic material, the nature of which has not been determined.

There appears to have been much speculation during the last century on the function of the dart. Among the theories advanced was that of Collinge (1890) to the effect that it is a degenerate weapon of defence and in the past was much stronger and more often used than now. However that may be, most workers have long accepted the view that, in those forms with functional darts, it provides a sensory stimulus to copulation (Moquin-Tandon 1855; Baudelot 1863; Ashford 1883; Cooke 1895).

It is extruded from the dart sac "just before or during the act of copulation" (Cooke 1895) and the pointed tip is used to prick the body of the partner, usually in the region of the genital atrium. During this process it frequently happens that the dart is torn from its attachment to the tubercle and may remain pendant from the skin of the wounded partner or be lost.

Ashford (1883, 1884) and other workers reported that foreign darts can often be found deeply embedded among the viscera of *Helix*. They may be found, at dissection, in almost any part of the body, and much ingenuity has been exercised in trying to account for their presence. It has been suggested that at copulation the dart is ejected from the sac, and with such force that it pierces the body wall of the partner to become lodged among the internal organs. That this occurs, however, was denied by Moquin-Tandon (1855), Taylor (1881), and others, and the idea has not been generally accepted, although it still appears in some textbooks (Borradaile *et al.* 1959). Ashford (1883) discounted the suggestion that the dart is thrust *in toto* through the body wall of the recipient. He put forward the following hypothesis: during the pairing process the dart comes in contact with, and adheres to, the extruded penis; when the latter is withdrawn, the dart accompanies it into the genital atrium, and from that position it "works its way" to almost any tissue of the animal's body.

Finally, it appears that the dart is not essential for copulation. There are reports of snails (*H. aspersa* among them) which did not possess darts at the time but were nevertheless found in process of pairing (Moquin-Tandon 1855; Taylor 1883).

II. Methods

The anatomical studies reported in Section III were carried out with the aid of a stereoscopic dissecting microscope. Both live and fixed material were used.

The experimental work reported in Sections IV and V was carried out using an electrically driven recording drum and perfusion apparatus. The perfusion bath was filled with Hédon-Fleig solution (Lee 1950; Goddard 1960*a*) through which air or

oxygen was bubbled. All the experiments, except those reported in Section IV(*a*)(8), were conducted at room temperature.

Most of the dissections were carried out so that certain tissues (e.g. the brain) were left attached to the penis sheaths—details are given where appropriate. For each experiment the penis sheath was mounted in the perfusion bath so that its longitudinal contractions were registered on the smoked drum. The distal end of the sheath,

Fig. 1.—Dissection of *H. aspersa* to show distribution of penial nerve. *1*, buccal mass (reflected forward); *2*, supra-oesophageal commissure; *3*, right cerebral ganglion; *4*, suboesophageal ganglionic complex; *5*, penial nerve; *6*, penis sheath; *7*, dart sac; *8*, penis retractor muscle; *9*, flagellum; *10*, vas deferens. *a*, *b*, and *c*, "fibres" of the penial nerve (see text, p. 221).

usually with the atrial region, brain, and penial nerve attached, was anchored to the oxygen tube at the base of the perfusion bath; the proximal end, with the flagellum attached, was secured by thread to the stylus of a "heart lever"; the only exception occurs in Section IV(*b*)(10), where the penis sheath was mounted in the reverse sense so that the tissues attached to the distal end (body wall etc.) were readily accessible at the top of the perfusion bath.

III. Innervation of the Penial Apparatus

According to some workers (Rzymowska 1914; Schmalz 1914; Bargmann 1930) the penial nerve of *Helix* is given off by the right cerebral ganglion. However, Pelseneer (1906) states that in most pulmonates the fibres of the nerve originate in the pedal ganglion and merely traverse the cerebral ganglion.

Schmalz (1914) gave a detailed account of the distribution of the penial nerves in *Helix pomatia*. There are usually two nerves involved, and they leave the right cerebral ganglion as noted above. They soon join to form a plexus. The plexus gives off several branches, some of which serve the vas deferens, penis sheath, and flagellum, whilst the others are distributed about the vulva and send a branch to the dart sac.

Although this distribution resembles that found in *H. aspersa*, the detailed picture in the latter species differs substantially from that given by Schmalz.

The penial nerve leaves the right cerebral ganglion, passing downward and back between the penis sheath and the vas deferens. Under the binocular microscope the "nerve" is revealed as consisting of several, more or less distinct, parallel "fibres". The fibres are carried in a delicate investment of connective tissue. They are variable in size, the largest being about one-third of a millimetre in diameter.

A short distance from the cerebral ganglion the fibres diverge. A large fibre passes anteriorly to the distal end of the penis sheath (*a* in Fig. 1). A few fibres (at least two) pass posteriorly and run alongside the vas deferens to which they are eventually distributed. Three or four fibres, including the fibre of largest diameter, pass laterally for a short distance and then branch; the major strands continue laterally to the penis sheath (*b* in Fig. 1), whilst the minor strands pass posteriorly to the vas deferens (*c* in Fig. 1).

There are a number of anastomoses between the various "fibres" constituting the nerve. The anastomosing strands are always small, and they have been ignored in this description.

Some animals show minor variations on the above pattern, but it might well be that such variations are more apparent than real, since the dissection is not an easy one, and under the microscope difficulties of interpretation appear.

In the experimental work reported in Sections IV and V the vas deferens was severed close to the point where it meets the flagellum and passes over into the penis sheath. The branches of the penial nerve running to the vas deferens were also severed. Some of these branches pass from the vas deferens to the penis sheath. It follows that the innervation of the penis sheath was partially disrupted in these experiments. However, there is reason to think that the disruption was of little consequence as far as the activity of the penis sheath reported here is concerned.

IV. Rhythmic Activity of the Penis Sheath

(*a*) *Procedures and Results*

A preliminary experiment involving the penis sheath of the snail gave the following results:

1(*a*) The snail was dissected so that the head region remained largely intact,* with the penis sheath attached to the genital atrium. Care was taken to ensure that

* A mid-dorsal longitudinal incision allows the body wall on either side of the head to be deflected and permits manipulation of the penis sheath.

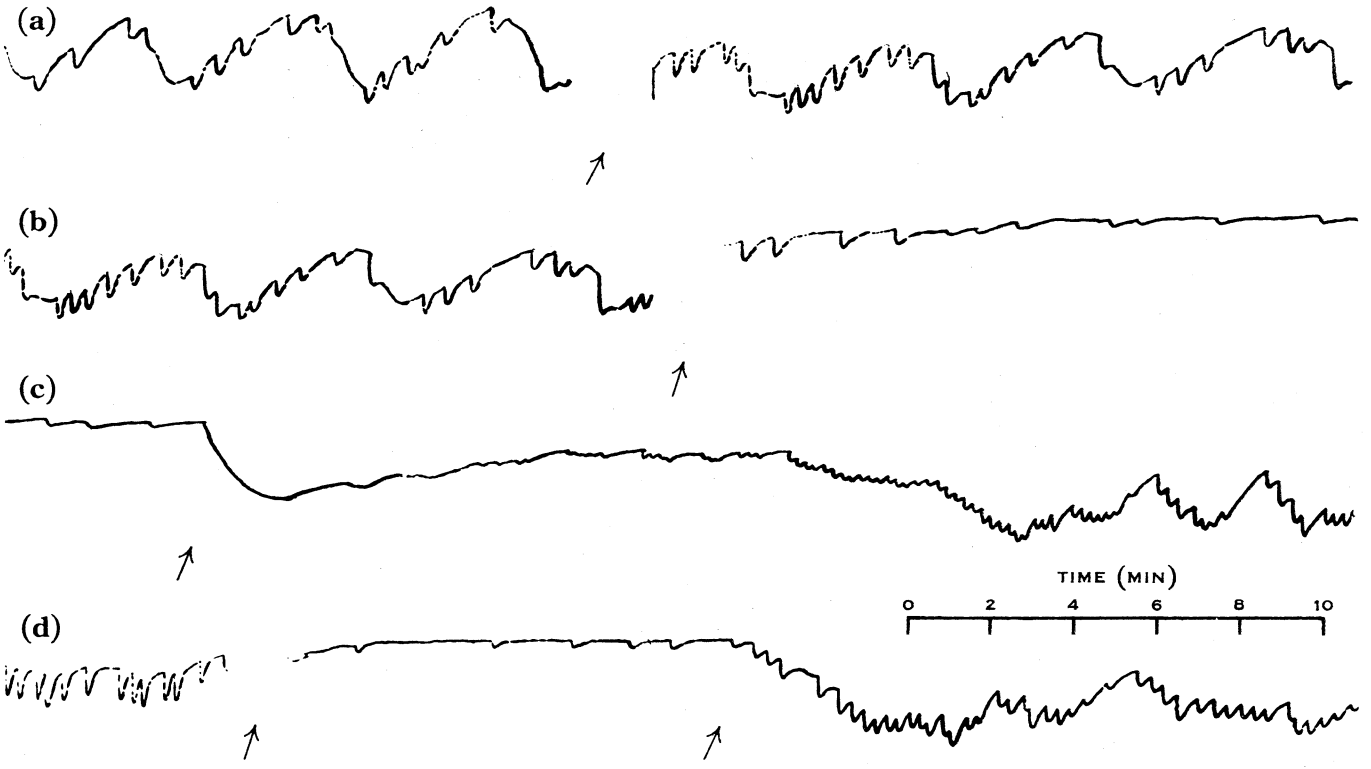

Fig. 2.—(*a*) Rhythmic contractions of penis sheath of *H. aspersa*. In all figures the traces read from left to right, and a downstroke indicates contraction of the longitudinal muscles of the penis sheath. At arrow all extraneous tissue except brain and penial nerve was removed. (*b*) Continuation of trace shown in Figure 2(*a*). At arrow the penial nerve was severed and the brain removed. (*c*) Continuation of trace shown in Figure 2(*b*). At arrow adrenaline (concn. 5×10^{-4}) was added to perfusion bath. (*d*) Continuation of trace shown in Figure 2(*c*). At first arrow adrenaline solution was replaced with saline; at second arrow adrenaline (concn. 5×10^{-4}) was again added. The response to adrenaline was more prompt than in Figure 2(*c*) but in both cases rhythmic activity was re-established. There is considered to be no significant difference between the rhythmic activity registered here and that registered in Figure 2(*a*). (A time base is given for all figures but as not all the traces have been reproduced at the same magnification, this base gives only a rough idea of the time intervals involved.)

the central nervous system (CNS)* and most branches of the penial nerve were not damaged. The preparation was mounted so that the longitudinal contractions of the penis sheath were registered on the smoked drum.

To ensure maximal leverage on the recording stylus, the vas deferens and penis retractor muscle were severed close to their junctions with the penis sheath. Note that the branches of the penial nerve running to the vas deferens, and thence to the penis sheath, were also severed (cf. Section III).

The muscle of the penis sheath registered pronounced rhythmic activity (Fig. 2(*a*), left of trace). The pattern of the rhythmic contractions will be considered later.

1(*b*) The preparation was removed from the perfusion bath for a short time (about 2 min) while further dissection was carried out. All tissue attached to the penis sheath, excepting the brain and penial nerve, was removed and the preparation was returned to the perfusion bath. The muscle of the penis sheath continued to register rhythmic activity (Fig. 2(*a*), right of trace).

1(*c*) The preparation was removed from the perfusion bath, the penial nerve was severed, and the CNS removed. When the penis sheath (now with no extraneous tissue attached) was returned to the bath, it was found that the rhythmic activity had disappeared (Fig. 2(*b*)); there were a few isolated contractions of small amplitude and a decrease in tonus of the muscle was noted.

1(*d*) Addition of adrenaline (concn. 5×10^{-4}) to the perfusion bath resulted in restoration of rhythmic activity (Fig. 2(*c*)). The latter disappeared when the adrenaline solution was replaced with saline and reappeared when adrenaline was again added (Fig. 2(*d*)).

The observations reported above were confirmed and supplemented by a series of experiments in which the following results were obtained:

(2) If the penis sheath is removed from the snail with the brain and penial nerve attached (vas deferens and penis retractor muscle severed), the penis sheath muscle shows a decidedly rhythmic pattern of contractions. The pattern is variable, but essentially it appears to consist of minor peaks superimposed on pulses of larger amplitude and period; the minor cycle is often obscure and tends to "fade out" before the major cycle. Some preparations show very complex patterns of activity, so much so that the above description cannot always apply; but, however complex the pattern, some sign of rhythm can always be detected.

(3) If the penis sheath is removed from the snail *without* the brain and penial nerve attached, the rhythmic pattern of contractions is found to be absent. Some such preparations show irregular contractions of small amplitude but there is no resemblance to the rhythmic pattern observed when the brain and penial nerve are present.

In the experiments reported below (Nos. 4–10) the penis sheaths were mounted *in situ*, i.e. attached to the genital atria and surrounding tissues.

(4) The penis sheath–brain preparation was mounted in the perfusion bath with the vas deferens and the retractor muscle severed close to their junctions with

* The terms "brain" and "central nervous system", as used here, are synonymous. They refer to the complex of ganglia surrounding the oesophagus.

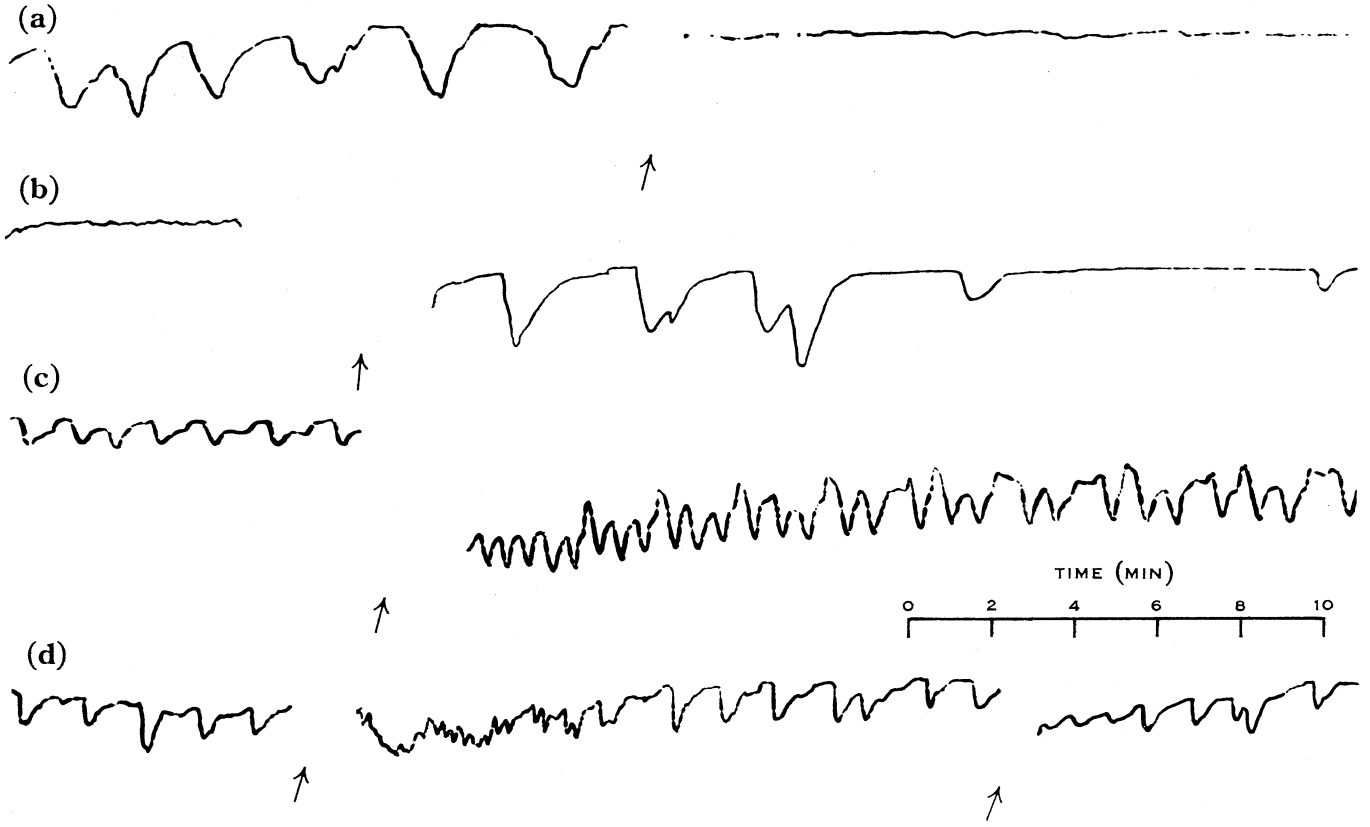

Fig. 3.—Penis sheath–brain preparation: (*a*) at arrow the penial nerve was severed. Rhythmic activity did not reappear. (*b*) At arrow some of the nerves running posteriorly from the suboesophageal ganglionic complex were severed. The induced rhythm (shown here) is prominent, although the trace was recorded some considerable time (about $\frac{3}{4}$ hr) after the "resting rhythm" had ceased. (*c*) Resting rhythm (left) flagging. At arrow the body wall about the genital atrium was pinched sharply with jewellers' forceps, breaking the integument. Other disturbance was kept to a minimum (e.g. tissue was not removed from perfusion bath for operation). (*d*) At first arrow the body wall about the genital atrium was pinched with jewellers' forceps, breaking the integument. At second arrow the body wall on the left of the head was similarly pinched. Tissue was not removed from bath for either operation.

the penis sheath. After the rhythmic contractions of the sheath had been recorded for some minutes, the tissue was removed from the bath and the penial nerve was severed. When the tissue was remounted, it was found that the rhythmic activity had ceased (Fig. 3(*a*)).

(5) The penis sheath–brain preparation was mounted with the vas deferens and the retractor muscle severed close to their junctions with the penis sheath and with the supra-oesophageal commissure of the brain transected. The rhythmic contractions of the penis sheath did not appear.

(6) It was observed that rhythmic activity of the penis sheath–brain preparation does not persist indefinitely. It may last for more than $2\frac{1}{2}$ hr, but usually fades out within 1 hr of completing the dissection.

(7) This series of experiments was carried out between autumn 1960 and autumn 1961. Traces recorded in April showed marked activity* of the penis sheath. Towards the end of autumn the contractions tended to diminish in amplitude and the duration of rhythmic activity tended to be less than in earlier traces. This tendency continued throughout winter and culminated in September, when two-thirds of the preparations showed no activity and the remainder registered very slight activity. By early October marked rhythmic activity was again apparent, and it persisted till mid-December. From mid-December to late March activity was again slight or non-existent.

(8) It appears that changes in temperature of the perfusion bath (12–26°C) do not affect the activity, or lack of activity, of the penis sheath–brain preparation. However, the evidence on this point is inadequate. The experiment has not been carried out at a period of maximal penis sheath activity. Only three traces are available, and these were obtained in winter and summer.

(*b*) *Further Observations and Conclusions*

It is concluded that the penis sheath of *Helix* is capable of rhythmic activity, but activity will not appear unless suitable stimuli are applied. The stimuli normally responsible originate in the CNS (presumably in the pedal ganglion—cf. Pelseneer 1906) and are transmitted to the penial apparatus *via* the penial nerve. Damage to the CNS, more specifically, severing of the supra-oesophageal commissure, abolishes the rhythmic activity. The effects of neural stimulation can be reproduced, in the absence of the CNS, by adding adrenaline to the perfusion bath. (See Section V(*b*)(8) for further information on the effect of adrenaline.)

The activity of the penis sheath is seasonal in occurrence, but is apparently not affected by transitory changes in temperature. It shows two annual maxima, in autumn and again in spring–early summer; in winter and most of summer the preparations registered little or no activity.

It is of interest that this cycle is not closely correlated with the pairing habits of the species as reported in the literature: Ellis (1926) states that in the northern

* The term "marked activity" signifies that the rhythmic contractions were of large amplitude and rhythm persisted for periods of the order indicated above (see No. 6). "Slight activity" signifies that the contractions were of small amplitude and the duration of rhythm was significantly less than indicated above (usually less than 15 min).

hemisphere "*Helix aspersa* pairs from April throughout the summer". However, a rough record has been kept of those animals in the Sydney area found in copulation. Although not many couples were found during the last two years, it is noteworthy that all cases on record were found during the "peak periods" for penis sheath activity. It is suggested that the pairing season for this species might be more restricted than is commonly thought and that there might be two such periods annually, corresponding to the peak periods for activity of the penis sheath.

(i) *Nature of the "Resting Activity"* of the Penis Sheath.*—It is known that spontaneous neural activity occurs in the central nervous systems of vertebrates, arthropods, annelids, and molluscs. Such activity "persists in isolated ganglia or pieces thereof for long periods under conditions which need not be regarded as abnormal or excitatory" (Bullock 1947). Superficially, it appears that the resting activity of the penis sheath of *Helix* is due to spontaneous neural activity of this kind. However, there is reason to think that this is not the case.

It has been observed that the resting rhythm does not persist indefinitely after dissection, although there is evidence that the preparations remain viable for some time. In fact, it has been shown that an "induced rhythm" can be evoked after the resting rhythm has ceased. The latter appears to be a transitory phenomenon associated with dissection. It would therefore seem that the neural impulses responsible represent an "injury discharge" from the CNS and are caused by the damage sustained at dissection. The following observation is relevant:

(9) The penis sheath–brain preparation was mounted in the perfusion bath with the vas deferens and the retractor muscle severed close to their junctions with the penis sheath. After the resting rhythm had ceased, the preparation was removed from the perfusion bath and the nerves running posteriorly from the suboesophageal ganglionic complex of the CNS were severed† close to the ganglia. Control traces had shown that removal of the tissue from the bath for a short time (less than 1 min) does not significantly affect the activity of the penis sheath. When the tissue was returned to the perfusion bath, it was found that rhythmic activity had recommenced and the tonus of the muscle had increased (Fig. 3(*b*)).

It is concluded that profound damage to the nervous system (as distinct from specific damage to the brain—cf. No. (5) above) will induce an injury discharge to the penial apparatus and cause rhythmic activity of the penis sheath. It seems that the resting rhythm is due to the same cause—injury to the nervous system sustained at dissection.

(ii) *The Calcareous Dart.*—The generally accepted view as to the function of the calcareous dart is that it provides a sensory stimulus to copulation. Mutual pricking with darts by two snails at copulation must result in generalized damage to the body walls, and it seems possible that such damage could result in an injury discharge to the penial apparatus. This hypothesis was tested as follows:

* The "resting activity" or "resting rhythm" is defined as the rhythmic activity which appears *at the outset*, when the penis sheath–brain preparation is first mounted in the perfusion bath (Section IV(*a*)(2). It will be convenient to distinguish between the resting activity and "induced activity" such as that due to adrenaline or that reported in Section IV(*b*)(9).

† These nerves had already been severed at dissection, but at some distance from the ganglia of the CNS.

(10) The penis sheath–brain preparation was mounted in the perfusion bath with the vas deferens and the retractor muscle severed close to their junctions with the penis sheath. Most of the anterior half of the animal remained attached to the preparation, care being taken to leave that portion of the body wall anterior to the lung *in situ*.* After the rhythmic contractions of the sheath had been recorded for some minutes, the body wall in the vicinity of the genital atrium was pinched sharply with fine forceps, causing localized damage to the integument.

This treatment caused an upset in the rhythmic pattern with an increase in tonus of the muscle. Eventually the tonus declined to the resting level and the contractions returned to a more "normal" pattern. The induced contractions were often of greater amplitude than the resting contractions they replaced (Fig. 3(*c*)). In one preparation the immediate response was a marked "double rhythm" of minor pulses superimposed on major. This gave way eventually to a pattern resembling that of the resting rhythm (Fig. 3(*d*), first arrow).

It should be pointed out that not all the preparations on which this experiment was carried out gave pronounced responses; it appears that the portion of the body wall pinched determines the measure of response.

When the body wall on the left of the head was similarly pinched, the effect was less marked (Fig. 3(*d*), second arrow). There was a slight upset in the rhythm, an increase in tonus, and a relatively rapid return to the pattern of the resting rhythm and to the original level of tonus.

Clearly an experiment like this is, to some extent, subjective. The degree of damage (intensity of the pinch), for instance, could well alter the result. An attempt was therefore made to hold this factor as constant as possible.

It is concluded that superficial damage to the nervous system (i.e. to the nerves in the body wall) is also capable of inducing an injury discharge to the penial apparatus. It seems that the damage caused by the dart at copulation might well be capable of evoking an injury discharge by the CNS and of thus affecting the function of the penial apparatus.

(*c*) *Discussion*

It will be noted that damage to the body wall in the region of the genital atrium has a more marked effect than damage to the body wall on the opposite side of the head. This is in line with what is known concerning the way in which the dart is used. It appears that the dart can be used to prick the partner at any point about the head, but is used mostly against the atrial region. The evidence indicates that the atrial region of the body wall is particularly sensitive to mechanical injury, and supports the view that the calcareous dart provides a "sensory stimulus" to copulation.

It remains to be seen how damage to the body wall could result in a discharge to the penial apparatus from a centre in the brain. Presumably the nerves responsible for exciting the neural centre are sensory and are distributed to tactile receptors in the skin. No doubt the frequency of the signals transmitted by these nerves is related, in the usual manner, to the intensity of the stimulus applied at the body wall. It is

* See footnote, p. 221.

suggested that normal tactile stimuli evoke signals whose frequencies are too low to excite the neural centre concerned with penial function; only drastic stimulation, to the point where damage results, will evoke frequencies of the order required to excite this centre.

It may well be that motor neurons also play a part in exciting the centre concerned with penial function. These could, possibly, contribute signals to the CNS by "reverse conduction" of impulses originating at the point of damage. However, the neural mechanism involved is not of present concern and will not be considered further.

If the resting rhythm is an artefact, in the sense that it is caused by injury received at dissection, the question arises whether its seasonal fluctuations can be regarded as signifying fluctuations in copulatory ability. It will be shown that the penis sheath–brain preparation is relatively insensitive to adrenaline in winter and summer, but highly sensitive at other seasons of the year (Section V(*b*)(8). It is concluded that the seasonal changes in the resting rhythm, which most probably reflect the seasonal changes in sensitivity to adrenaline, are valid indications of seasonal changes in copulatory potential.

V. Assay of Effects of Tissue Extracts etc. on Penial Activity

(*a*) *Methods*

An attempt was made to assay the effects of certain tissue extracts, "neuro-humours", etc. on the penial activity described in Section IV.

For each trace a penis sheath–brain preparation was mounted in the perfusion bath filled with Hédon-Fleig saline, with the vas deferens and the penis retractor muscle cut close to their junctions with the penis sheath; the snail's head was left, to a large extent, intact.* With the exception of those reported in Section V(*b*)(8), the assays were carried out on preparations showing "resting" rhythmic activity, so that most of this work was done in autumn and spring (cf. Section IV(*a*), experiment 7). The adrenaline assays were conducted intermittently throughout the year; most of the preparations used showed resting activity, but some did not.

The extracts were prepared by grinding known weights of tissue in known volumes of Hédon-Fleig saline in a mortar. Grinding was continued until the coarsest particles of tissue could be passed through a hypodermic needle of $\frac{1}{2}$ mm internal diameter. The final concentrations of the extracts (i.e. after addition to the perfusion bath) will be expressed as wet weight in grams of tissue extracted per 100 c.c. of Hédon-Fleig saline.

In three assays blood was used instead of tissue extract (expt. (7) below); the blood samples were mixed with a small amount of saline when drawn and added to the perfusion bath with as little delay as possible. These precautions were taken to prevent clotting. The concentrations are expressed as percentages (c.c. blood/100 c.c. saline).

It was thought that certain tissues of the reproductive tract might produce substances capable of affecting penial activity. The other extracts assayed (e.g. liver) were intended to serve as controls.

* See footnote, p. 221.

(*b*) *Results*

(1) Extract of common duct of reproductive tract: one trace; concentration 0·158 g common duct material/100 c.c. saline. No effect on trace.

(2) Extract of mucous glands of reproductive tract: one trace; concentration 0·024 g/100 c.c. saline. No effect on trace.

(3) Extract of albumen gland: results equivocal; of a total of 20 traces, three registered responses to the extract (Fig. 4(*a*)). Concentrations 0·092–0·247 g/100 c.c. saline. (*Note*: the concentrations eliciting responses were well below the maximum.)

(4) Extract of liver (digestive gland): five traces; concentrations 0·134–0·292 g/100 c.c. saline. Responses registered on all traces (Fig. 4(*b*)). Minimum concentration capable of eliciting a response not determined.

(5) Extract of gonad (gonad dissected free of surrounding liver tissue): five traces; concentrations 0·052–0·400 g/100 c.c. saline. Responses registered on all traces (Fig. 4(*c*)). Minimum concentration capable of eliciting a response appears to be about 0·05 g/100 c.c. saline.

(6) Extract of liver and gonad (no attempt made to separate the two tissues): seven traces; concentrations 0·158–0·356 g/100 c.c. saline. Responses registered on all traces (Fig. 4(*d*)). Minimum concentration capable of eliciting a response not determined.

Comment: The responses recorded under Nos. 4, 5, and 6 showed much variation, as can be seen from inspection of Figs. 4(*b*)–4(*d*); however, there is considered to be no significant difference between the response to liver extract and that to gonad extract. If liver or gonad extract or extract of both tissues is administered when a resting rhythm is present, the result is usually a decrease in frequency and an increase in amplitude of the major pulses. The effect on the minor cycle, when one is present, is not clear.

If the extract is administered after the resting rhythm has ceased, the result is a persistent increase in tonus of the muscle, accompanied by a reappearance of rhythmic activity. It seems that the strength of the response is determined to some extent by the age of the preparation (i.e. time elapsed since dissection).

(7) Blood: three traces; concentration 2% in all cases. No response except a slight and sustained decrease in tonus (Fig. 4(*e*)).

(8) Neurohumours, etc.: (i) adrenaline; 48 traces; concentrations $2{\cdot}5\times10^{-5}$–$2{\cdot}5\times10^{-4}$. Lowest effective concentration was $1{\cdot}25\times10^{-5}$, but this figure is meaningful only when related to the time at which the trace was made (October). (ii) acetylcholine; two traces (June); concentrations 5×10^{-5} and $3{\cdot}5\times10^{-5}$. No effect, although the preparations showed prominent resting rhythms and were decidedly sensitive to 5×10^{-4} adrenaline. (iii) histamine; one trace (August); concentration $2{\cdot}5\times10^{-4}$. No effect, but the preparation was relatively insensitive (resting rhythm of small amplitude and duration; 5×10^{-4} adrenaline caused only a slight increase in tonus).

Comment: For a given dosage level the preparations showed maximal responses to adrenaline in autumn and spring and minimal responses in winter and summer. There is therefore some correlation between sensitivity to adrenaline and seasonal changes in the resting rhythm (cf. Section IV(*a*)(7)). It appears, moreover, that any

preparation showing a resting rhythm will respond to adrenaline, but not all preparations responding to adrenaline necessarily show a resting rhythm.

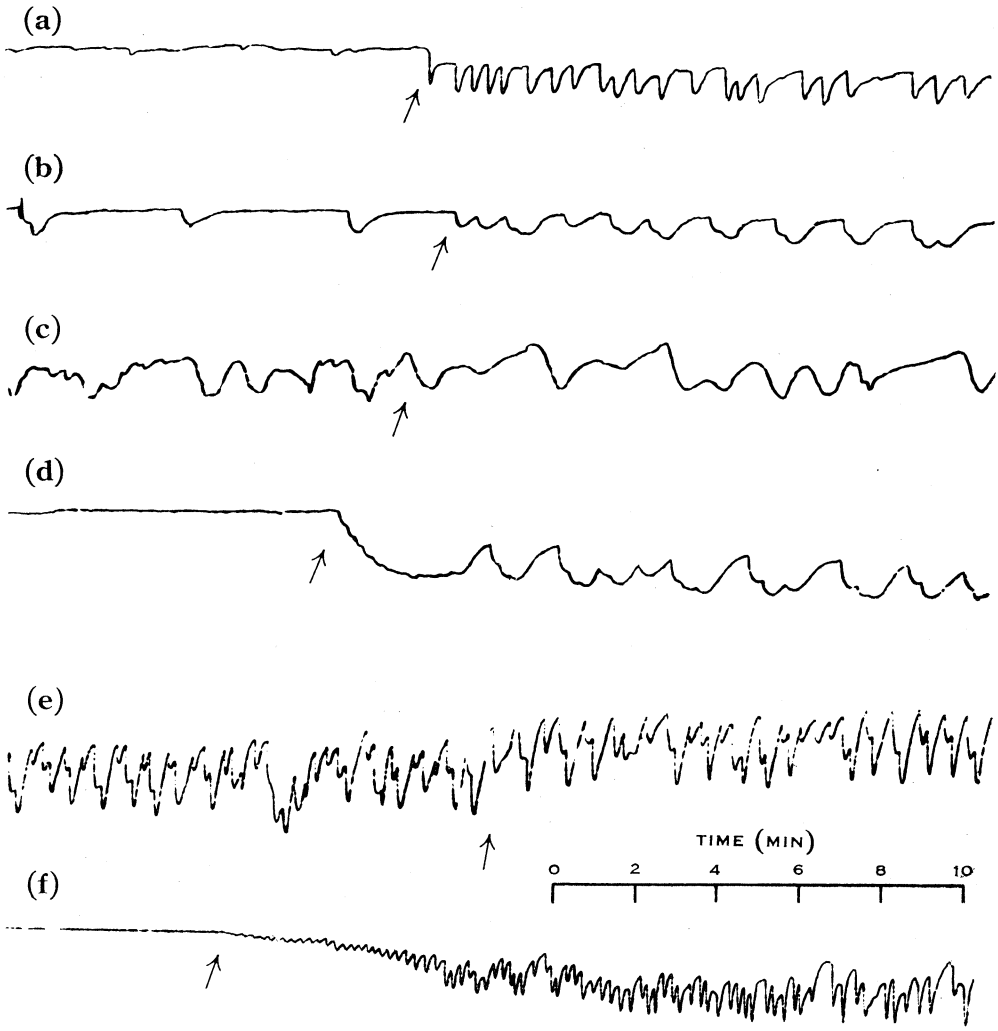

Fig. 4.—Penis sheath–brain preparation: (*a*) after cessation of resting rhythm. At arrow albumen gland extract (concn. *c.* 0·13 g/100 c.c. saline) was added to the perfusion bath. (*b*) Liver extract (arrow; concn. 0·134 g/100 c.c. saline) added to bath when resting rhythm had almost disappeared (pulses intermittent and of small amplitude left of trace). (*c*) Showing effect of gonad extract on resting rhythm: extract (concn. 0·065 g/100 c.c. saline) added to bath at arrow. (*d*) Liver/gonad extract added to bath (arrow; concn. 0·158 g/100 c.c. saline) after resting rhythm had ceased. (*e*) At arrow blood (concn. 2%) was added to the perfusion bath. (*f*) Adrenaline added to bath (arrow; concn. 5×10^{-4}) $2\frac{1}{2}$ hr after cessation of resting rhythm. Trace recorded in June.

In general, adrenaline causes an increase in tonus and an induced rhythm (if administered after the resting rhythm has ceased), which more or less resembles the resting rhythm (Figs. 2(*c*), (2*d*), and 4(*f*)). With the less sensitive preparations the

induced contractions are of small amplitude, and at the height of winter and summer the only effect of adrenaline treatment may be an increase (sometimes a decrease) of tonus.

(*c*) *Discussion*

Nerves with cholinergic and nerves with adrenergic properties occur in molluscs. Moreover, acetylcholine has been extracted from molluscan tissues and there is evidence for the presence of adrenaline in certain members of the phylum (Krijgsman and Divaris 1955). The response of the penis sheath of *Helix* to adrenaline suggests that the latter is the neurohumour released by the penial nerve, but the evidence on the point cannot be regarded as conclusive.

The concentration of adrenaline required to affect the penis sheath is high. However, much depends on the method of application. There is little doubt that with the method used here the tissue was inadequately perfused, so that the threshold concentration is probably a good deal lower than $1 \cdot 25 \times 10^{-5}$.

Histamine is widely distributed in vertebrate tissues, and perhaps also in invertebrate. In vertebrates its effects are somewhat similar to those of adrenaline. In *Helix* it exerts a depressive effect on the heart (Krijgsman and Divaris 1955), but it does not appear to be effective on the penial apparatus in the concentration tested.

It is apparent that aqueous extracts of liver or gonad or of both are capable of affecting the activity of the penis sheath, whereas most of the other extracts are not capable of doing so, at least not in the concentrations tested. The activity detected in some of the albumen gland extracts could be attributed to active principle derived from the liver or gonad. The albumen gland is closely surrounded by liver and is connected with the gonad by the hermaphrodite duct, so that accumulation of active principle by diffusion from the liver or by transport from the gonad seems not unlikely.

It seems improbable that the substance found in the liver arrives there by diffusion from the gonad, as care was taken to select liver tissue as far removed as possible from the gonad, and there are indications that active principle is present in the liver in high concentration. There is reason to think, therefore, that active substances are produced by both the liver and the gonad.

It is not clear what role the liver could play in penial function. It seems probable that the ability to influence the penial apparatus is a "side effect" of some substance produced by the liver and normally confined to the alimentary canal.

The effect of gonad extract deserves closer attention. The gonad could reasonably be expected to produce a substance capable of affecting penial function, and it is clear that it does affect the penial apparatus under the experimental conditions used here. Whether it does so under natural conditions is another matter.

It seems that the observed effect of gonad extract could be due to an endocrine secretion. The evidence that the gonad of *Helix* functions as an endocrine organ is scanty (see review by Goddard 1960*b*), but that it does so may be allowed. Moreover, it appears that the minimum concentration of gonadal material required to affect the penial apparatus is about 0·05 g/100 c.c. When we bear in mind the inertia of the apparatus, the crudity of the extraction and perfusion methods, and the fact that an aqueous solvent was used (the substance concerned might not be water-soluble to any marked extent), it becomes probable that the true minimum

is well below this figure. In other words, the effective concentration of extract is small, and may well be within "physiological limits".

However, the results do not exclude the possibility that the observed effect is due to an exocrine secretion, since the cephalic sensory structures were present in the preparations used. Conceivably an exocrine secretion, released *via* the genital ducts, could affect the penial apparatus by way of the sensory nerves and the CNS. In point of fact, there is no evidence that the observed response is due to a secretion (*sensu stricto*), so that it is by no means established that the gonad influences the penial apparatus, under natural conditions, by either of these methods.

In a critical assessment the assay results must be regarded as interesting but inconclusive. It is not proposed, however, to continue with this investigation and it was decided to publish the results as they stand.

VI. Acknowledgment

The author is indebted to Dr. A. K. O'Gower, of this School, for help in preparation of the photograph and traces.

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