The mechanism of locomotion of *Agriolimax reticulatus*
(Mollusca: Gastropoda)

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(With 1 plate and 2 figures in the text)

The locomotion of *Agriolimax reticulatus* is described and the musculature directly responsible for producing the pedal waves has been identified. The probable mechanism of locomotion is discussed.

Contents

Introduction

There have been few attempts to correlate the anatomy of the pedal musculature of gastropod molluscs with the type of pedal locomotory waves occurring on the sole and no adequate account of the mechanism of pedal locomotion of pulmonates has been published. The present work was undertaken to attempt to identify the musculature directly concerned in locomotion, namely that which causes the waves to pass along the foot, and to ascertain its probable mode of action.

Most terrestrial pulmonates, including *Agriolimax reticulatus*, crawl utilizing one set of muscular waves which pass along the sole of the foot from posterior to anterior as the animal crawls forwards. Thus, according to Vlès' (1907) classification of pedal muscular waves, the waves of *A. reticulatus* are direct monotaxic. The muscular locomotory waves in *Agriolimax* are restricted to the median third of the foot, the two lateral thirds exhibiting no muscular waves. The centre strip is darker in colour than the two lateral strips and the locomotory waves are usually paler than the background colour. Lissmann (1945) showed that any point on the sole is stationary between waves, and moves forwards during waves.

Of the few workers who have attempted to explain the locomotory waves of pulmonates in terms of the anatomy of the pedal musculature, Simroth (1878) could do so only by postulating that the muscle fibres involved actively expand, rather than, as is the case for all other known muscle fibres, actively contract. Carlson (1905) disproved this and suggested that the galloping locomotion of *Helix dupetithouarsi* Deshayes was produced by the antagonistic action of transverse and longitudinal muscles of the head/foot on the contained blood spaces. This is probably correct for galloping but not for the normal

pedal sole locomotion which continues unabated during galloping. Trappmann (1916) similarly suggested an antagonism between transverse and longitudinal muscles in the sole but he was under the impression that the waves were convexities of the sole, not as was conclusively proved by Lissmann (1945, 1946), concavities. Parker (1911) proposed that the combined action (not antagonistic) of longitudinal and dorso-ventral muscles in the foot produced the waves of *Helix* and *Limax*. Parker further suggested, in the light of the evidence of Jordan (1901) and Biedermann (1905), that the muscles work against the elastic, fluid-filled cavities of the foot. The chief objections to this mechanism seems to be that there are relatively few longitudinal muscles actually near the sole and that, in *Helix*, there are very few distinct blood spaces in the foot. This apparent lack of blood in the foot may be a consequence of the method of fixation. Clark (1964) contains a convenient discussion of this earlier work.

Runham & Hunter (1970) have proposed a similar mechanism for terrestrial slugs to that suggested for *Patella* by Jones & Trueman (1970) but the configuration of the wave shown by Runham & Hunter cannot apply to slugs for they show the sole as being stretched as a wave passes. Direct waves are always longitudinally shortened. Jones & Trueman did suggest that direct waves during rearwards locomotion in *Patella* are produced by dorso-ventral muscles, there being only transverse, dorso-ventral and a few "longitudinal" fibres in the foot. They further suggested that the waves of pulmonates, specifically *Helix*, might similarly be produced, but presented no evidence in support of the latter suggestion.

Material and methods

Visual observations and cine film of locomotion were used to ascertain the method of locomotion. Slugs were allowed to crawl on a sheet of Perspex with a 5 mm grid etched on. This also enabled observations to be made on the situation at the commencement of locomotion.

To obtain sections of the foot during locomotion specimens were put through the following procedure. They were allowed to crawl on pieces of filter paper which were hanging from a horizontal bar by a single strand of cotton thread. When the slug was actively crawling the thread was burned through by playing a flame from a bunsen burner on it. This avoids any tactile stimulus due to cutting that might stop the animal from crawling. The severing of the thread allowed the animal and filter paper to drop into a vacuum flask containing liquid nitrogen, which boils at $-196°C$. This freezes the animal very quickly, evidenced by the fact that in frozen specimens the optic tentacles are usually only about one third retracted. The specimens were then transferred to alcoholic Bouin's fixative maintained at $-20°C$ where they were kept for one week. The specimens were then subjected to standard histological treatment, serially longitudinally sectioned at 6 μm and stained in Mallory's triple stain. In addition a relaxed but non-crawling specimen was longitudinally sectioned to compare the musculature of a stationary animal with that of a crawling one.

Results

When a specimen of *Agriolimax* commences to crawl the first pedal wave to appear does so near the anterior end of the sole, about a quarter of the distance between head and tail. This is similar to *Helix* (Lissmann, 1945). Previous workers, notably Lissmann (1945; 1946) have shown that each wave is a region of longitudinal compression that is lifted from the substrate. So that at the commencement of locomotion a wave of longitudinal compression appears towards the front of the foot. This wave then moves anteriorly

and other waves appear moving forwards behind the first wave until the centre strip of the sole has some six or seven waves passing along it, new waves subsequently propagated only at the posterior of the foot. Each wave advances any point on the sole by about 0·66 mm for some 15 waves traverse any point as the animal crawls 1 cm.

Figure 1 is a series of hypothetical diagrams which illustrates the situation at the commencement of, and during subsequent locomotion. The principles involved apply to any animal which crawls using direct waves. In the diagrams the length of each wave and the distance between each wave is roughly proportional to the equivalent distances of a crawling *Agriolimax*, though the whole "foot" in the diagrams is proportionately only
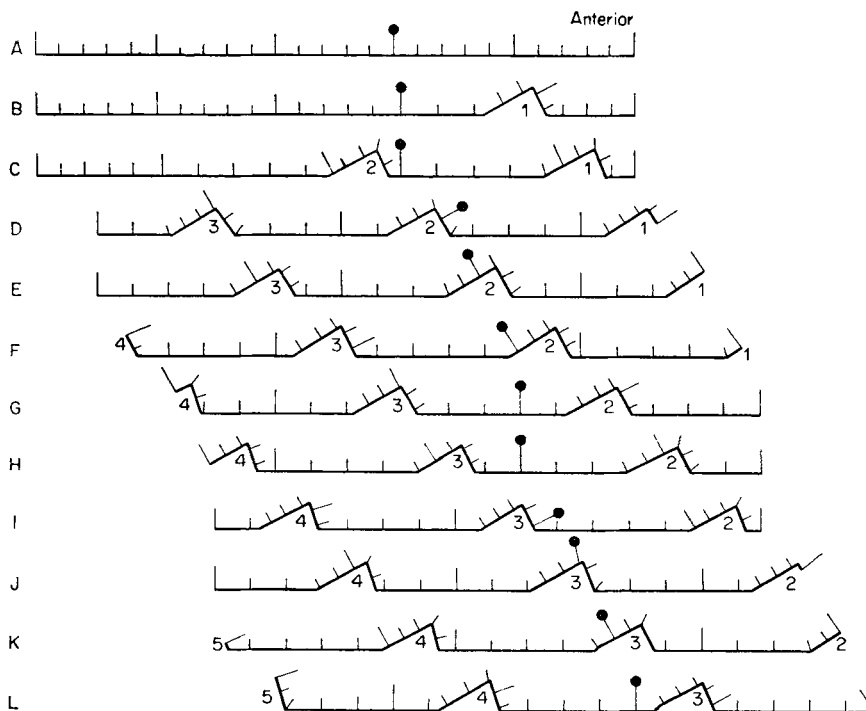

FIG. 1. A series of hypothetical diagrams to illustrate the commencement of locomotion and subsequent movement in an animal such as a slug which uses direct locomotory waves. For explanation see text.

half the length of the foot of a slug for the "foot" in the diagrams has three or four waves on it at once whereas the foot of *Agriolimax* has six or seven.

The "foot" has been divided (A) into equal arbitrary sectors, every fifth intersector being emphasized and one being individually marked with a dot. We have seen that the first wave to appear does so near the front of the foot and that it is a wave of longitudinal compression. This situation is shown in B. During the wave (1) the arbitrary sectors are compressed from their original resting length and this and the simultaneous uplifting will cause stretching of the sectors immediately behind the waves from their original length. Two separate pieces of experimental evidence in support of this relative stretching and compressing will be presented below.

The first wave moves anteriorly (C) and another wave of compression (2) forms a little way behind the first. Similarly this second wave stretches the sole behind itself. Both of these waves move forwards (D) and a third wave (3) appears behind the first two.

At this stage and in the subsequent diagrams E–L each sector of the "foot" is either stretched from its original length, whilst it is stationary, or compressed from its original length, whilst it is uplifted and moving forwards. In these diagrams each sector during the passage of a wave is compressed to half the length that it is between waves. This degree of relative compression is approximately that observed in the results described below.

In the remaining diagrams (E–L) new waves appear only at the posterior end of the "foot", when each preceding wave has moved some way forwards.

It can be seen with reference to a single point on foot (marked with a dot) that any point on the foot only moves forwards when a wave of compression moves over it. Further it can be seen that a point moves forward during the entire period it is off the ground, even during the trailing side of each wave (E–F and J–L). It is stationary between waves of compression (G–H).

We have just seen that any part of the foot is either stretched or compressed from its resting length. This means that a crawling slug may remain almost the same length as a stationary slug, for if the foot was not stretched between the waves of compression then a crawling slug would be appreciably shorter than a stationary one. This certainly is not the case. In fact the overall length of an animal fluctuates slightly depending on the precise position of the waves relative to the anterior and posterior of the foot. This is illustrated in the diagrams where I is 91 % of the length of A, and F and K are 103 % of A, other diagrams being between these extreme values. In *Agriolimax* the fluctuations will be about half of this (95·5 % and 101·5 % respectively) for the foot of a slug is relatively twice as long as in the diagrams.

The sections obtained from rapidly frozen animals exhibit upliftings at regular intervals along the sole of the foot, six or seven in number, which may be taken as the locomotory waves. The waves are about 1·8 mm apart and it is important to remember that the waves are passing forwards (to the right of all diagrams and plates) during locomotion.

The sole of the foot (Plate I) consists chiefly of muscle fibres in a haemocoelic cavity. Ventrally it is bounded by the pedal epithelium and dorsally it is bounded by the large supra-pedal mucous gland (SPMG). This gland opens anteriorly and supplies the mucous trail on which the animal crawls. The thickness between the epithelium and the mucous gland is about 0·5 mm.

The musculature of the sole has a very distinct orientation and distribution. Immediately ventral to the mucous gland the muscle is longitudinally orientated, though there are some transverse fibres. This layer of longitudinal muscle (Plate I, LM) is quite compact and is about 0·1 mm thick. The rest of the thickness, about 0·4 mm, is a fairly open meshwork of fibres running down obliquely from the longitudinal muscle to the pedal epithelium. These oblique fibres are continuous at their dorsal end with the fibres of the layer of longitudinal muscle. Half of the oblique fibres run forwards and downwards from the longitudinal muscle. They thus exert a posteriorly directed upward force on the epithelium. These will be called posterior oblique fibres (Fig. 2 and Plate I, POF). The other half of the oblique fibres run backwards and downwards from the longitudinal muscle and thus exert an anteriorly directed upward force on the epithelium. These will be called anterior oblique fibres (AOF). There are also transverse fibres in this part of the foot and relatively
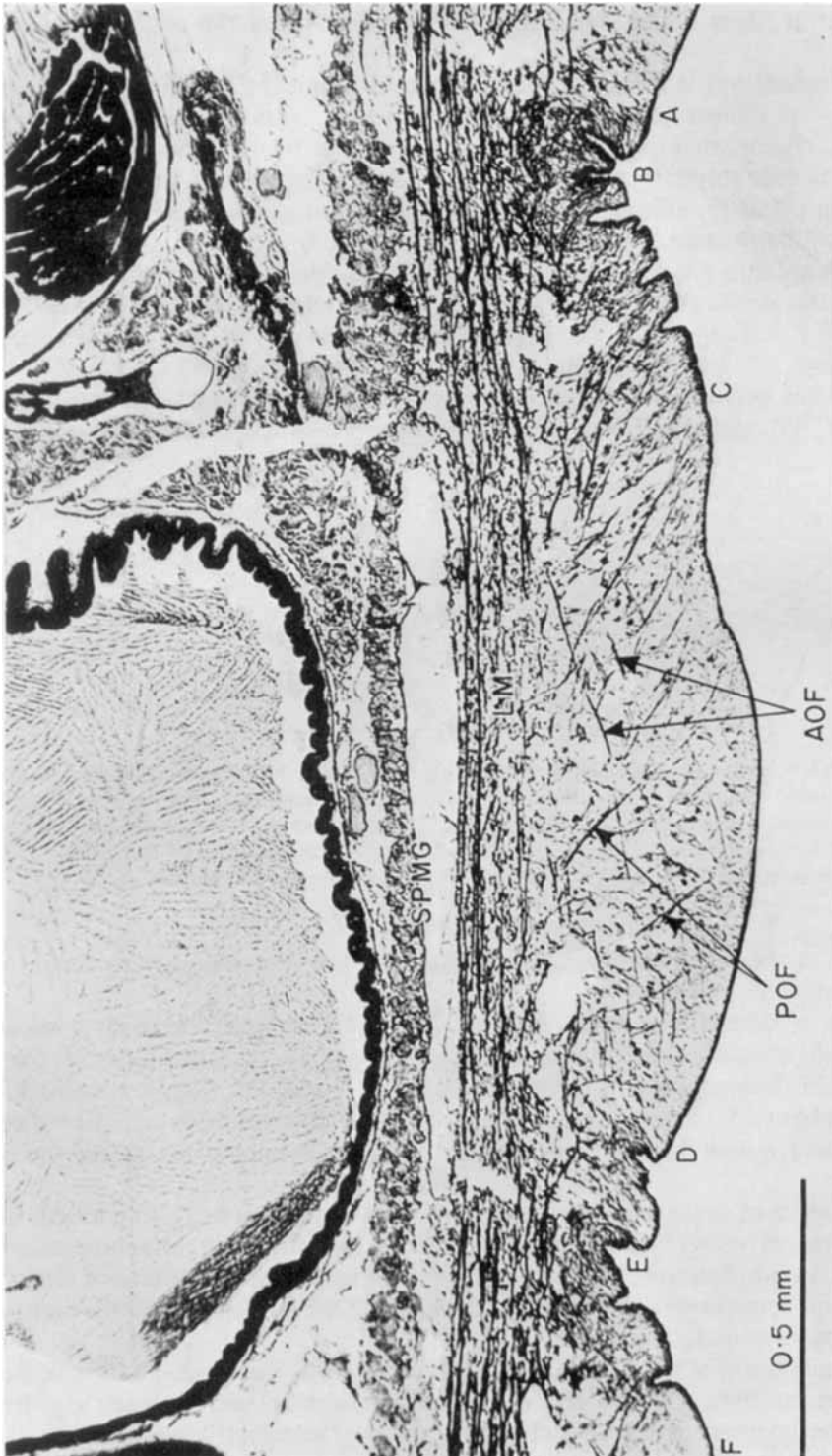
PLATE I. Part of a longitudinal section of an *Agriolimax* frozen whilst crawling. Anterior is to the right. SPMG Supra-pedal mucous gland; LM, longitudinal muscle. Other lettering as in Fig. 2 with which this should be compared.

few dorso-ventral fibres which are considerably thinner than the other muscle fibres of the sole.

Each wave (uplifting) is about 0·2 mm deep and about 0·5 mm long. The haemocoel above the wave is almost completely occluded and the pedal epithelium is thrown into irregular folds. Each wave is asymmetrical in that the front slope of the concavity is steeper than the rear slope, this is shown diagrammatically in Figs 1 and 2 and is seen in the micrograph (Plate I), except for the wave at the front of the foot, which is a more or less symmetrical depression.

Both sets of oblique muscles are attached onto the pedal epithelium at varying angles depending on the position of the pedal wave (Fig. 2 and Plate I). Immediately behind a wave (at C and F in both Fig. 2 and Plate I) the anterior oblique muscles are attached at an angle of about 30°, but immediately in front of a wave (at A and D) they are attached at about 20°. Thus between two successive waves when the sole to which they are attached is stationary (C–D), the anterior oblique fibres increase in length. That is assuming that
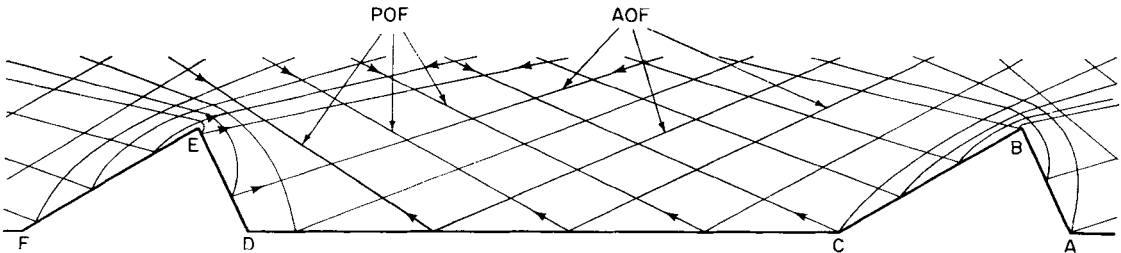

Fig. 2. Diagram for comparison with Plate I summarizing the situation during locomotion on part of the sole. Anterior is to the right. Contracting muscle fibres are indicated with arrowheads. The posterior oblique muscle fibres (POF) contract between waves (C–D) pulling the body forwards relative to the sole and exerting a backward thrust on the substrate. The anterior oblique fibres (AOF) contract to produce waves (A–B–C and D–E–F) and to pull the sole forwards relative to the body. For a more detailed description see the text.

the dorsal ends of the fibres in the longitudinal muscle remain roughly equidistant and do not alter their relative position.

The anterior oblique fibres then, increase in length between successive waves. The opposite happens to the posterior oblique fibres. The angle of attachment to the sole is greater, about 35° immediately in front of a wave (at A and D), than it is behind a wave (at C and F), about 25°. This indicates that the posterior oblique muscle fibres contract *between* successive waves, but that the anterior oblique fibres contract *during* the passage of waves.

As a consequence of this and of the stretching of the sole from its resting length between waves, the fibres of either set of muscles diverge towards their attachment onto the stationary sole. In the sections of the non-crawling animal the oblique fibres of either set are parallel. This confirms the earlier statement concerning the stretching and the compressing of the sole from its original resting length.

During the passage of a wave (Fig. 2 and Plate I, A–B–C and D–E–F) the sole becomes compressed and the fibres of both sets of oblique muscles are much closer together. The result is that the haemocoel above each wave is almost completely occluded.

Discussion

The most important fact to emerge from the results is that the two sets of oblique muscle fibres would appear to contract at different stages. The anterior oblique muscles shorten during the passage of a wave and it is probable that it is these fibres that are responsible for producing the pedal wave. They contract and pull the pedal epithelium upwards and forwards. They then probably remain contracted while the wave of initial contraction continues forwards. This allows the sole to be pushed downwards after being moved forwards.

The means whereby the sole is pushed downwards is probably a combination of high haemocoelic pressure and low pressure beneath the wave. Terrestrial molluscs require high blood pressure to maintain body turgor and to keep themselves and their appendages erect (Jones, 1970). Direct measurements, using methods similar to those used by Jones (1970) and (1971), of the cephalic blood pressure of both *Helix aspersa* and *H. pomatia* show that the pressure is about 17 cm of water (Dale & Jones, in prep.). The cephalic pressure in four freshwater snails was between 6 and 11 cm of water. The ventricles of *H. aspersa* weighs 0·22 % of the body weight (less shell) but that of *Lymnaea stagnalis* (with a cephalic pressure of about 7 cm of water) weighs 0·13 % of the body weight (less shell). This suggests that it is the ventricle that is producing the high blood pressure. The ventricle of *Agriolimax* is as sturdy as that of *Helix* and it is probable that the blood pressure is similarly high in *Agriolimax*. Isolated tentacles of *Helix pomatia* require internal pressure of at least 15 cm of water to be erect in air (Dale & Jones, in prep.) and the tentacles of *Agriolimax* must require a similar internal pressure. In *Agriolimax*, therefore, there is probably a relatively high internal pressure, and this pressure may assist in pushing the sole downwards as the anterior oblique fibres relax.

In addition to high pressure produced by the ventricle it is possible that local high haemocoelic pressure may be produced in the pedal haemocoel by the waves themselves. We have seen that the haemocoel is almost completely occluded above each wave, and as each wave moves forwards it may force a region of high pressure in front of itself. However because the waves are restricted to the centre third of the foot it is probable that any high pressure may escape laterally. In addition to high blood-pressure it is possible that the pressure beneath each wave falls as the foot is lifted. This has been demonstrated for *Patella* (Jones & Trueman, 1970) but the drop in pressure is probably not as marked as with *Patella* due to the comparatively large amount of mucus beneath the foot of *Agriolimax*. Any low pressure thus produced will help to pull the sole downwards after the muscles have contracted.

The asymmetry of most of the waves indicates that the epithelium is being pulled forwards by the anterior oblique muscles, and further the symmetry of the wave near the front of the foot indicates that at the front the epithelium is not being pulled forwards. There is, of course, no muscle at the front of the foot which could pull the epithelium forwards. At the front of the sole the wave of muscular contraction may be propagated forwards by the few dorso-ventral fibres and the front extremity must be pushed forwards solely by haemocoelic pressure.

This far the pedal epithelium has been lifted, moved forwards relative to the rest of the slug and re-applied to the substrate, due only to the contraction of the anterior oblique muscle fibres. Once the sole is re-applied to the substrate it is probable that the posterior oblique fibres contract for they can be seen to be shorter just in front of a wave than they

are just behind one. This will pull the layer of longitudinal muscle, and thus the rest of the slug, forwards relative to the sole.

The means whereby the epithelium remains firmly attached to the substrate between waves may be a consequence of the relative forces exerted by the two sets of oblique fibres. The anterior oblique fibres contract over the relatively short region of a single wave, but the posterior oblique fibres contract in the comparatively wide region between waves. Thus approximately the same force is being exerted anteriorly during a wave in a small area as is being applied over a large area between waves by the posterior oblique fibres. There is, therefore, a larger force per unit area being applied to the epithelium by the anterior oblique muscles than by the posterior oblique muscles, and the larger the force per unit area the more likely the epithelium is to lift.

The whole process of locomotion is continuous, for the body of the slug and probably the layer of longitudinal muscle in the sole, moves along at constant speed. It is only the pedal epithelium that is alternately accelerated and decelerated. This allows for high efficiency and is similar in principle to the use of limbs in arthropods and vertebrates where it is mechanically advantageous to have the minimum mass possible being subjected to alternate acceleration and deceleration (see Gray, 1968).

Because the whole process is continuous and the longitudinal muscle is probably moving forwards at constant speed, the dorsal ends of the oblique fibres are continually moving forwards. This permits the sole during the latter part of each wave to move forwards, for if the anterior oblique fibres attached here remain the same length and their dorsal extremity is moving forwards, then the ventral extremity, the pedal epithelium, also may move forwards.

The situation is summarized in Fig. 2. The posterior oblique fibres (POF) contract between waves (C–D) and this exerts a backwards thrust on the substrate whilst moving the body forwards. This backwards thrust has been detected by Lissmann (1945, 1946) using light levers and a bridge apparatus.

The musculature of the foot of other terrestrial slugs is similar to that of *Agriolimax*. Simroth (1879) describes similar oblique muscle fibres in the sole of *Limax cinereoniger* as does Schmidt (1965) in *Arion rufus*, and it is probable that the mechanism described above applies to all terrestrial slugs. Both of these workers describe each set of oblique muscle fibres as being parallel, as they are in a non-crawling slug.

The musculature of the foot of terrestrial snails is considerably more complex than that of slugs and it is uncertain whether a similar mechanism may apply. Certainly there are muscles orientated as the oblique fibres in slugs (Trappmann, 1916) but there would appear to be no haemocoelic cavity as open and as extensive as that in the foot of *Agriolimax*, the whole foot of both *Helix aspersa* and *H. pomatia* being almost completely muscular (pers. obs.).

Mucus, which is such a characteristic feature of all slugs and snails plays an important role during locomotion. The mucus involved in locomotion comes from two sources. A comparatively thick tenacious mucus is produced from an orifice below the mouth by the supra-pedal mucous gland. This gland in *Agriolimax* lies above the foot musculature and is completely devoid of any musculature. This is similar to the situation in *Milax sowerbii* (Barr, 1926) but in *Helix* and *Limax* the gland is embedded in the musculature of the foot (Barr, 1928). *Arion ater* has the gland lying on top of but attached to, the pedal musculature (Barr, 1928).

The mucus from the supra-pedal gland is produced in very large quantities during locomotion, but the means of this rapid production and discharge is obscure. The duct of the gland is ciliated and this must help in the discharge of the mucus but it is difficult to believe that the cilia are the only means employed. In *Helix* and *Limax* where the gland is embedded in the foot the musculature of the foot may assist in discharging the mucus, but this cannot be so in *Agriolimax* and *Milax*. Barr (1926) suggests that once the mucous trail is established then the mucus will be drawn out of the gland by its own tenacity and this seems to be the most likely explanation.

Machin (1964) has shown that the rate of mucus production depends on the hydrostatic pressure of the blood, and it has been suggested above that the hydrostatic pressure in the foot may increase during locomotion. Runham & Hunter (1970) consequently suggested that the rate of mucus production in slugs might thus increase during locomotion.

There are numerous long-necked unicellular mucous glands in the entire surface of the sole and these produce a much thinner, less tenacious mucus than the supra-pedal gland. Several workers have noticed that many species of slugs cannot attach themselves to a vertical surface solely with the epithelial mucus, whereas this is accomplished with relative ease when the supra-pedal mucus is secreted. The thin mucus produced by the sole probably fills the concavities under the pedal waves, and as was suggested above this may be at reduced pressure.

The haemocoel above each wave is occluded and thus blood must be squeezed out of this part of the foot. It is possible that this mucus may also be squeezed out of the glands of the sole as each wave passes. As a consequence of the forward movement along the foot by the wave it is likely that the thin mucus will be carried forward to the front of the foot by the waves themselves. This tendency is however opposed to some extent by the cilia on the sole which beat in a posterior direction and transport mucus posteriorly (Barr, 1926).

As each pedal wave passes along the foot the sole of the foot is compressed and then re-expanded. On re-expansion the haemocoel above the sole must refill with blood. This is possibly supplied from the compression of the following wave. If the mucous glands of the sole have been emptied by the compression then they too must re-expand as each wave passes. It is possible that some mucus may actually be resorbed into the glands as the foot expands or that more mucus fills the gland from the surrounding blood, for mucus is an ultrafiltrate of the blood (Burton, 1965).

During the course of the experiments reported here it was noticed that specimens of *Agriolimax* that were brought into room temperature, about 20°C, during winter were unable to adhere to vertical surfaces, even porous ones, and even with the mucus from the supra-pedal gland. During the summer months adhesion was readily attained at room temperature. After some hours at room temperature the winter slugs could adhere quite efficiently. These observations suggest that the slugs can control the viscosity of the mucus according to the environmental temperature, for viscosity decreases with temperature and at a higher temperature a more viscous mucus is needed to compensate for this effect. If this is so then presumably tropical snails and slugs secrete thicker mucus than temperate animals to compensate for the higher temperature.

Summary

During locomotion waves can be seen passing forwards over the median third of the sole. There are six or seven waves on the sole at any one time. At the commencement

of locomotion the first wave to appear on the sole does so near the front of the foot.

Each wave is a region of longitudinal compression and this causes a compensatory stretching of the sole between waves. This allows a crawling slug to be no shorter than a stationary one.

By dropping crawling slugs into liquid nitrogen the locomotory waves have been fixed. Examination of longitudinal sections of such slugs has enabled the locomotory musculature to be identified. This is principally the anterior and posterior oblique muscle fibres of the sole, the anterior fibres move the sole forwards relative to the body and then the posterior fibres contract to pull the body forwards relative to the sole.

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