

OBSERVATIONS ON THE BURROWING OF *ARENICOLA MARINA* (L.)

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INTRODUCTION

The hydrostatic skeleton of *Arenicola marina* has been investigated in some detail both by Chapman & Newell (1947) and by Wells (1954) and our knowledge of the habits of this species is broadly based on the extensive researches of the latter author (1961). When a new method of continuous pressure recording became available to me I immediately tried it out on *Arenicola* as an established standard for comparison before working on pressure in the haemocoel of molluscs. The results obtained, which are described in this paper, throw new light on the hydrostatic mechanism and the co-ordination of activity in *Arenicola*. Wells (1961) comments that our knowledge of how, mechanically, *Arenicola* works is very rudimentary. It is hoped that this contribution will advance our understanding of the mechanism of burrowing.

MATERIAL AND METHODS

It was most convenient to use *Arenicola* of 15-20 cm. in length which were sent to Hull from the Marine Station at Millport, local specimens being rather small for experimental purposes. These specimens travelled well during the winter, would burrow rapidly on arrival and could be satisfactorily kept in sea-water aquaria while work was proceeding. Confirmatory and more extensive experiments on freshly collected material have subsequently been made at Millport. The substrate used for recordings of burrowing was either sand from the east coast of Yorkshire (Fraisthorpe) in which *Arenicola* lives, or from Kames Bay, Millport.

The major advance which this work presents is the continuous recording of pressure changes synchronously with the taking of ciné film in an attempt to relate muscular changes to pressure peaks. Pressure-recording was carried out on an E. and M. Instrument Co. Inc. four-channel pen recorder, named the 'Physiograph', one channel being coupled to a Bourdon-type pressure transducer and amplifier. On some occasions a second channel was used to record the movement of the worm by means of a light nylon thread tied lightly round the body and taken to a sensitive myograph which could be used for this purpose.

The Bourdon transducer used in conjunction with the Physiograph has a maximum sensitivity of 5 cm. of water pressure per cm. pen deflexion although it was generally more convenient to operate at a lower amplitude so that a full-scale deflexion of the pen was equal to 80 cm. water pressure. Attached to the gauge by a short length of rubber pressure tubing was either a hypodermic needle or glass cannula which could

be inserted into the worm. In most of this work a glass cannula of internal bore 2.5 mm., cut obliquely at the end, was found more convenient than a sharp hypodermic needle. Insertion of the cannula was generally made in the dorsal body wall in the posterior trunk region, the cannula passing forward as far as 2 cm. anterior to the point of insertion. Complete burial of the lugworm could be satisfactorily achieved in periods of 4 min. with the cannula thus inserted.

Calibration of the pressure gauge was carried out before and after each recording using a burette to which a thin rubber tube and clamp were attached at the outlet. The cannula or hypodermic could be pushed through the tubing at the same level as in the experimental animal and the pen deflexion and head of water could be recorded. Zero pressure could be checked during recording by means of a T-junction near the cannula with an adjustable side tube and valve. The rate of reaction of the transducer to change in pressure was determined as this is very relevant to investigation of sudden pressure changes in the worm. Application of a pressure of 64 cm. of water under the same conditions of amplification and cannulation as used when recording changes in a burrowing worm gives a linear response to 61 cm. pressure in 0.12 sec. and reaches 64 cm. in an additional 0.05 sec. At maximum amplification 18 cm. pressure is attained in 0.1 sec. Using hypodermic needles in place of the glass cannula gives a generally satisfactory response when their bore is of 1 mm. or more but at lower internal diameters the response is slower; for example, with a 0.5 mm. bore needle there was a 3 sec. delay in reading a peak pressure of 40 cm. although a 60% reading was attained in 1 sec. Accordingly only needles of relatively wide bore were used.

Previous methods of recording hydrostatic pressure in invertebrates do not often appear to include means of continuous recording. These range from the indirect method of applying known pressures and observing the results in relation to proboscis extrusion in *Arenicola* (Wells, 1954), to the use of a spoon gauge by Newell (1950) on the earthworm, to the refined manometric technique of Batham & Pantin (1950) to record accurately pressures of less than 1 cm. in *Metridium* at 15 sec. intervals, and to the electronic condenser manometer of Cottrell (1962).

The technique upon which most of the previous work on the hydrostatic skeleton of *Arenicola* is based is that of Chapman & Newell (1947) using a capillary manometer and recording pressures at minute intervals. The results obtained with this device were somewhat at variance with those of the Bourdon transducer so that a comparison was made between the two methods. Two hypodermic needles of about 0.75 mm. bore were inserted into the posterior trunk region of an *Arenicola* simultaneously and the readings on the manometer were taken at the maximum and minimum readings which occurred alternately at between 5 and 10 sec. intervals. The manometer was turned on with a standing pressure of 18 cm. of water, which Chapman & Newell considered to be of the order of pressure in resting worms. While the transducer recorded fairly normal peaks of pressure (Fig. 1) of the order of 45 cm. of water, the manometer showed peaks of around 5 cm. above the 18 cm. of water first inserted. This applied pressure gradually decreased and after about 7 min. the rises in pressure in the manometer were from near zero.

Pressure (30 cm.) was then applied to the manometer needle by means of the burette and rubber tube, as for the calibration of the transducer. A delay of 30–40 sec.

occurred before the manometer gave the full pressure reading, obtained with a similar needle on the Physiograph in just over 1 sec. Since the pressure peaks are of not more than 4 sec. duration their small amplitude on the manometer is hardly surprising. The delay of recording of the manometer is obviously a function of the diameter of the needle and tubing and the rate of flow of fluid in or out of the manometer.

One of the advantages of the Bourdon transducer is its relatively low volume displacement of $1.15 \text{ mm.}^3/\text{cm.}$ water pressure, which enables readings to be taken with very little volume change to the worm; that is, nearly isometrically. Indeed this transducer should prove suitable to determine pressures where there is very much less fluid available, as in the earthworm (Newell, 1950). Chapman & Newell (1947) considered that the volume changes in their manometer were insignificant when compared with the total volume of the worm, estimated by them as 1.5 ml. The displacement of water in a manometer tube of 0.1 cm. diameter caused by a pressure of 60 cm. of water is just less than 0.5 ml., which is by no means insignificant in relation to the total body fluid.

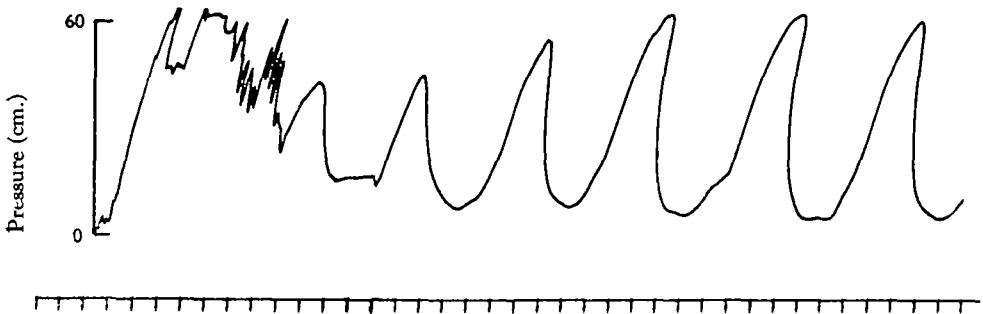


Fig. 1. Pressure changes in the coelom of *Arenicola* during burrowing into sand. A glass cannula was inserted in the posterior trunk region at the commencement of the recording (left) and the worm reacts to this for the first 7 sec. before a regular series of peaks commences. Time-mark 1 sec. The pressure is measured throughout in centimetres of water.

Some of the advantages of making a continuous recording of pressure changes with the Bourdon transducer have been pointed out above together with a comparison of the manometric technique previously used in order that the results reported in this paper may be more easily understood and interpreted in relation to previous findings.

EXPERIMENTAL OBSERVATIONS

(a) Introduction

A recording such as Fig. 1 is obtained when a Bourdon transducer is used to record the hydrostatic pressure of the coelomic cavity of a lugworm while it is burrowing into sand. Immediately after cannulation there is some reaction and a high pressure is built up for not more than 10 sec. This is followed by a series of peaks of pressure as burrowing proceeds. Each peak is of about 2 sec. duration and they are commonly of the order of 70 cm. amplitude, while between the peaks the pressure drops to a much lower level, around 6 cm. pressure in the example figured but commonly of the order of 2 cm. pressure. The effect of the initial high pressure and the mean value of

the subsequent peaks possibly accounts for the pressure values obtained by Chapman & Newell (1947) in a manometer whose rate of reaction is relatively slow.

The method of production and the significance of these peaks is discussed below.

(b) *Pressure determination in worms at rest*

One of the difficulties encountered in this part of the work is to decide when a worm is at rest. It may be considered to be at rest between the peaks of pressure shown in Fig. 1, but the overall picture whenever a worm in good condition is placed on sand is that of a burrowing *Arenicola*. Indeed if the worm does not begin to burrow fairly soon one considers it to be spent and generally unsuitable material. However, worms

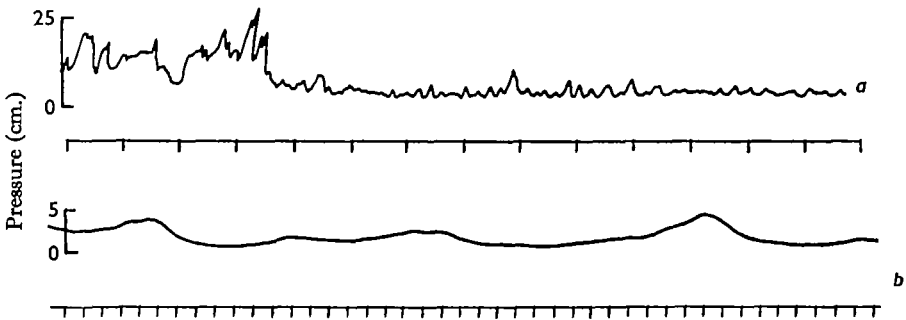


Fig. 2. Recordings of resting pressures in relatively inactive non-burrowing *Arenicola*. *a*, Effect of cannulation for the first 3 min. followed by low undulating pressure without major pressure peaks (time-mark, 30 sec.). *b*, Normal resting pressure in gently wriggling worm (time-mark, 1 sec.).

placed in glass dishes without sand or with a layer of wax on the bottom cannot burrow and become quiescent to varying degrees shortly after cannulation. Fig. 2*a* shows a typical recording with the lugworm wriggling gently on a wax surface. First there is a period of 3 min. when the worm contracts violently after cannulation, setting up pressures as high as 25 cm. of water. This is followed by a period of 9 min. (not all shown on the figure) when the worm wriggles gently, giving a maximum pressure of 5 cm. and a minimum of 1.5 cm., the average pressure for the whole period being about 2 cm. of water. In all the observations made on the coelomic pressure of whole *Arenicola* the pressure only very rarely fell below 1.0 cm. and in general 2 cm. of water may be taken as a reasonable mean value for the hydrostatic pressure in a relatively inactive worm. To such an internal pressure the term 'resting pressure' will be applied for the remainder of this paper.

After a lugworm had settled down after cannulation the coelomic pressure generally gently undulated as in Fig. 2*b*. The only marked exception was on the occasions when the worm was against the side of the dish and made some more considerable exertion to become free of the cannula, when pressures of up to 20 cm. were recorded. It appeared as if the worm required an extra surface upon which to obtain leverage before these higher pressures would be exerted. The value of 2 cm. cited is markedly different from the 14 cm. given by Chapman & Newell (1947) but it agrees with that of Wells (1954, 1961) determined by the indirect means of observing the effect of

applied pressures on proboscis extrusion. The function of the resting pressure is to support the body wall of the worm and to maintain the body turgor so that the interaction of the antagonistic muscles may take place. The weight of a medium-sized *Arenicola* in water is of the order of 0.5 g. and if the animal is lying on its side the maximum weight of tissue that the body fluid must support is 0.5 g. if all this weight is attributed to the trunk region. In a worm of length 20 cm. and diameter 1.2 cm. approximately 12 cm. may comprise the trunk segments. Considering a horizontal longitudinal section of the worm, a maximum cross-sectional area of 1.2×12 cm.² supports a weight of 0.5 g., equivalent to a maximum water pressure of only 0.035 cm., which is small in comparison to the resting pressure. The principal function of the resting pressure would thus appear to be the maintenance of muscle tonus.

More violent wriggling than that discussed above, involving the turning and twisting of the entire trunk, produced no greater pressures. In this type of activity it was difficult to relate body movements to pressure changes but it was clear that for body movements involving contraction and relaxation of the different components of the muscle system pressures of more than 5 cm. are not required. There would appear to be no necessity for high hydrostatic pressures in the fluid/muscle system for normal changes of shape in *Arenicola*. Relatively low tensions in the antagonistic circular and longitudinal muscles are adequate to provide turgor of the body wall and elongation and contraction of the trunk segments (see Discussion below).

(c) *Minor fluctuations in pressure*

Wells (1954) and previous authors (e.g. Just, 1924) have observed peristaltic waves running forward from the posterior of the trunk along the branchiate region during active burrowing. These waves also occur when the worm is at rest and are quite obviously rhythmical in occurrence. They are closely related to the small pressure peaks shown in Fig. 2. Wells remarks that these waves are one of several phenomena which are seen during vigorous burrowing, each one affecting pressure conditions in the coelom and potentially interfering with the others. A close examination of the waves was made by direct observation of a gently wriggling worm and by filming burrowing activity. Their rate of movement was generally of the order of 2 cm./sec. and they occurred at intervals of 5–10 sec. The exact relationship between the wave and the small pressure increase was difficult to determine by observation but it appeared to correspond to the passage of the wave into the head segments. Comparison of the recording of pressure with film synchronized to the pen-recorder confirmed this. The diameter of the worm was measured in two places anteriorly near the entrance of the worm into the sand, which gave similar results (Fig. 3c) and at the point of insertion of the cannula (Fig. 3b) 6 cm. posterior. Both of these sets of measurements show successive dilations in phase with the pressure peaks. Dilation B₁ at the cannula passes forward to make dilation B₂ anteriorly at the rate of 2 cm./sec. and may be considered to set up the pressure fluctuation B (Fig. 3d) when it reaches the burrow. Pressure fluctuation A is similarly related to dilation A₂ (Fig. 3c). The corresponding dilation posteriorly (Fig. 3b) is not recorded, being before the start of the graph, and dilation C has not yet progressed to the anterior segments (Fig. 3c). The dilation of the worm anteriorly lags by about 3 sec. and precedes the pressure increase by 1 sec. The distance between the diameters measured is 6 cm., so that the

velocity of the wave is around 2 cm./sec., which corresponds to the previous direct observation.

As each wave passes along the body the tensions in the muscles of the body wall change. Recordings of body-wall movement made from a preparation of *Arenicola* similar to that of Wells (1949*b*, Fig. 6) correspond with the movement of the wave through the segments being used for recording. Each peristaltic wave accordingly

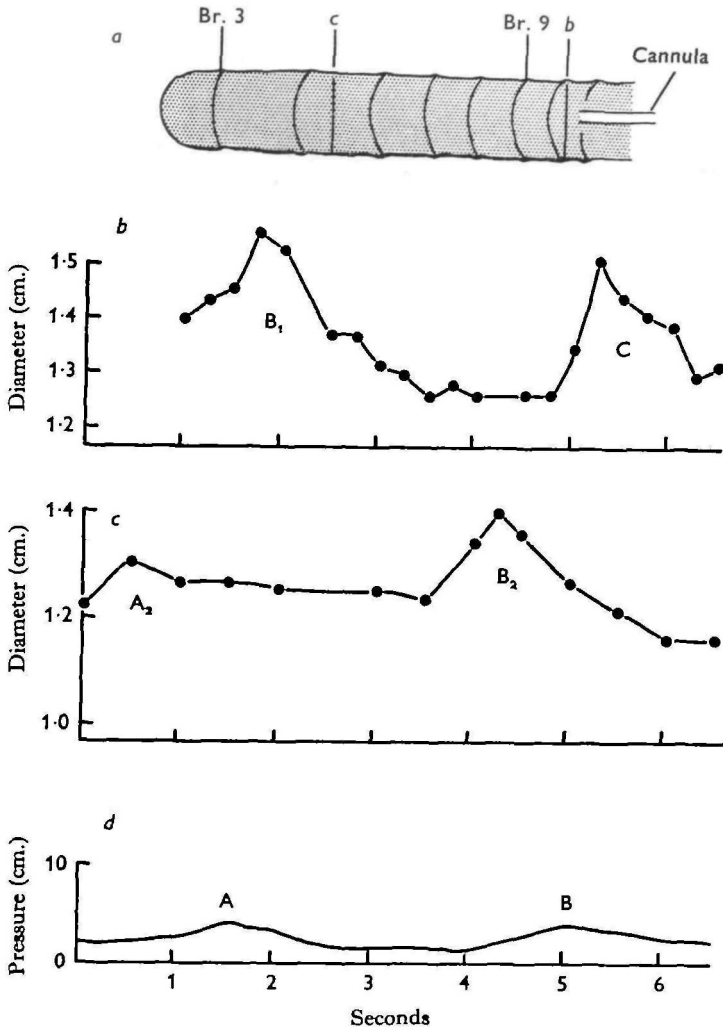


Fig. 3. Graphs showing relation of movement of peristaltic waves down the trunk of *Arenicola* and pressure. *a*, Diagram of worm used, segments anterior to the 3rd branchial annulus (Br. 3) are beneath the sand to the left; position of cannula, 9th branchial annulus (Br. 9) and the diameters measured in *b* and *c* are indicated, *b*, and *c*, Changes in diameter as dilations A₂ and B₁ and B₂ pass along the trunk from the posterior. These correspond to the pressure fluctuations below (*d*, A and B). Further explanation in the text.

corresponds with one of the series of contractions described by Wells (1949*a*, *b*; 1950*b*) as the irrigation-defecation cycle. The passage of the wave has been observed in these preparations to continue along the body up to the most anterior chaetigerous

segments and in some recordings, particularly when the body-wall contraction is nearly maximal, contraction of the anterior body-wall segments immediately precedes the contraction of proboscis muscles. This is not always apparent but it is obvious that there may at times be some close relationship between proboscis extrusion, the peristaltic wave and the pressure in the coelom. This is discussed below.

(d) Pressure change during burrowing

Fig. 1 gives a characteristic picture of the changes in coelomic pressure during burrowing. Between each peak of high pressure there is a fall to a resting pressure of not less than 1 cm. but usually of around 2 cm. The maximum pressure recorded during burrowing was 110 cm., though peak readings of 60–80 cm. were more

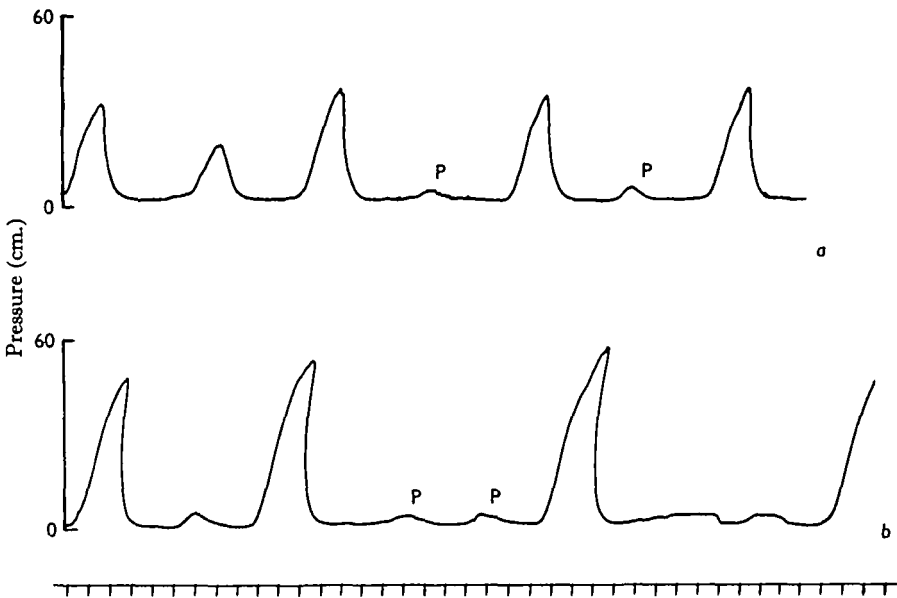


Fig. 4. Reduction in amplitude of major pressure peaks (P) after an *Arenicola* has burrowed for 1½ min. a, Single fluctuation between successive major peaks followed 20 sec. later by b, with two fluctuations. Time-mark, 1 sec. Further information in text.

common. This may be dependent on the efficiency of cannulation, for loss of coelomic fluid can result in the reduction of pressure. The peak lasts for 3–4 sec. and a pressure of over 40 cm. is rarely sustained for more than 2 sec. The shape of the peak in the recordings shows a gradual increase and a sharper decline of pressure. This effect is exaggerated in Figs. 1, 4 and 6, due to the pen tracing an arc, but when the recording is replotted so as to correct for this the general form of the peak still shows the features described above (Fig. 9). Since the response of the Bourdon transducer to 64 cm. pressure is complete in less than 0.2 sec. it is not likely that the recording device has much other effect on the shape of the pressure spike.

The recurrence of the pressure peaks at regular intervals during burrowing is always clearly visible in recordings. The recurrence frequency of peaks is of the order of 1/5 or 6 sec. and is seen to persist until the worm is buried, when recording is

terminated because of the loss of the cannula. Thus a worm which completely burrowed, except for the last part of the tail, in 4 min. would produce about forty peaks of pressure. But in many worms in the laboratory the frequency of the major peaks was reduced after perhaps 1 or 1½ min. The form of recording then obtained was similar to that shown in Fig. 4*a*, where between successive major pressure peaks a small fluctuation (P) occurs and in this particular instance two minor peaks (P) are later seen between successive high pressure peaks (Fig. 4*b*). It will be observed that the overall frequency of minor and major peaks is the same and that the minor peaks are probably occasions when the full pressure is not developed. This was too general an occurrence to be accounted for as a blockage of the cannula and may be interpreted as lack of development of the full muscular power of the worm possibly because of fatigue. The occurrence of one, then two, and in some instances three, minor peaks between successive high-pressure peaks as burrowing progresses supports this view. Withdrawal of 0.5 ml. of the coelomic fluid (about one-third of the total) before burrowing also produces this effect so that possibly the leakage of coelomic fluid from around the cannula may account for the small peaks. In general, however, burrowing worms showed no loss of turgidity until the cannula was withdrawn and the gut was everted through the cut body wall.

The minor peaks resemble those described in the previous section in relation to the peristaltic wave passing down the body of the worm. The frequency of their occurrence is certainly very similar. The major peak of pressure commences at the same time as a minor peak is due. Films of burrowing show clearly that peristaltic waves may develop into the major pressure peaks when they reach the anterior segments of the worm. Each major peak is related to certain muscular changes in the body wall, described below, but which only occur fully in response to each peristaltic wave when the animal is actively burrowing.

It has been observed above that a worm giving only normal resting pressures can produce some peaks of 20 cm. amplitude when pushing against the wall of a glass dish. If the first three or four segments of *Arenicola* are allowed to enter a glass tube of a slightly greater diameter than the body, pressure peaks of up to 50 cm. are set up. These peaks appear to enable the worm to press against the tube and to progress into the tube. Indeed the high-pressure peaks only occurred when part of the body of a worm was in a tube or buried in sand. Chapman (1950) has also observed that maximum pressures occur in *Arenicola* when it is confined in a glass tube and concludes that greater pressures can be exerted by the longitudinal muscles than by the circular.

A noteworthy exception to the idea of confinement of the worm for production of maximum pressures was obtained by immersion of an average-sized worm in isotonic potassium chloride (Fig. 5) and a pressure of at least 180 cm. was recorded. All muscles appeared to contract violently. This was the maximum pressure recorded in *Arenicola*, and only with difficulty was the worm retained on the cannula; but the proboscis was not everted.

A close study of the pressure changes in the coelom during the actual process of burrowing supports the hypothesis that high pressures are only normally produced during confinement of part of the body. This was carried out by filming the early part of burrowing and synchronizing the camera to the pressure record. A typical recording is shown in Fig. 6, in which a pressure of more than 60 cm. occurs following

cannulation. As early as this, before pressure is reduced, the head turns down on to the substrate and penetration is made by proboscis extrusion, discussed below. Indeed immediately after being placed on sand after cannulation (only 2 sec. in some filmed instances) the worm already has the first chaetigerous annulus beneath the surface of the sand. Only after the penetration of the second annulus do the major pressure peaks occur. These steadily increase in amplitude as more segments become buried.

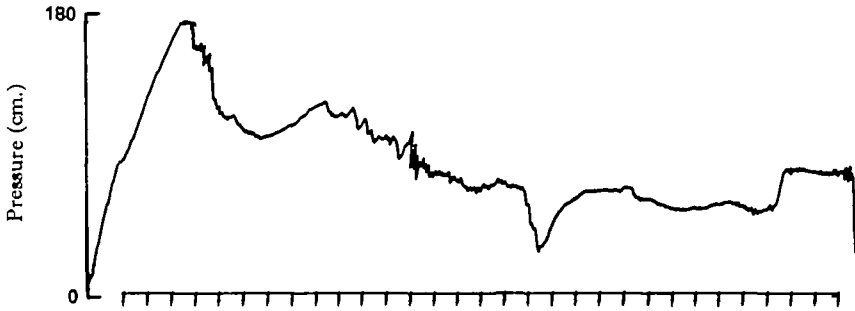


Fig. 5. Pressure developed by *Arenicola* when immersed in isotonic KCl. KCl was added at the beginning of the recording, evoked strong muscular contraction and produced the maximum pressure recorded. The cannula was ejected from the body cavity at the end of the recording. Time-mark, 1 sec.

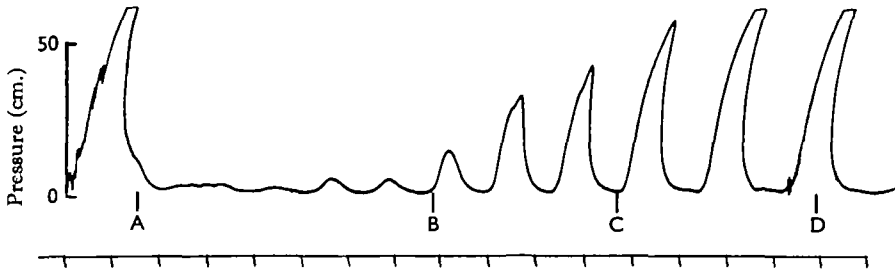


Fig. 6. Pressure changes in the body cavity of *Arenicola* during the commencement of burrowing into sand. Major pressure peaks are only observed after several segments have entered the burrow. The effect of insertion of the cannula is seen at the start of the recording and the following changes in burrowing are marked. A, Head down on to sand, followed by repeated proboscis eversion and penetration. B, C and D, 2, 3 and 4 chaetigerous annuli respectively beneath the surface. The flat top of the last two peaks is due to limitation of the travel of the pen at the amplification used. Time-mark, 5 sec.

After about six peaks, corresponding to the entrance into the sand of at least three or four chaetigerous segments, maximum pressures are recorded. The pressure developed by the worm, in initial stages of burrowing at least, is related to the amount of penetration. The high-pressure peak can be related to at least two possible functions, namely to extend the anterior end of the worm, causing movement of the head into the sand, and to dilate the anterior segments so that they press against the sand and allow the maximum grip by the chaetae. If the former is exclusively true then it is surprising that greater pressures are not obtained at the very initial stages of burrowing. Chapman (1950) observes that the longitudinal muscles are more important than the circular in producing great pressures and this view is developed in the following

section. It would appear from this later discussion that the gripping of the anterior segments in the sand and the pulling into the sand of the posterior segments are very closely related to the pressure peaks.

It has been observed by Chapman (1958) that the intestine in the caudal portion of *Arenicola* serves for the passive accumulation of faeces and he concludes that this process is aided by the existence of a low-pressure region in the caudal segments. This is achieved by the presence of a succession of septa separating it from the relatively high-pressure region of the trunk coelom. To check on the pressure changes in this region during burrowing a cannula was introduced into the coelom of the tail region, about half-way along, pointing forwards. On introduction of the cannula a maximum pressure of 12 cm. was observed for about 15 sec. followed by fluctuations in pressure

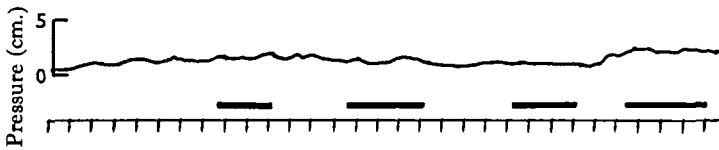


Fig. 7. Pressure in the middle tail segments of an *Arenicola* during burrowing. Horizontal lines indicate pressure peaks in the main body cavity, which show little effect on the caudal region. Time-mark, 1 sec. Further information in the text.

of 4 cm. peak-to-peak amplitude. Burrowing then commenced and there was little change in pressure. The recording in Fig. 7 was taken as the terminal trunk segments entered into the sand and there were no major changes in pressure in the tail then or at any time during the burrowing. The amplitude of the pressure change in the tail was very small even though the tail was continually wriggling. Such pressure changes as could be observed did not correspond to the pressure peaks of the general body coelom and were probably directly related to movement of the tail. It is of interest to note that the standing or resting pressure in the tail region was about 2 cm., which corresponds with that of the anterior coelom. These observations bear out those of Chapman even though the caudal septa are perforated by ventro-lateral clefts (Wells, 1961). Although it is not known whether these are capable of being closed the hydrostatic isolation of the tail region, demonstrated above, would seem to depend upon their closure. Wells (1961) also suggested that the tail is adapted for the support of the rectum during defaecation. This would seem to infer incomplete isolation of the caudal region from the anterior part of the body. No observations have been made during this work on the pressure changes during defaecation but there is no reason why the caudal segments should not have multiple functions.

(e) *Muscle activity in relation to pressure*

Previous authors (for example, Newell, 1950) have found it difficult to correlate pressure changes in worms with muscular contractions and relaxations. Using the continuous recording of pressure by the Bourdon transducer as a basis, direct observations of the activity of *Arenicola* in burrowing were made and the recordings were marked manually by a key. From these it was clear that the worms were thrusting forward into the sand to a maximum when the pressure rose to a peak and that on retraction from the sand the pressure fell sharply. More reliable recordings of this

were made by tying a loose loop of thread around the body of the worm just behind the insertion of the cannula and attaching the other end to a light spring which operated a mechano-electric transducer. This preparation is similar to that described by Wells (1949a, Fig. 4) for recording the irrigation cycles of the body wall of *Arenicola*. A typical recording is shown in Fig. 8, where the upward deflexion in the upper trace indicates a movement into the burrow corresponding to an increase in coelomic pressure in the lower recording. The movement out of the burrow, downward deflexion of upper trace, similarly corresponds to the sudden drop in pressure; and when the pressure-recording is corrected for the arc described by the pen, pressure may be observed to commence to fall synchronously with retraction.

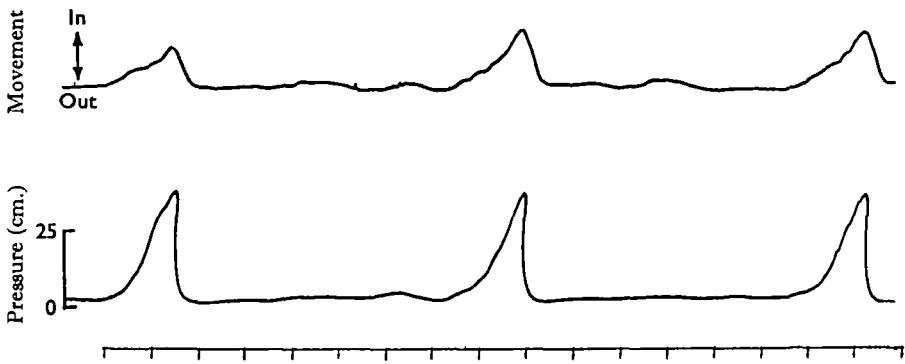


Fig. 8. Simultaneous recording of movement (above) and pressure (below) in a burrowing *Arenicola*. Movement into burrow (upward deflexion) occurs with increase in pressure and retraction (downward deflexion) accompanies the fall in pressure. Time-mark, 5 sec.

The sudden increase in pressure of a burrowing *Arenicola* cannot be adequately related to muscular changes by such a method as that described above. Accordingly, film was taken simultaneously with the pressure recording and it was synchronized by a manually operated key which both marked the recording and moved a lever in the field of the camera. The pressure recorded was subsequently indicated on each frame of the film by means of a line whose length was varied in proportion to the pressure. Thus pressure changes and movement could be viewed together repeatedly. This was followed by the analysis in detail of several of the filmed sequences, by measurement of segmental length and diameter, at intervals of $\frac{1}{4}$ sec., over the trunk region and more especially over the most anterior segments visible above the surface of the sand. Segments could usually be recognized and followed throughout a sequence by means of their chaetae. These were used for measurement of segmental length and of movement forward or backwards in relation to a fixed point in the field of the camera.

Figs. 9, 10 and 11 give some results of these measurements on different worms, filmed while they burrowed into sand in the laboratory. The contraction of the circular muscles at the commencement of the pressure peak is clearly shown in Fig. 9b and c, I. This thinning of anterior trunk segments is followed, during the pressure peak, by dilation (c, II and III), and a subsequent more minor contraction (IV) before dilation (V) prior to the next pressure peak. These last two phases (IV and V) appear to be

in the nature of a recovery process. The changes in diameter are most marked in the most anterior region of the trunk visible as the worm progresses into the sand. The major contraction (I) and dilation (II) always occur simultaneously with the raising

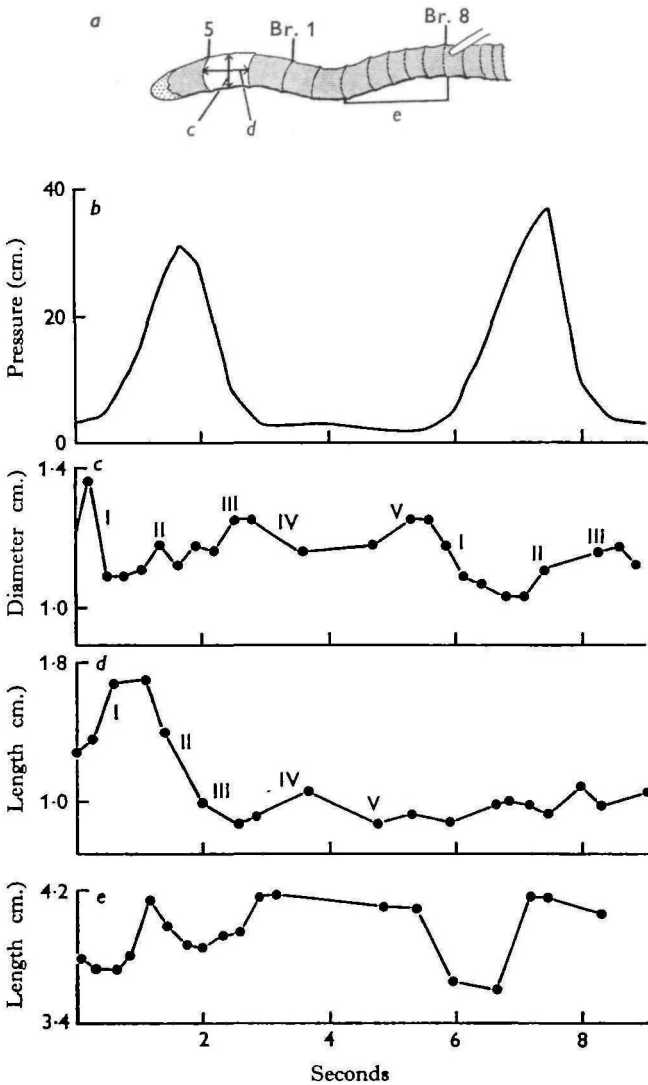


Fig. 9. Simultaneous graphs of coelomic pressure (b), segmental diameter (c) and length (d and e) in an *Arenicola* (a) during burrowing. These are obtained by use of pen recorder and synchronized film. The location of the measurements and the cannula are shown in a, the anterior four annuli being beneath the sand on the left. 5, Br. 1 and Br. 8 indicate the 5th chaetigerous annulus and the 1st and 8th branchial annuli respectively. I-V indicate the phases of muscular activity as described in the text and Fig. 10. The pressure peak coincides with contraction of the circular muscles (I) followed by the longitudinal muscles (II).

of the pressure. From the appearance of segments just entering the sand and from side views of the whole worm during burrowing the dilation with increase of pressure tends to be greater beneath the sand than above the surface. The impression is gained

that the pressure is withstood by the circular muscles only above the sand, whereas beneath the surface the sand itself takes the strain.

While these changes are proceeding in the circular muscles inverse changes of length are in general taking place in the longitudinal muscles, which act antagonistically to the circular muscles by means of the coelomic fluid. Fig. 9*d* shows the length between the 5th and 6th chaetigerous annuli, the most anterior complete segment visible in this recording. As the recording commences on the extreme left of the graph the segmental length is short while the circular muscle is relaxed. Elongation (Fig. 9*d*, I)

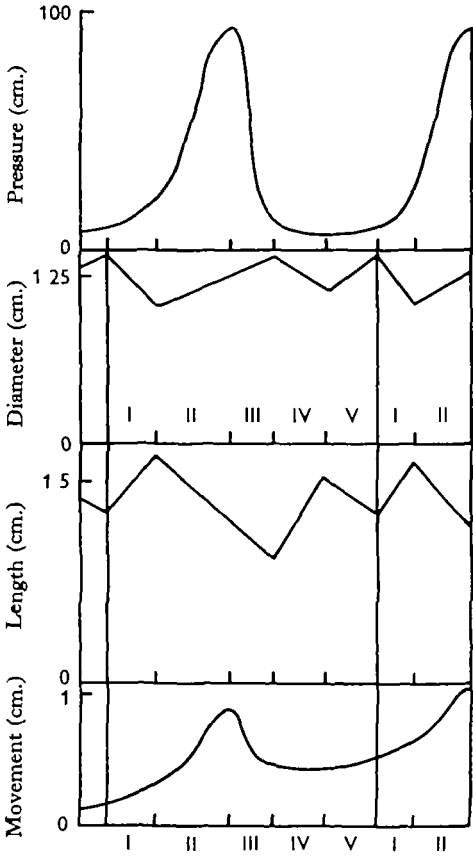


Fig. 10

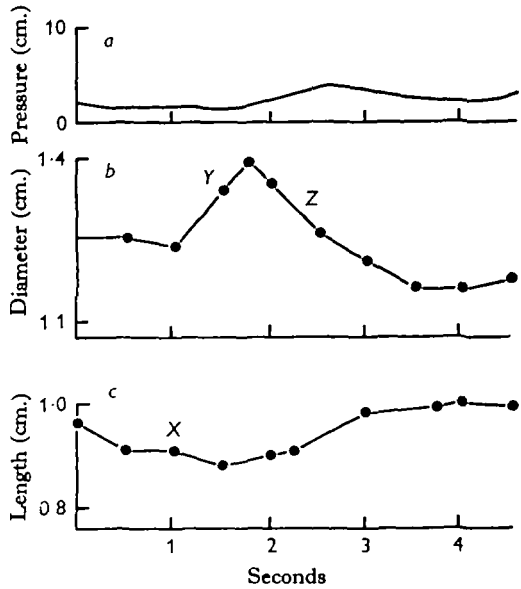


Fig. 11

Fig. 10. Generalized diagram showing the principal changes in coelomic pressure in a burrowing *Arenicola* (above), movement into the sand (below) and the corresponding changes in diameter and length (centre) of a typical segment close to the entrance of a burrow. The phases of muscular activity (I-V) show a complete cycle for each pressure peak. In the bottom graph movement forward into the burrow and retraction are shown, respectively, by the rise and fall of the curve, the net results of each pressure peak being a move forward. Overall time of the diagram is 8 sec. Further information in the text.

Fig. 11. Simultaneous graphs of coelomic pressure (*a*) and segmental diameter (*b*) and length (*c*) between the 4th and 5th branchial annuli of an *Arenicola* at rest. Some contraction of the longitudinal muscle (*X*) precedes extension (*Y*) and the subsequent contraction (*Z*) of the circular muscles. This gives rise to only a small fluctuation of the resting pressure in the absence of a major contraction of the longitudinal muscles (*c*).

occurs as the pressure increases, due to circular contraction to be followed by a major contraction of the longitudinal muscles coincident with the pressure peak (*d*, II and III). During this shortening the circular muscles relax and dilation reaches a maximum as the pressure falls (III). This is followed by elongation (IV) and a subsequent secondary contraction of the longitudinal muscles (V).

When high-pressure peaks occur in rapid succession (Figs. 1, 9, 12) it is clear that a cyclical series of muscular changes occurs in the body wall of the worm. An attempt has been made in Fig. 10, at the risk of over simplification, to represent schematically the more important changes occurring in a single anterior branchial segment of *Arenicola* after the first four or five chaetigerous segments have penetrated into the sand. This representation is derived from a detailed study of twelve pressure peaks in filmed and recorded sequences in several different worms, and from general observations on many more. There are five principal phases of activity associated with a single high-pressure peak. These follow each other cyclically as in Fig. 10, phase I returning after phase V. In phase I thinning and elongation occur due to contraction of circular muscles with some relaxation of the longitudinal muscles. This is the principal contraction of the circular muscles, simultaneous with the commencement of the pressure peak and the worm pushing forward into the burrow. As the circular muscles attain their maximum contraction the longitudinal muscles, now considerably elongated, contract strongly (II). The result is the high pressure in the coelomic fluid. This pressure peak drops suddenly (III) when the circular muscles, under great tension from the pressure derived from the longitudinals, relax sufficiently to allow maximum dilation (III-IV). This is followed by a secondary contraction of the circular muscles which stretches the longitudinals (IV-V). During phase V the latter make their second contraction of the cycle, causing dilation of the body wall prior to phase I. The recurrence frequency of the peaks depends on the exact length of the cycle and phases IV and V may be protracted. Both circular and longitudinal muscles undergo two contractions and relaxations for each pressure peak and these changes are most easily considered graphically as in Fig. 10. The principal or primary contraction of each muscle group has a major effect on the hydrostatic pressure of the coelom while their secondary contractions (IV and V) are possibly concerned with recovery before the next pressure peak. The curves shown in Fig. 10 are generalized from examination of film of *Arenicola* burrowing. Segmental muscles do not always operate in exactly the same way for each peak of pressure. An example of this is shown in Fig. 9 where at the time of the second pressure peak there is only a small change in length of the 5th-6th chaetigerous segment (*d*). While it is possible that the longitudinal muscles of this segment may be exerting pressure on the hydrostatic system in isometric contraction, there is marked shortening of the 4th to 9th branchial segments (*e*). The muscles of the whole body wall may thus be involved in the production of high pressures. This is one of the advantages of a continuous body cavity, for the extent to which muscles of different regions contract at each pressure peak clearly varies. Certainly the circular muscles of the whole of the trunk, apart from those buried in sand, must withstand the strain of the high pressure each time it is applied, but the shortening of the longitudinals coincident with the production of high pressure may take place remote from the point of application of the pressure. In this way the body of *Arenicola* may be considered as a hydraulic system in which the

force applied by posterior muscles can be transferred to the anterior end of the body for use in burrowing.

It is evident then that the function of the longitudinal muscles is not the same for each segment in all contractions. Usually, however, the segments nearest the burrow behave more uniformly, become more greatly dilated at the passing of each peak and are always more elongated than the posterior segments. This is represented in the sketch of the worm used in the recordings of Fig. 9. In general there is an inverse relationship between the circular and longitudinal muscles of the body wall. Contraction of one gives some extension of the other as in Fig. 10, but it would be surprising if this relationship was exact when one segment only is considered. The most marked exception is when a major pressure peak is produced, for although the segments near the burrow entrance dilate, the circular muscles of the more posterior trunk segments withstand the strain apparently in isometric contraction. The posterior trunk becomes extremely turgid at the pressure peak and can be often observed to be pulled erect off the sand when a firm attachment has been made by the anterior segments. Retraction of the worm only occurs when this adhesion is lost.

The changes in segmental dimensions in the anterior region of a worm have also been observed when no large pressure peak has been produced (Fig. 11). Longitudinal shortening (Fig. 11*c* at *X*) causes dilation of the body wall (at *Y*) prior to contraction of the circular muscles. This marked circular contraction (Fig. 11*b* at *Z*) corresponds to the minor increase in pressure, but the longitudinals show no major contraction such as produces the large pressures shown in Fig. 9. This supports the previous observations on the role of the longitudinal muscles in producing high pressures. Such a low-amplitude pressure fluctuation as in Fig. 11 resembles that of a simple peristaltic wave passing down the body. Longitudinal shortening and dilation of the body wall (Fig. 11, *X* and *Y*) similar to that preceding all pressure peaks (Fig. 10, V-I) appears to occur during the passage of a peristaltic wave along the body from the posterior trunk region. The development of a major pressure peak from a small fluctuation in pressure, such as the peristaltic wave produces, only additionally requires the shortening of the longitudinal muscles. This appears to occur rapidly after the peristaltic wave passes into the segments beneath the surface of the sand.

Retraction of the worm from the burrow clearly commences as the pressure falls (Figs. 10, 12). To see what was happening beneath the sand, observations were made of a worm's burrowing immediately behind a glass plate. Retraction is accompanied both by protraction of the chaetae and by flanging of at least the first three chaetigerous annuli in the manner described by Wells (1950*a*) for a worm in a glass tube. The effect of burrowing on the sand adjacent to the head of a worm is discussed below but it is obvious that the amount of retraction is related to the grip or hold that the anterior end can obtain on the substrate. The flanging of the anterior segments certainly increases the hold of the worm considerably. At the commencement of burrowing it has been shown that the pressure peaks increase in amplitude with depth of burial (Fig. 6). This may well be related to the adhesion of the head to the substrate, for only when the longitudinal muscles contract fully can maximum pressures be achieved. These muscles cannot contract fully without causing marked retraction unless good adhesion of the head is obtained.

Observations have been made of the commencement of burrowing in sand before

the pressure peaks have exceeded 20 cm. and before more than three chaetigerous annuli have passed into the sand. Movement in and out commences even before the pressure peaks occur, and when the small peaks are recorded they are out of phase with the protraction-retraction cycle. The relationship described above (Fig. 10) only occurs when more than three segments are in the sand and large pressure peaks are produced. It is clear that something different is happening at the initial entry of the worm into the sand. High pressures are not involved in initial penetration but proboscis extrusion as described by Wells (1954, 1961) is certainly concerned and this will be discussed below.

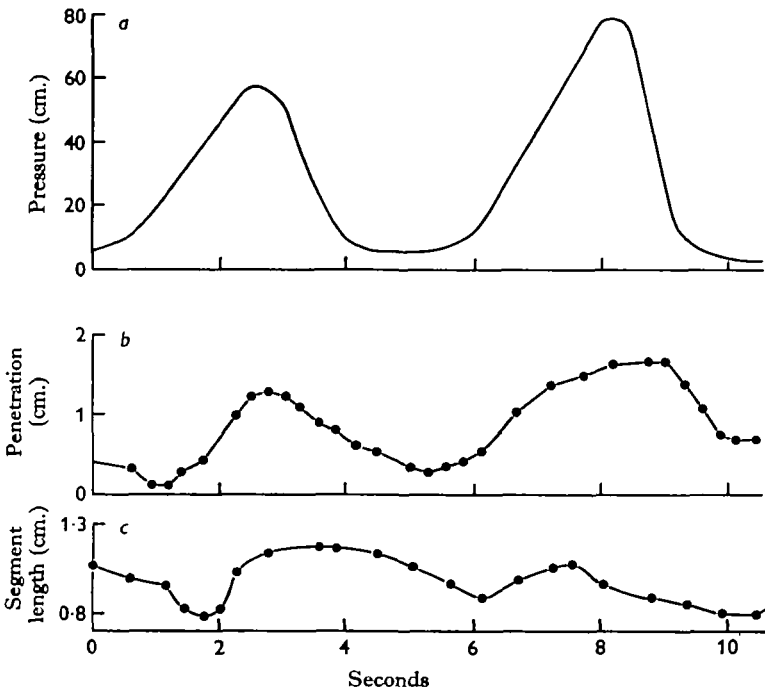


Fig. 12. Graphs showing coelomic pressure (a), movement of worm into burrow (b), and length of 1st branchial segment (c) of a burrowing *Arenicola* after the first five chaetigerous segments have passed into the sand. Movement of the 1st branchial parapodium (b) shows maximum penetration with peak pressure and retraction at the fall in pressure. Further information is in the text.

Also relevant to burrowing activity in the lugworm are the movements of the body into the sand in relation to the pressure peaks. Although this has been recorded mechanically, synchronous film recordings have the advantage of also giving additional information regarding segment length and thickness (Fig. 12).

Comparison of the pressure record with the graph of movement of the first branchial parapodium shows similar features to the movements recorded mechano-electrically. The greatest movement forward into the sand corresponds with the high pressure and backward movement coincides with the fall in pressure (Fig. 12a, b). The worm is only still, for not more than 1 sec., at the maximum and minimum pressures recorded. The movement of the 5th branchial parapodium was also graphed and its

movement forward and backward was similar to that shown in Fig. 12*b* except for rather lower amplitude. This may have been due to the restricting effect of the cannula since this reduction of movement was not so apparent in worms completely free to burrow. Indeed in such worms the movement forwards is often greater and the withdrawal less than in the cannulated specimens, producing, as one would expect, more rapid burrowing. The changes in length of the first branchial segment (Fig. 12*c*) indicate that although the contraction of the anterior longitudinal muscles corresponds with some forward movement into the sand, maximum protraction of the head occurs during lengthening of at least the anterior end of the worm.

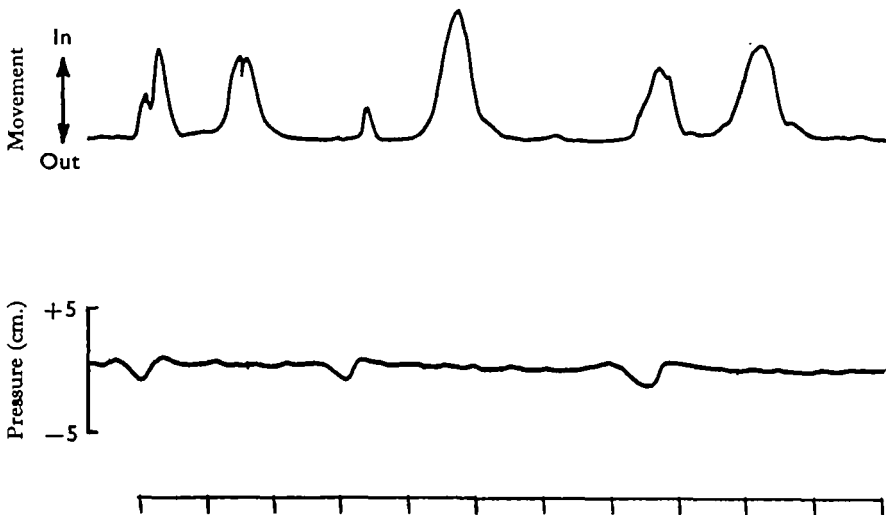


Fig. 13. Recordings of the movement of a burrowing *Arenicola* (above) and the pressure changes in the adjacent sand (below). Movement into the sand is indicated by an upward deflexion of the trace and sometimes corresponds to a negative pressure in the sand. This is discussed in the text. Time-mark, 5 sec.

(f) Observations on the effect of pressure on burrowing

When an *Arenicola* burrows in sand by the side of a glass surface, movement of the sand particles may be observed and filmed. At the time when the maximum pressure is exerted the sand is clearly pushed away from the worm, moving outwards from most of the body situated beneath the sand. This gives the impression of pressure being exerted not only in front of the head but also on most of the substrate adjacent to the worm. During retraction the opposite may be seen to happen. Annulation of at least the anterior three chaetigerous segments takes place in order to anchor the head of the worm, and any retraction of front segments tends to give rise to a flow of sand to fill the cavity which would otherwise be produced.

An attempt was made to measure the pressure imparted to the sand by the burrowing worm. For this purpose a hole was drilled in the side of a Perspex tank, was covered with nylon mesh (25/sq.in.) and connected to the Bourdon transducer. At the same time a lugworm was tied to a mechano-electric transducer by a looped thread so that movements of the worm could be recorded simultaneously with external pressure. With only one pressure transducer it was not possible to record body pressure at the

same time, but the movements of the worm have already been shown to correspond to pressure peaks (Fig. 8). A typical recording of sand pressure and movement of the worm is shown in Fig. 13, and it may be observed that not all the movements into and out of the sand made an effect on the pressure. This may have been due to the loop of thread around the trunk of the worm, through which the worm gradually wriggled during the recording. When pressure does vary, a small negative pressure (1 cm.) occurs even before movement of the worm into the sand. At the maximum forward movement the pressure is swinging positive and this positive pressure slowly returns to zero. While this positive swing is probably due to the pressure applied by the worm to the substrate, the negative pressure was unexpected. However, poking a finger down into the sand near the pressure tube gave a similar negative pressure so that the explanation may lie in the dilatant properties of the sand. In a consideration of the thixotropic properties of sand, Chapman & Newell (1947) point out that the whitening of sand on a beach under a footfall is due to the applied force upsetting the original packing of the particles in the sand-water system. The available water no longer suffices to fill the enlarged spaces between the particles and is withdrawn from the surface. This whitening effect provides a ready test for a dilatant sand-water system and the sand being used experimentally was shown to be dilatant in this manner on the beach.

Accordingly a reasonable explanation for the negative pressure could lie in the dilatant property of the sand-water mixture. The force being applied by the hydrostatic pressure of the body cavity disturbs the packing of the sand-water system and tends to draw in water. This exerts a negative pressure on the water in the adjacent tube which passes to the transducer. I have observed a similar effect with the foot of the sand-burrowing bivalve, *Tellina tenuis*, which utilizes the hydrostatic pressure of the haemocoel to obtain adhesion of the foot in the sand. It should be noted that a medium which is dilatant is one which shows increased resistance to increased rate of shear. Thus the application of pressure to the substrate alongside the worm must make the sand more resistant. The chaetae can grip and the body wall adhere to form a firm anchor for the pulling forward of the worm. The pressure peak can thus be thought of as carrying out at least two functions. First it provides pressure for the elongation of the anterior end of the worm against the resistance of the substrate, which is dealt with more fully below, and secondly provides a firm anchor for the anterior segments when the remaining trunk and tail are pulled downward and forward into the sand. This adhesion occurs with only a few segments buried and as more chaetae enter the burrow the adhesion of the worm probably becomes less critical.

Retraction takes place when the internal pressure falls and adhesion tends to be lost. It is at this stage that flanging of the anterior segments may be observed to take place. Wells (1944*a*) has described the structure of the anterior annuli and how they cause flanging. Each annulus contains a circular coelomic canal which becomes inflated all at once in the flanging process. The process of flanging does not seem to be closely related to high coelomic pressure, for annulation has been observed to occur in an open glass dish when only resting pressures were being recorded. There was no obvious relationship between pressure fluctuations at rest and during flanging.

Retraction from the burrow might be expected to give a negative pressure, as must happen in Wells's (1944*a* and 1961) experiment in which *Arenicola* was pulled from

the neck of a filter funnel, but this was not recorded with *Arenicola* in sand. Withdrawal of a finger from a similar position in sand does however give a negative pressure and its absence on retraction of a worm is puzzling. It could be avoided by slower retraction and by sand-water flowing inwards rapidly enough to replace the retracted volumes. If this is so the retraction is closely related to the exact physical condition of the sand-water mixture adjacent to the worm.

(g) *The mechanism and role of proboscis extrusion in burrowing*

Wells (1944, 1948) has demonstrated that the proboscis functions in burrowing not as an animated cork borer but by pushing the sand aside and backwards. He disagrees with the view of Chapman & Newell (1947) that burrowing is due to the physical properties of wet sand and the hydrostatic pressure of the body cavity. When the burrowing worm is watched through glass, proboscis eversion and sand movement can be seen to correspond to Wells's descriptions. Initial stages of burrowing involves proboscis extrusion and retraction but no great hydrostatic pressures are used until several segments have penetrated the sand. Hence the views of Wells on the functioning of the proboscis are fully confirmed.

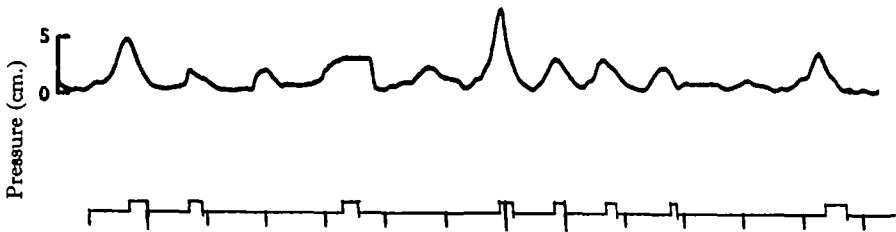


Fig. 14. Coelomic pressures of *Arenicola* in a glass dish during a period of active extrusion of the proboscis. All proboscis extrusions are marked above the 5 sec. time-mark. Further information in the text.

Wells has also described the mechanism of proboscis extrusion and retraction in some detail (1954, 1961). It is based on the cyclical contraction and relaxation of the circular and longitudinal muscles of body wall and buccal mass, proboscis extrusion depending on an appropriately timed release by the sudden relaxation of the circular muscles around the mouth and of the whole musculature in the wall of the buccal mass. This allows the internal pressure to evert the buccal mass, the power being obtained by a levy from the tonus of the whole body-wall musculature. In this paper an attempt is made to see how far pressure changes are involved in proboscis extrusion. Recordings of the hydrostatic pressure in the trunk of a worm in a glass dish were made in the usual manner with proboscis activity marked on the recording by pressing a key at extrusion and releasing it at withdrawal. The recording (Fig. 14) shows the pressure during a series of complete proboscis eversions. The pressure-recording resembles records of normal resting pressures, and the eversion of the proboscis corresponds to the peak pressures. Examination of recordings shows that the proboscis everts at maximum pressure or as the pressure is falling. The pressure peaks are not all of the same amplitude and for some peaks there is no eversion. Thus exact values of these pressures would appear to be immaterial in eversion and there is no clear-cut

threshold level. Whilst making the recording shown in Fig. 14 peristaltic waves were clearly observed to pass along the worm and to correspond with the raising of coelomic pressure when they reached the anterior segments. This relationship was not always so clearly defined, but it would be reasonable to expect that the proboscis could evert when the pressure is increased by the peristaltic wave although this by no means occurs for each wave.

Wells (1954) describes a preparation of the anterior end of *Arenicola* where the worm is tied on to a cannula and extrusion of the proboscis is observed at different pressures which could then be applied to the preparation. He states that the pressure required for full extrusion is low; a head of anything from 2 to 10 cm. of sea-water suffices. While this is in accord with the pressures recorded in the whole worm a further investigation of the anterior preparation was carried out. In some recordings the pressure transducer was coupled to the preparation by means of a T-piece in the

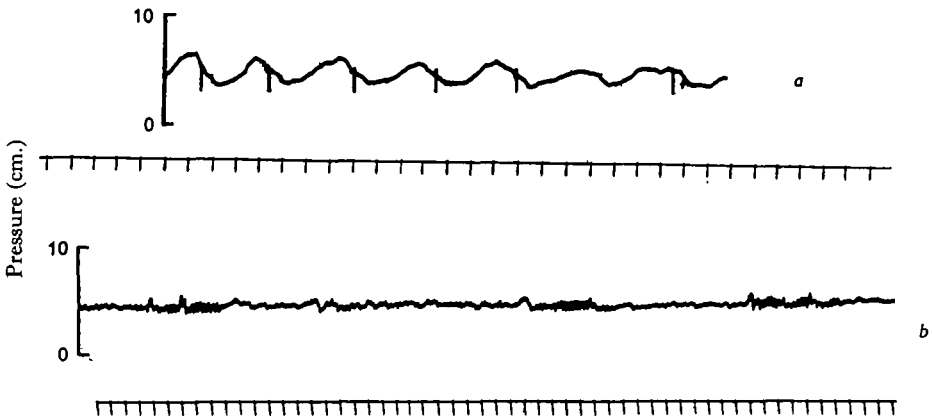


Fig. 15. Pressure recording from the cannula of an anterior preparation of *Arenicola* (Wells, 1954) showing (a) pressure fluctuation and proboscis extrusion (at vertical lines) and (b) outbursts of pressure fluctuations at the recurrence frequency of the feeding cycle. Time-marker (a) 5 sec., (b) 30 sec. Further information in the text.

tube connecting the pressure head to the cannula, in others by a hypodermic needle inserted into either the main coelomic cavity or the coelomic cavities of the head. Wells observed that for the present purpose only the most anterior head coelom is isolated from the main coelom. Insertion of a hypodermic needle into the head coelom always resulted in lack of eversion but insertion between the second and third septa gave, as expected, similar results to insertion into the general coelom. Fig. 15a shows the fluctuations of coelomic pressure when a head of 4 cm. was applied to the preparation. This was ligatured to the cannula at the 6th annulus so that the body-wall musculature of the more anterior segments produced the fluctuation of 2.5 cm. in pressure. The point of each proboscis eversion is marked and clearly coincides with the dropping of coelomic pressure, although it does not occur every time the pressure fluctuates. Without being able to measure the pressure of the head coelom it is impossible to say what forces are involved in the release of the proboscis. The rise in pressure which occurs first before extrusion may well initiate a build-up of pressure in the head coelom prior to release. Anterior segment preparations are able to produce these

waves of pressure fluctuations irrespective of what pressure head is applied between 1 and 20 cm., but at the higher pressure the amplitude of fluctuation is smaller and the proboscis extrusion is not coincident with the fall of pressure. This confirms Wells's (1954) findings that at 15 cm. and over the body wall shows little or no movement during the cycle and that extrusion is often jerky. Optimum conditions for proboscis extrusion appear to be at between 1 and 5 cm. pressure in *A. marina* and this species clearly depends for full extrusion on fluid delivered under pressure from the general coelom.

The regular fluctuations in pressure were recorded in the anterior segment preparations (Fig. 15*b*) as a short period of vigorous activity alternating with periods of rest. The cycle occupies about 8 min. and corresponds with the period of proboscis and oesophageal activity described by Wells (1937, 1950*b*) and by Wells & Albrecht (1951) as the feeding cycle. They observe that the rhythm of activity can spread to the body-wall muscles of at least the first three segments. It is thus apparent that in these anterior segment preparations the feeding cycle was influencing the body-wall musculature, and it is not unexpected that the proboscis extrusion corresponds with pressure fluctuations. These observations were made in anterior preparations without the presence of the posterior trunk region. When the latter was present, as in the whole worm (Fig. 14), the peristaltic wave reaching the head was observed to correspond to the pressure changes, and to proboscis extrusion. This peristaltic wave may be taken to represent changes in the body wall of the trunk described by Wells & Albrecht (1951) as the irrigation-defecation (i-d) cycle under the control of a pacemaker in the posterior trunk segments. Only when the peristaltic waves were easily noticeable were fluctuations of the magnitude shown in Fig. 14 observed in the resting pressure. It may be suggested that only when the contractions of the i-d cycle were strong did they have an effect on the proboscis and anterior body wall. At other times proboscis and anterior body wall would be under the control of the feeding cycle.

Preliminary experiments, using the preparation of Wells (1949*b*) to record proboscis and body-wall contractions simultaneously, did show contractions passing forward to the three most anterior segments of the body wall but only when the contractions of the body-wall muscles were maximal. Proboscis extrusion was then commonly synchronized with body-wall contraction. This is in accord with the conclusion of Wells & Albrecht (1951) that the integration of activities which the pacemakers determine probably depends on variation in the extent to which their influences spread though the neuromuscular system. They appear to compete for territory. Maximal contraction of the body wall, which may be experimentally induced, can be considered to influence the proboscis because of its spread. In a similar manner the feeding cycle can spread into the body wall (Wells, 1954, Fig. 5) and affect the pressure as has been demonstrated in the anterior segment preparation. The dominant rhythm in the proboscis and anterior body-wall region presumably controls activity, and dominance may vary cyclically as Wells (1950*b*) has demonstrated.

The role of the proboscis in the early stages of burrowing is clearly to effect the first penetration into the sand by successive extrusion and withdrawals. No great hydrostatic pressures are used in this phase but as the segments move inwards and the pressure peaks become of considerable amplitude the conditions of extrusion must change. As observed above, pressures of over 100 cm. are encountered during

normal burrowing or by immersion in isotonic KCl. In the latter case the proboscis was not extruded so that the worm is clearly able to withstand normal burrowing pressures without forced extrusion of the proboscis. This is presumably prevented by the circular muscles of the mouth. On the other hand extrusion of the proboscis would be very powerful if caused by such high pressures. Observations of proboscis activity while the worm is burrowing beneath the sand are difficult to obtain even when it is moving against a glass plate. The recordings obtained on some occasions, but not on all, showed proboscis extrusion with the pressure peak. High pressure in the coelom certainly appeared to be applied to the substrate both with and without proboscis eversion. The isolation of proboscis extrusion from the activity of the posterior trunk segments is perfectly feasible according to the observations of Wells & Albrecht (1951) but it is not surprising that the near-maximal contraction of the body wall involved in the production of a pressure peak spreads anteriorly to influence proboscis activity. Further investigations are clearly required into the problem of interaction of the rhythmic patterns of the two pacemakers.

Wells (1954) remarks that the work of the extrusion of the proboscis is carried out by drawing a levy from the tonus of the whole body-wall musculature. But this is a very small levy in relation to the strength of the body wall. Extrusion occurs at an optimum at pressures not exceeding 7 cm. while the body wall can generate pressure of up to 15 times this value.

DISCUSSION

This paper on the hydrostatics of the body fluid of *Arenicola marina* has established a number of new findings in regard to the resting pressure, the pressures used when burrowing and extruding the proboscis and the muscular activities of the body wall. It is appropriate to consider these findings briefly in the light of previous researches. It is to be regretted that so far I have been unable satisfactorily to record pressures when a worm is below the sand in a normal burrow. Wells (1961) considers that *Arenicola* must be able to 'blast' out a vertical channel to the surface of the sand for a headshaft. In large worms at a depth of 30 cm. Wells (1945) has shown that the pressure head required is rather higher than the depth of sand and that the pressure for steady irrigation is half as great. Pressures of this order should not pose any great problem even to a medium-sized *Arenicola*.

The resting pressure of about 2 cm. is of the same order as Wells (1954) considered likely on the basis of experiments on proboscis extrusion. This author (1961) considers that in a motionless *Arenicola* the circular muscles are under a greater tension than the longitudinals. But for any resting pressure there must be some tension imposed on the longitudinal muscles, and the worm would stretch slowly unless the tension was absorbed by the connective tissue of the body wall or unless these muscles contracted. Recordings (Fig. 2) show that there is a continuous fluctuation in the resting pressure. It is hardly ever steady and this probably involves some rhythmical contractions and relaxation in the antagonistic circular and longitudinal muscle system. No greater pressures than the fluctuating resting pressure appears to be required for movements on the surface of sand or for extrusion of the proboscis but in these movements both sets of muscles are involved.

The method used by Batham & Pantin (1950) to determine the tension in *Metridium*

muscle may be used to make some estimate of the tension in the muscles of body wall of *Arenicola*. For a cylinder of infinite length the tension per cm. in its wall, across a line parallel to its axis, is equal to the internal pressure multiplied by the radius of the cylinder. A worm obviously has not infinite length but the relation should hold for the greater part of the trunk. The worms used had a trunk of mean diameter of 1.2 cm. which gives a tension at resting pressures of only 1.2 g./cm. of the body-wall, but at a peak pressure of 100 cm. the tension would be 60 g./cm. With a maximum resting pressure of 6 cm. the tension in the body is under 4 g./cm. or less than 7% of the maximum tension in the body-wall, a rather lower figure than that recorded for *Metridium*. It is difficult to be sure of the thickness of the circular muscle layer in *Arenicola* after fixation but an average figure of 0.02 cm. was used in the determination of tension per cm.² of cross-section of the circular muscles. A longitudinal section of the circular muscles in the body wall 1 cm. long thus has a cross-sectional area of 0.02 cm.². If the body wall can exert 1.2 g./cm. at rest then the muscle must produce a tension of 60 g./cm.², while at peak pressure of 100 cm. the tension exerted would be 3 kg./cm.². But this tension is only maintained for a maximum of 2 sec. during which some stretching of the circular fibres is apparent (Fig. 9). This figure is significantly less than that for *Metridium* (40 kg./cm.²) but it is comparable to the figures quoted for other muscles such as *Helix* columella muscle or frog skeletal muscle (Batham & Pantin, 1950). The figure of 3 kg./cm.² is comparable to the values I have obtained for muscle-strip preparations of *Arenicola* body wall in isometric contraction. It is hoped to publish these results in detail shortly but it is already apparent that the muscles of the body wall are well able to exert adequate tension to produce the pressures recorded.

The power stroke in the production of the large hydrostatic pressure is the contraction of the longitudinal muscles as is evidenced in Figs. 9 and 10. This has been recognized by various authors in the past, notably by Wells (1950a, 1961) who comments that the massive longitudinal muscles used in phasic contraction never maintain maximum tension for long. This view is substantiated by the short duration of the pressure peak. Chapman (1950, 1958) observes that in an earthworm the longitudinal muscles can exert a pressure on the coelomic fluid of about ten times that of the circular and comments that this implies that the former do not exert their maximum pressure in a free unconstrained worm. The longitudinals contract to their full extent only when the worm is supported by the walls of its burrow or in response to adverse stimuli. This interpretation is hardly likely to hold for *Arenicola* where the trunk coelom is continuous and the pressures recorded in the coelom must be withstood by the circular muscles of all trunk segments outside the burrow. The more likely explanation of the absence of high pressures until some segments are beneath the sand or in a tube is that an anchor is necessary at the anterior end as a holdfast before major contraction of the longitudinal muscles occurs to produce high pressures. The actual pressures produced in *Arenicola* may be just as dependent on the adhesion of the chaetae in sand as on the absolute strength of the longitudinal muscles. A somewhat similar condition exists in respect of the foot of bivalve molluscs. High pressures are not normally recorded except to anchor the foot in the sand immediately prior to contraction of the pedal retractor muscles (personal observation, to be published shortly). It is also of interest to note that in blowflies the development of high

pressures involved in normal digging is not dependent on the fly being enclosed (Cottrell, 1962).

During burrowing a wave of peristaltic contraction and dilation passes along the trunk from the posterior segments to the anterior of the worm where it may be developed into a high-pressure peak by the synchronous contraction of the longitudinal muscles of the trunk. Previous authors (Just, 1924) have observed this feature, while Chapman & Newell (1947) point out that coelomic fluid is forced into the anterior end of the worm before activity. The latter authors have recorded different pressures at anterior and posterior ends of the worm and consider that there is a mechanism for the provision of temporary bulkheads across the body cavity. Observations in the course of this work confirm the latter suggestion but show that in general there is no difference in amplitude of the pressure peaks in anterior or posterior coelom. This is to be expected with the longitudinal musculature of the whole of the trunk being involved in burrowing. The circular muscles, on the other hand, only dilate and contract at the beginning of the pressure peak (Fig. 10, I) when the anterior five or six segments are considered. Further back this dilation and contraction occurs earlier, presumably as the peristaltic wave passes, although the dilation during the pressure peak (Fig. 10, III) may effect the entire trunk segments.

Based on the experiments described in this paper and the muscular activity summarized in Fig. 10, the burrowing activity in *Arenicola* on a suitable sandy substrate consists of the following stages:

- (1) Probing forward by the head, mouth turned downwards and proboscis extruding. Movements largely confined to the anterior segments, no large pressures recorded.
- (2) Three or four chaetigerous annuli buried in sand by proboscis extrusion cycle and elongation of anterior segments, forming an anchor in the sand.
- (3) The longitudinal muscles of the whole trunk contract and high hydrostatic pressure bears on the substrate. This is repeated cyclically (Fig. 10).
- (4) During the high pressure the anterior end of the worm is forced forward, forms a new anchor so that subsequent contraction of the longitudinal muscles draws the worm into the substratum.

This closely agrees with the fundamental method of burrowing into a substratum by soft-bodied animals suggested by Clark (1964).

The principal function of the proboscis in burrowing is to open a way into the sand into which the animal can move. It does this by eversion and scraping away the sand as described by Wells (1961), not as a rod or cork borer pushed into the substrate. After initial penetration it is presumably everted with considerably more pressure than before to clear a way through the substratum. It is of interest to consider what pressure can be applied to the substrate from the extruded proboscis by analogy with the pressures determined by Newell (1950) for the earthworm. According to Wells (1954) only the anterior septum, separating the head from the trunk coelom, effectively divides the coelom anteriorly in *Arenicola marina*. The diameter of this septum could be taken to be approximately the same as that of the proboscis or perhaps rather smaller, so that the maximum thrust available at the end of the extruded proboscis would hardly be much more than 100 g./cm.² at the pressure peaks. According to the results of a 'penetratometer' study by Chapman & Newell (1947) this pressure would not be enough to penetrate sand unless it had been first disturbed. This certainly does

not occur at initial penetration when only low pressures are available, so that mechanically the proboscis would not appear to be an effective device for penetration except by scraping. Certainly the termination of an *Arenicola* in the form of a cone, as described by Newell (1950) for the earthworm, could effect penetration by virtue of the large thrust (600–1000 g./cm.²) which might then be available, but with a relatively large proboscis this can hardly happen.

The function of the pressure peaks during the burrowing of *Arenicola* is twofold: first to ensure strong forward pressure and secondly to anchor the anterior end firmly during contraction of the longitudinal muscles. Since the pressure peak is directly associated with this muscular contraction, muscular tension and adhesion to the burrow rise together. Maximum tension in the muscle system is not sustained, the pressure drops after 2 sec. and as an immediate consequence some retraction of the worm always occurs (Figs. 8, 10, 12). That the fall of pressure means loss of adhesion to the sand of the burrow is evidenced by the marked annulation occurring at retraction. The anterior end of the worm should be thought of as being pressed firmly against the substrate each time the pressure increases, making use of the dilatant properties of the sand for adhesion. Loss of adhesion, as shown by retraction of the entire anterior end of the worm, occurs as the pressure drops.

Batham & Pantin (1950) have emphasized that the performance of any act involves the whole muscular system of an animal whose skeleton consists of a single reservoir under pressure. While this is undoubtedly true the effect may be reduced if the volume of the body is great in relation to the part changing shape. The extrusion of the proboscis, for instance, seems to have little direct effect on the coelomic pressure although eversion does occur during a fall in pressure (Fig. 15*a*). While an attempt has been made in this paper to relate pressure to muscular changes and to proboscis eversion, the mutual interference of different activities has not been much in evidence presumably because of the relatively large volume of the body cavity and because of co-ordination between the changes in body form. Septa dividing the coelom allow the localization of reciprocal muscle actions and in the same manner the tail segments of *Arenicola* are not affected by the high pressures of the trunk (Fig. 7). There are two advantages of a single large body cavity, which must be over-riding in *Arenicola*. First the coelomic fluid which acts as a hydraulic system allows the pressure produced by the muscles of the posterior trunk to be transferred to the anterior end of the worm, and secondly the work done in any movement may be derived from the tonus of the whole body wall, a small levy on each part of the musculature.

SUMMARY

1. Continuous recordings of the hydrostatic pressure in the coelom of *Arenicola marina* show a resting pressure of about 2 cm. of water in a non-burrowing worm. During burrowing a series of pressure peaks is produced and these gradually increase in amplitude up to 110 cm. as burrowing progresses.

2. The pressure peaks are of 2 sec. duration, occur at intervals of 5–7 sec., and for each there is a major contraction of the circular muscles followed by the shortening of the longitudinal muscles. The main power stroke in producing the high pressure is the contraction of the longitudinal muscles of most of the trunk segments.

The sequence of muscular contractions and the phases of burrowing are considered.

3. The pressure is utilized at the anterior end of the worm both to aid passage through the sand and to anchor the head while the posterior segments are pulled into the burrow.

4. At maximum pressures the tension developed in the circular muscle of the body wall is estimated to be 3 kg./cm.², while the resting pressure corresponds to less than 7% of this.

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