THE PUMPING MECHANISM OF THE NEMATODE ESOPHAGUS

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ABSTRACT The radial orientation of the myofilaments in the nematode esophagus raises interesting questions as to how such a structure can function as a pump. A physical model of the esophagus of *Ascaris lumbricoides* was developed and the membrane theory of shells applied in order to relate the observed dimensional changes to myofilament force, pressure stresses, and membrane elastic constants. By stressing the excised esophagus passively with osmotic pressure, the esophagus was shown to be elastically anisotropic with the ratio of circumferential to longitudinal elastic constants, $E_\psi/E_l \simeq 2.74$. When this value was incorporated, the model predicted the ratio of the respective strains, ϵ_ψ/ϵ_l, to be 0.52 during an equilibrium contraction of the esophagus. This agreed with the experimental value, 0.46 ± 0.10, measured during occasional, prolonged muscle contractions. When measured during normal pumping, on the other hand, the value of ϵ_ψ/ϵ_l was 0 ± 0.10. This indicated that a nonequilibrium condition normally occurs in which a greater myofilament force per unit area of lumen membrane is not balanced by internal pressure and therefore acceleration of the lumen contents and negative intraluminal pressure occurs.

The pumping action of esophagi dissected from *Ascaris* was observed to be normally peristaltic and periodic. Contraction was initiated by a spontaneous depolarization that propagated at 4.0 ± 0.20 cm/s along the esophageal membrane. A wave of localized increases in the internal pressure of the muscle and localized changes in external dimensions was observed. A subsequent spontaneous repolarization, which propagated at 5.8 ± 0.23 cm/s, triggered relaxation of the muscle during which the localized pressure and dimensional changes returned to resting values. A mechanism was deduced in which fluid is drawn into and moved along the lumen by the wave of contraction. During the wave of relaxation, the lumen contents are pressurized and injected into the intestine by elastic restoring forces.

INTRODUCTION

The nematode esophagus pumps fluid and food particles from the environment at atmospheric pressure to the high hydrostatic pressure existing in the intestine and pseudocelom (1, 2). Though nematode esophagi vary interspecifically in overall shape (3, 4), all are constructed of an approximately cylindrical layer of muscle cells, one cell thick, which surrounds the lumen (Fig. 1). The myofibrillar apparatus is radial,

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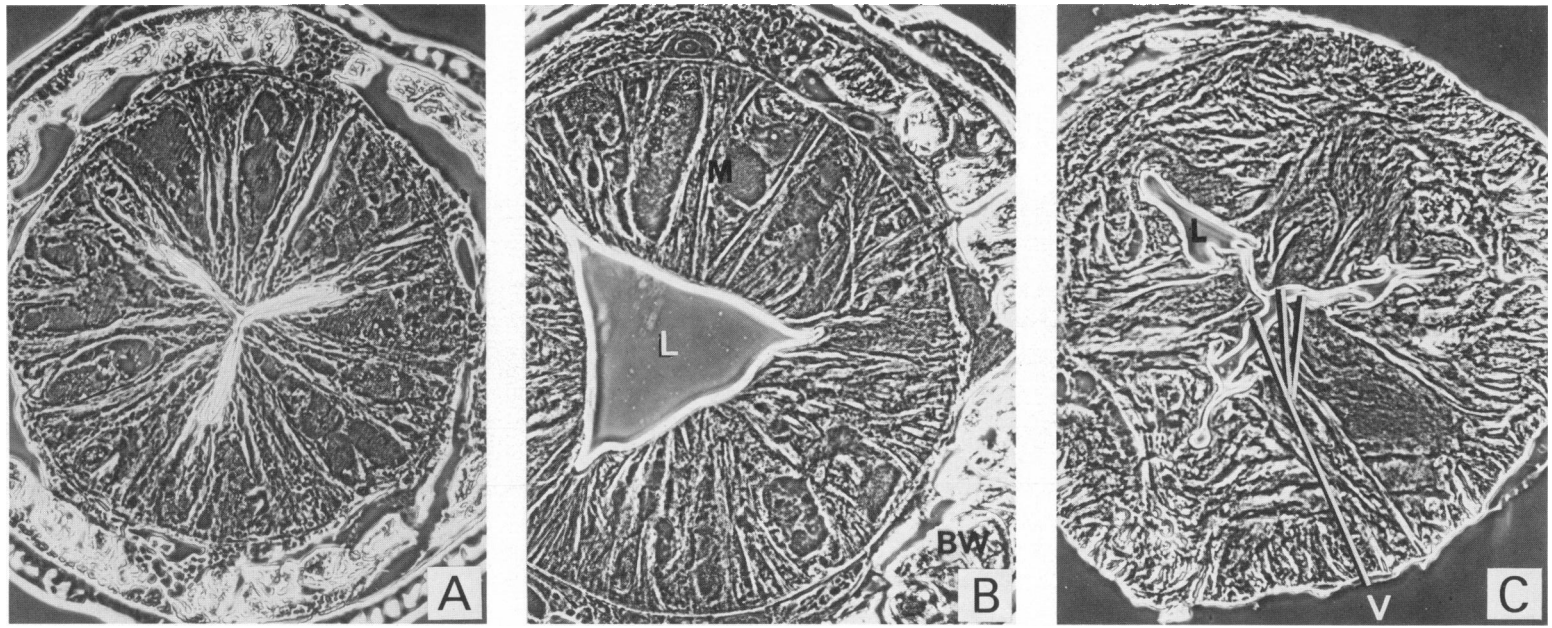

FIGURE 1 Phase micrographs of transverse sections through the esophagus showing lumen shape and radial bundles of myofilaments (M). (A) Anterior region of relaxed esophagus, triradiate configuration of lumen. × 230. (B) Anterior region, triangular configuration. × 190. (C) Complex lumen structure of posterior region. × 140. BW, body wall; L, lumen; V, structure associated with esophagointestinal valve.

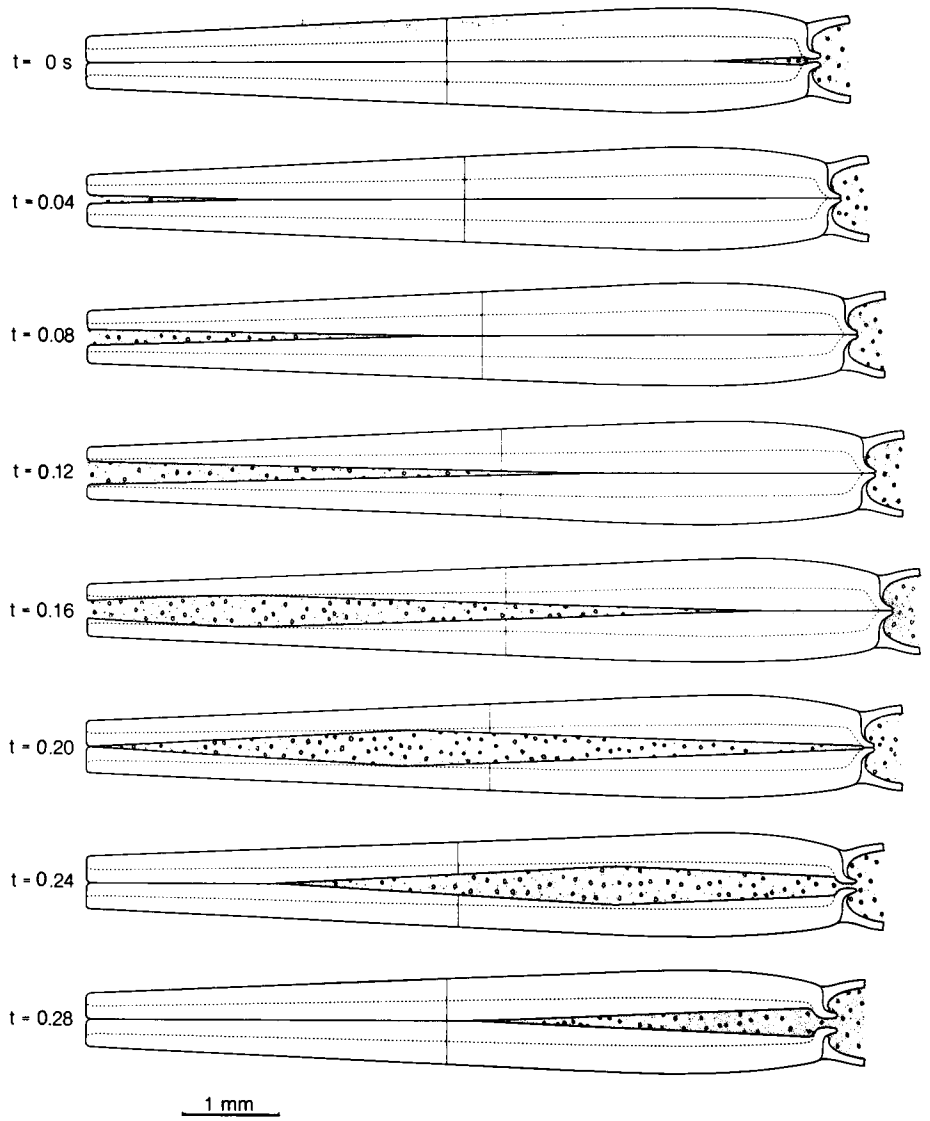

FIGURE 2 Reconstructed normal pumping sequence showing the approximate position of the lumen contents as a function of time. At $t = 0$, contraction is triggered near the anterior end. During the next 0.18 s fluid is drawn in by the advancing wave of contraction along the anterior 70% of the pharynx. From $t = 0.20$ to 0.32 s the elastic restoring forces pressurize the contents and inject them through the pharyngointestinal valve into the gut. The cycle repeats at $t \simeq 0.32$ s. The action of the esophagointestinal valve depicted is hypothetical. The dotted lines indicate the lumen opening probably existing after secondary phase of prolonged contraction. The vertical line indicates the movement of the midpoint of the esophagus, which is out of phase with that of the posterior end.

extending from the cell membrane adjacent to the lumen to the external cell membrane (5, 6).

The question of how a radially contracting esophagus can function as a pump was first examined by Martini (7). On the basis of morphology only, he proposed a two-dimensional model in which myofilament contraction shortens the distance between the luminal and outer membranes, and the displaced volume raises the hydrostatic pressure within muscle cytoplasm. This forces the elastic outer membrane to expand radially. As a consequence, the lumen opens and fluid is drawn inward. During relaxation, the elastic forces, via the internal pressure, restore the resting dimensions, pressurize the lumen contents, and inject them into the intestine.

The evidence for this model has continued to be indirect at best. Mapes (8) found that fluid is ingested by *Ascaris lumbricoides* in gulps, 0.125 s in duration, followed by an equal noningestive period. He proposed a peristaltic pumping action that assumed the mechanism proposed by Martini. Del Castillo and Morales (9) observed that when the anterior end of the *Ascaris* esophagus is ligated, a negative intraluminal pressure is maintained throughout the depolarized phase of the action potential and returns to zero on repolarization. In another approach, Doncaster (10) and Mapes (11) chose several nematode species having a transparent body wall and esophagus, to describe the movement of fluid and particles along the esophagus of intact nematodes. They did not, however, investigate the change in the outer radius of the esophagus predicted by Martini's model.

In this paper we describe directly observed localized changes in external dimensions of the *Ascaris* esophagus which support the essence of Martini's two-dimensional model, except that the expansion of the external membrane was longitudinal rather than radial. Therefore, it was necessary to develop a three-dimensional physical model. Elastic constants determined by experiment were incorporated. Comparison of predicted and measured strains provided support for the model and led to useful predictions.

Because the body wall and esophagus of *Ascaris* are both opaque, the movement of fluid along the esophageal lumen could not be observed directly, either in vivo or in the isolated esophagus. The peristaltic pumping[1] sequence proposed in Fig. 2 was deduced, therefore, from measurements of static dimensions, changes in external dimensions and internal pressure, and the velocity of propagation of the waves of contraction and relaxation.

THE MODEL ESOPHAGUS

To explain how the radial forces generated by the myofibrils lead to an increase in cytoplasmic pressure, lengthening of the esophagus and opening of the lumen, a three-dimensional physical model was developed. The model, Fig. 3, describes any portion

[1] Here defined as the propulsion of fluid through a tube by the action of a wave of alternate opening and constriction proceeding along the tube.

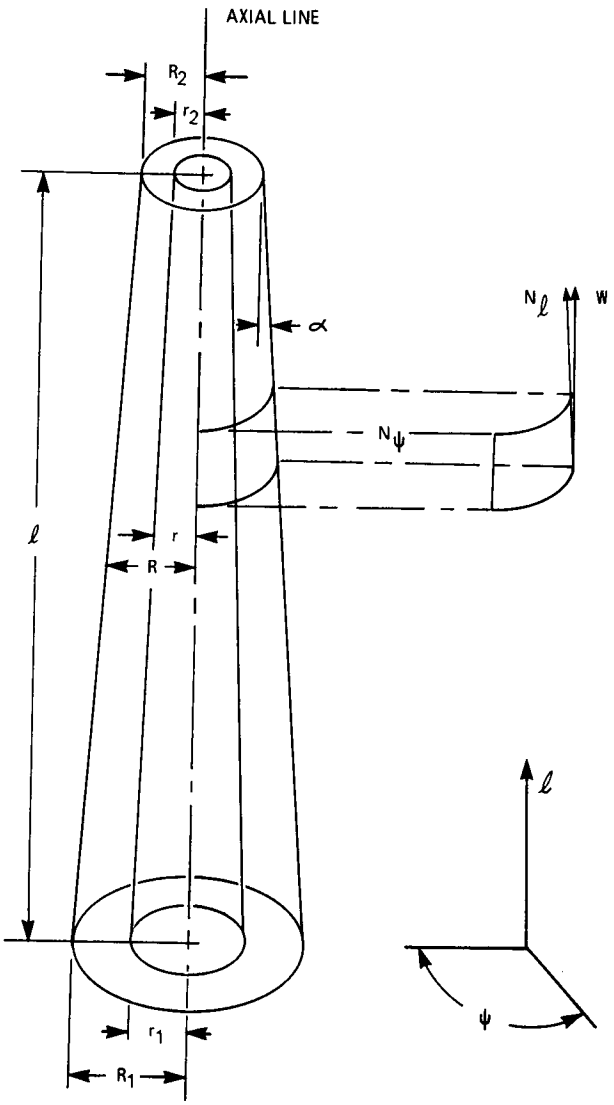

FIGURE 3 Schematic representation of the model esophagus. The length, l, may represent the whole length of the conical region of the esophagus or any portion thereof. R, R_1, and R_2 represent instantaneous esophageal radii, and r, r_1, and r_2, instantaneous lumen radii. The cut-out defines the direction of N_ψ and N_l, the circumferential and longitudinal stress resultants, and W, the resultant force parallel to the axial line. The semiangle of each cone, α, is drawn larger than the actual value, for purposes of illustration.

of the conical, anterior 70% of the esophagus. The action of the posterior region is complicated by the presence of the esophagointestinal valve. Also, the dimensional changes in that region were too small to be measured with adequate precision. The model is sufficiently general that the length, l, may represent the whole length of the tapered region of the esophagus or any portion of that length. The model should be

used in the latter context in interpreting the localized dimensional changes that were observed.

The shape is assumed to be equivalent to two coaxial truncated cones with closed ends, variable radii (r and R), and variable length (l). For simplicity a circular lumen cross section is assumed, though the lumen may actually assume other shapes with triradiate symmetry. The model, however, is independent of lumen shape, lumen volume being the important parameter. The coaxial cones are given the same vertex because in the measurements on transverse sections, $r/R = r_1 = r_2/R_2$ is constant for a given-sized opening, and $R_1/R_2 = r_1/r_2 = \rho$ is constant for a given l. The cone semiangle of the outer cone, 5.5°, is that measured for the tapered portion of the esophagus. The inner cone radius reduces to zero to represent the closed lumen.

For simplicity, both longitudinal and circumferential elastic force components were assumed to reside in the external membrane. In the case of the circumferential component this is probably a good approximation. The other possible location, the lumen membrane, is folded when closed (Fig. 1 A) and therefore little circumferential elastic force should develop there until it opens beyond a fully extended, circular cross section. In the case of the longitudinal component, the actual location makes little difference in the analysis, and the external membrane is chosen for simplicity. Other possible locations will be discussed later.

Constraints on Changes in Dimension

Constraints are imposed by the dimensions of the esophagus and by the incompressibility of cytoplasm. When the muscle is relaxed, $r = 0$, $R = R_0$, and $l = l_0$, and the

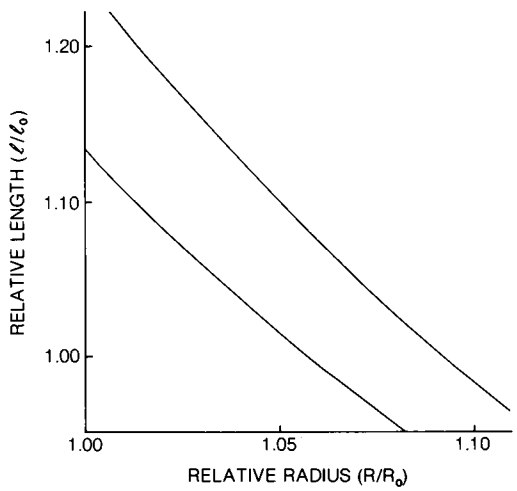

FIGURE 4 Relative length as a function of relative radius for two magnitudes of lumen opening according to Eq. 1. Upper line: locus of points for lumen open to maximum circular configuration possible without circumferential stretching of the lumen membrane ($r = 0.44\ R_0$). Lower line: locus of points for lumen open to a cross-sectional area equivalent to the maximum triangular configuration possible without circumferential stretching (equivalent radius = $0.34\ R_0$).

volume enclosed by the outer cone is then $V_0 = (\pi/3)l_0R_0^2f(\rho)$ where $f(\rho) = \rho^2 + \rho + 1$. When the lumen is opened, total volume, $V = (\pi/3)lR^2f(\rho)$, lumen volume, $V_L = (\pi/3)lr^2f(\rho)$, and the cytoplasmic volume (between the cones) is $V_C = V - V_L = (\pi/3)l(R^2 - r^2)f(\rho)$. Because the cytoplasm is incompressible and none is lost during pumping, $V_C = V_0$, and one obtains the relationships:

$$V/V_0 = R^2/(R^2 - r^2) = (R/R_0)^2(l/l_0) \tag{1a}$$

$$(l/l_0)^{-1} = (R/R_0)^2 - (r/R_0)^2. \tag{1b}$$

The possible dimensions for two values of (r/R_0) are graphed according to Eq. 1*b* in Fig. 4. The locus of any point on the graph represents the radial and longitudinal strains expected when the lumen is opened uniformly (r/R_0 is constant) along the length, l, over which the strains are measured. In cases where the actual opening is nonuniform over a large l, R/R_0 and r/R_0 in Eq. 1b are those corresponding to average cross-sectional area.

Pressure- and Myofilament-Induced Stresses

Local changes in dimensions are induced by changes in the myofilament force acting on the inner and outer membranes and the balance between pressures across the membranes. Acting on the outer membrane of the esophagus are the external pressure, P_e, the myofilament force per unit area, F_R, and the opposing esophageal internal pressure, P_i. In the excised preparation, $P_e = 0$ (atmospheric pressure), whereas in vivo it is the pseudocelomic pressure. Acting on the inner membrane (lining of the lumen) are the lumen pressure, P_l, myofilament force per unit area, F_r, and the opposing pressure, P_i.

The esophagus outer membrane is flexible and therefore P_i must always exceed P_e for the esophagus to maintain the normal "inflated" appearance observed in the excised preparations of *Ascaris* and in transparent species in vivo. If the membrane is punctured, the cytoplasm oozes out of the relaxed esophagus and, upon muscle contraction, the outer membrane collapses inward. The lumen membrane must also be flexible, and P_i must also exceed P_l when the esophagus is relaxed for the lumen to remain closed. Because P_i increases during contraction, P_e remains constant, and P_l, if anything, decreases, it is evident that P_i must be greater than P_e and P_l throughout the contraction sequence.

In transverse sections (Fig. 1 A, B), the angle of insertion of the myofilaments onto the outer membrane appears approximately 90° to the tangent regardless of position. However, the insertion angle onto the lumen lining depends on the point of insertion onto the rays of the triradiate lumen. This relationship is depicted in Fig. 5, sector 3. The force per unit area exerted by a myofilament on the lumen lining will be proportional to the sine of the insertion angle, and will be at a maximum where the myofilament is perpendicular to the lining as in a circular region (Fig. 5, sector 2).

During an (hypothetical) equilibrium contraction, the lumen starts to open when the myofilament normal force per unit area F_r just exceeds the pressure difference P_i –

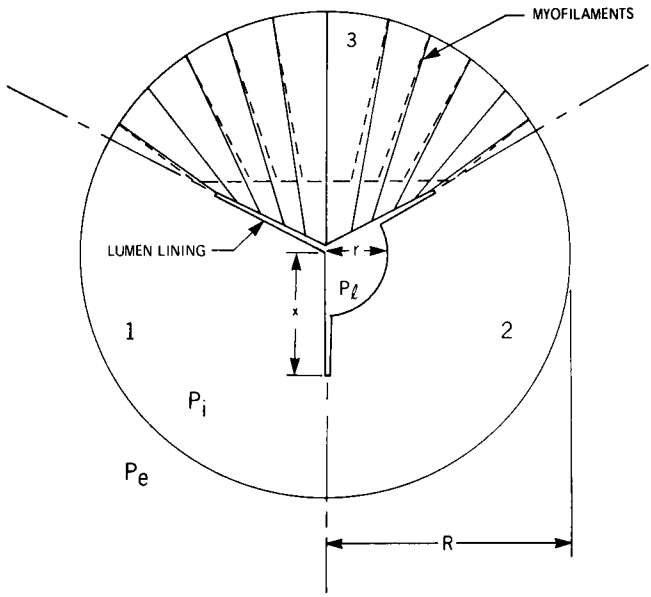

FIGURE 5 Schematic cross-sectional view of the nematode esophagus. Sector 1, the triradiate, closed configuration. The length of one ray is x. Sector 2, the postulated lumen configuration during equilibrium contraction. Myofilaments would be perpendicular to the circular, open portion of the lumen. Sector 3 solid lines, the lumen and myofilaments in the relaxed state. Sector 3 dashed lines, a possible lumen and myofilament configuration during rapid contraction.

P_l. As a result of the angle of insertion of the myofilaments, this will occur first at the "V" or midpoint of each sector of the triradiate closed lumen. The opening force will move the lumen radially at the "V," flexing the lumen. The flexing of the lumen will change the angle of nearby myofilaments to near normal, and they may then contribute to lumen opening. A partially circular lumen will result (Fig. 5, sector 2). The opening of the lumen is accomplished only at the expense of an increase in P_i, and the consequent elastic deformation of the esophagus. Thus, for any constant myofilament force greater than the minimum force required to initiate opening, there will be a unique, stable, open configuration of the lumen, approximately circular in cross section. In the following derivation, a circular lumen is assumed. This ignores the small effect of tension due to myofilaments pulling on the unopened portion of the lumen membrane.

In a thin cross-sectional slice of esophagus with a thickness Δl, the myofilaments exert a total force ΔF. At the outer membrane, the force per unit area, $F_R = \Delta F/2\pi R\Delta l$. The force ΔF maintains the lumen open to a radius r (Fig. 5), and the force/area at the inner membrane, $F_r = \Delta F'/2\pi r\Delta l$, where $\Delta F'$ is the normal force exerted by those myofilaments inserted on the open region of the lumen. The fraction of myofilaments inserted on the open region of the lumen, for any radius r, is $2\pi r/6x$ where x is the length of one ray of the triradiate lumen (Fig. 5). Thus $\Delta F' = \Delta F(2\pi r/6x)$ and $F_r = \Delta F/6x\Delta l$ and:

$$F_r = (2\pi R/6x)F_R. \tag{2}$$

R/x is measurable from transverse sections such as Fig. 1 A and B. The equation was obtained from geometrical consideration of the model esophagus and is based on a number of assumptions. It is valid for equilibrium contraction of the esophagus when the circumferential strain is not large ($R \simeq R_0$) and the myofilaments insert at $\sim 90°$ to an approximately circular lumen opening.

Formulation of the Relationship between Strain and Stress

To relate circumferential and longitudinal strain to the stresses due to myofilament forces and esophagus internal pressure, equations were derived from the membrane theory of shells (12) for the case of a single conical shell. The bending stress resultant is neglected in membrane theory, and all other shears or twisting resultants are zero for axial symmetry. The stress resultants, N_l and N_ψ (Fig. 3) are line forces analogous to surface tension, but are not constants as for a liquid. W, the resultant force parallel to the axial line, and α, the cone semiangle are also shown in Fig. 3.

The longitudinal and circumferential strains, $\epsilon_l = \Delta l/l_0$ and $\epsilon_\psi = \Delta R/R_0$, are assumed to be linearly related to the corresponding stresses, σ_l and σ_ψ, thus $\sigma_l = E_l\epsilon_l$ and $\sigma_\psi = E_\psi\epsilon_\psi$, where E_l and E_ψ are the elastic constants. All of the elastic forces are assumed to reside in the external membrane of uniform thickness τ, therefore $N_l = \sigma_l/\tau$ and $N_\psi = \sigma_\psi/\tau$, and the equation relating strains to the membrane stress resultants is:

$$\epsilon_\psi/\epsilon_l = (N_\psi/N_l)(E_l/E_\psi). \tag{3}$$

The membrane stress resultants are related to F_R, P_i, and P_e by the membrane equations for conical shells. These are derived by consideration of the equilibrium of forces acting on a small surface element of the shell (12). Because in the esophagus the centrifugal force is zero and the weight of the membrane is negligible, the body forces are zero or negligible, and the equations simplify to become:

$$N_\psi = -P_nR/\cos\alpha, \tag{4a}$$

$$N_l = W/\cos\alpha, \tag{4b}$$

$$WR = -\int P_aR\,dR + L. \tag{4c}$$

The applied axial load, L, is zero in the case of the isolated esophagus. Because the force per unit area in the axial direction, $P_a = P_e - P_i$, is independent of R, Eq. 4c becomes:

$$WR = \tfrac{1}{2}(P_i - P_e)(R^2 - r^2) \tag{4d}$$

for the model esophagus. In the absence of myofilament force, the force per unit area normal to the membrane P_n is equal to P_a, but in the general case, $P_n = P_e - P_i + F_R\cos\alpha = P_e + P_i + F_R$ (because α is small). Substituting for P_n and utilizing Eqs. 4a, b, and d, Eq. 3 becomes:

$$\epsilon_\psi/\epsilon_l = 2\frac{E_l}{E_\psi}\frac{R^2}{R^2 - r^2}\left(1 - \frac{F_R}{P_i - P_e}\right). \quad (5)$$

Adding the condition that cytoplasmic volume remains constant during contraction, by substituting Eq. 1*a*, one obtains:

$$\frac{\epsilon_\psi}{\epsilon_l} = 2\frac{E_l}{E_\psi}\frac{V}{V_0}\left(1 - \frac{F_R}{P_i - P_e}\right). \quad (6)$$

Case of Pressure-Stressed Esophagus

To measure E_l/E_ψ conveniently, it was useful to measure the dependence of ϵ_ψ on ϵ_l when the internal pressure was raised in the absence of muscle contraction (by exposing the esophagus to hypoosmotic solutions). In this case, because cytoplasmic volume varies, Eq. 5 applies, and because $F_R = 0$ and the lumen is closed ($r = 0$) by the pressure ($P_i - P_l > 0$), Eq. 5 simplifies to $\epsilon_\psi/\epsilon_l = 2E_l/E_w$.

Case of Equilibrium Contraction

In this case, myofilament force is sufficient to open the lumen and is equal to or slightly greater than the difference in pressure across the lumen membrane. Substituting Eq. 2 for F_R and $F_r = P_i$, Eq. 6 becomes:

$$\frac{\epsilon_\psi}{\epsilon_l} = 2\frac{E_l}{E_\psi}\frac{V}{V_0}\left(1 - \frac{3x}{\pi R}\frac{P_i}{P_i - P_e}\right). \quad (7)$$

This equation can be tested if the external pressure $P_e = 0$ as for the exposed esophagus. P_i then cancels and all other terms are measurable.

METHODS

Collection and Storage of Ascaris

Ascaris lumbricoides was chosen because it is the largest available nematode with a simple esophageal morphology. Specimens were collected from the small intestine of freshly killed pigs and placed in a Thermos bottle containing, at 38°C, a storage solution composed of 30% artificial seawater (13, 14) plus 1 g/liter glucose. Within 2 h, the animals were placed in fresh storage solution, 6–12 per 500-ml jar, and kept in the dark at 36–38°C during the day and 30–32°C at night, changing the storage solution twice per day. The practice of lowering the storage temperature at night and including glucose in the storage solution kept the nematodes in good condition (active movements and active esophagus preparations) for 3–4 days. Most experiments were completed within 36 h of collection.

All experiments on the esophagus were done under artificial perienteric fluid as formulated by del Castillo and Morales (13), except that isethionate ion was substituted for acetate because acetate is postulated to be an intermediate of the modified glycolytic pathway in *Ascaris* (15).

Preparation of the Esophagus

The anterior 2–3 cm of the worm was pinned to a paraffin block, covered with artificial perienteric fluid, and cut along one of the lateral lines to within < 1 mm of the lips. The body wall was

carefully dissected away from the esophagus and pinned back. Special care was taken to avoid tearing the outer esophageal membrane and stretching the internal esophageal structures. For conduction-velocity and pressure measurements, the freed body wall was cut off circumferentially, 1.0–1.5 mm from the anterior end, leaving a short segment of the body wall still attached to the esophagus. The temperature of the experimental chamber was regulated at 37°C by water circulated from a constant temperature bath. For conduction-velocity and pressure measurements a Plexiglas electrode apparatus formed the bottom of the chamber, but for all other experiments the bottom was covered with black paraffin to which the esophagus could be fastened by pinning through the attached segment of the body wall.

Measurement of Esophageal Dimensions

Static dimensions of the whole esophagus were recorded with a single-lens reflex camera and measured from the prints using a traveling microscope. Changes in dimensions during pumping were recorded at 50 frames/s with a Beaulieu 2008s camera using SO-105 super 8 Ektachrome film. Measurements made on projected images used a movie projector with a single-frame advance.

Dimensions of the lumen were measured from phase micrographs of unstained serial transverse sections. Preparatory to sectioning, the esophagus was fixed in 2% glutaraldehyde in 15% seawater (~300 mosM) at 5°C for 3 h, dehydrated in an ethanol series and xylene, and embedded with wax. As a control against possible shrinkage during processing and possible distortion as a result of dissection, the measurements were checked on sections of whole *Ascaris* anterior ends fixed in acrolein and embedded in methacrylate. Esophageal dimensions in these sections were not significantly different from those of the dissected and wax-embedded esophagi.

Electrical Measurement of Propagation Velocity

The propagation velocity of esophageal action potentials was measured with four external electrodes placed at 2-mm intervals along the esophagus. The 0.13-mm silver wires were embedded in a Plexiglas trough, 2 mm apart, so that only a 0.5-mm length was exposed at the bottom of the trough. The esophagus, partly submerged in artificial perienteric fluid, was held gently against the electrodes by surface tension. Experiments were done at 37°C. When required, stimulating pulses were applied through a Bioelectric Instruments IS2B isolator (Bioelectric Instruments, Inc., Hastings-on-Hudson, N.Y.) intracellularly through a low-resistance glass microelectrode, or more usually, extracellularly through an insulated pair of fine wires. Oscilloscope traces of the action potentials were recorded on Polaroid film, and the time required to travel between electrodes was measured from the prints with a traveling microscope.

Detection of Pressure Changes

Transient changes in esophageal internal pressure during contraction were monitored externally with an indentation gauge. The depth of the indentation in the external membrane caused by an insect pin was monitored with a ceramic phonograph cartridge whose output voltage was displayed on an oscilloscope screen.

RESULTS

Static Dimensions of the Esophagus

Mapes (8) described the *Ascaris* esophagus as being cylindrical. Closer examination revealed, however, that the anterior 70% is conical (Fig. 6) with a cone semiangle of 5.5°. The average shape of the esophagus was obtained by measuring 20 whole

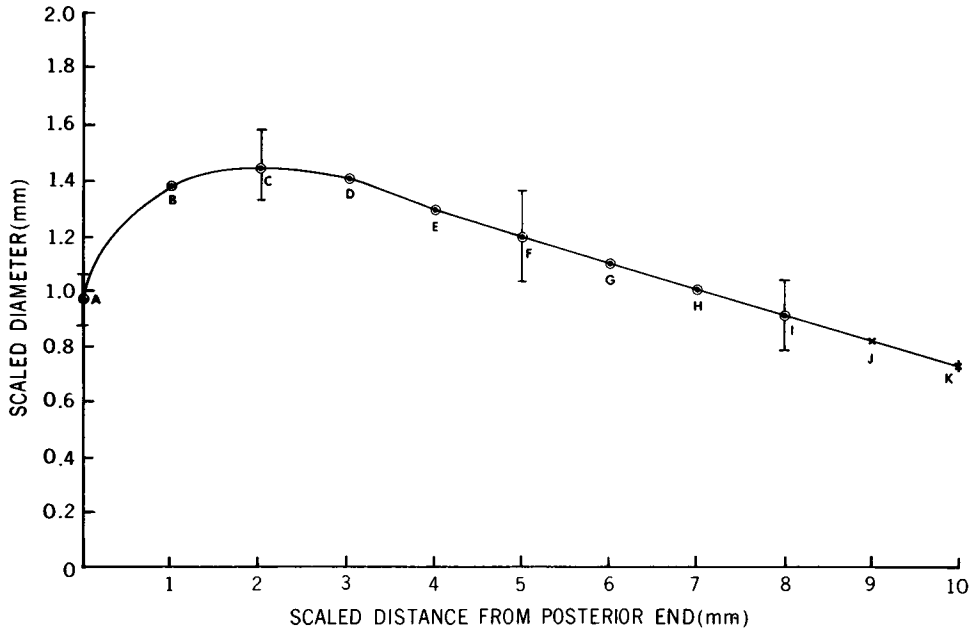

FIGURE 6 Mean external diameter as a function of position determined from measurements of photographs of 20 relaxed esophagi. The original lengths (5.5–8.6 mm) were scaled to 10 mm. The uncertainty bars indicate ± SEM. The points indicated by × were obtained from measurements of serial transverse sections.

esophagi ranging from 5.5 to 8.6 mm long (mean = 7.5 mm). For each esophagus, the diameter was measured at millimeter intervals along its length, multiplied by a scale factor to normalize the dimensions to a 10-mm length, and plotted on graph paper. A best-fit smooth curve was drawn through the data points, and the scaled diameter was read off at each millimeter of length. This procedure, which resulted in nine scaled diameters A_1, B_1, ... I_1, was repeated for 19 other esophagi. The data points A_1 through A_{20}, B_1 through B_{20}, ... I_1 through I_{20}, were averaged and plotted (Fig. 6, data points A, B, ... I). Points J and K could not be obtained directly because this region was hidden by a portion of anterior body wall still attached at the anterior end. Therefore, the curve was extended on the basis of measurements of serial sections on other esophagi.

By studying transverse serial sections of the esophagus, the simple, triradiate shape of the lumen (Fig. 1 A, B) was found to be maintained throughout the conical region of the esophagus. In this region, the ratio of ray length, x (Fig. 5, measured as 1/6 of the lumen circumference), to the external radius was constant ($x/R = 0.46 \pm 0.01$ [SEM], $n = 15$). In the posterior, nonconical region, the ray structure becomes complex as in Fig. 1 C. This modified lumen structure probably contributes to the action of the esophagointestinal valve (2). It is apparently this modified region which Reger (16) has illustrated in his Fig. 1, giving the incorrect impression that the lumen is shaped like this along its entire length.

Electrical Activity and Contraction

The spontaneous, diphasic action potentials we observed were essentially as described by del Castillo and Morales (9). However, the prolonged depolarizations were usually observed in preparations that had been left in artificial perienteric fluid for extended times. In these preparations, the repolarizing phase occasionally failed to initiate in the normal time, in which case the muscle remained in the contracted state at a membrane potential near zero until repolarization occurred. In fresh preparations, the action potentials usually recurred at regular intervals, and the repolarizing phase followed the depolarizing phase within 0.1–0.2 s. The initiation site was located at a different longitudinal position in each preparation, usually near the anterior end. It appeared to be stationary in a given preparation.

Changes in External Dimensions during Contraction

Changes in the length and external diameter of the esophagus were observed by high-speed cinematography. Length and diameter normally changed cyclically during pumping with a repetition rate of 2.3/s. The changes in dimension are localized and consecutive, as indicated by the results of Fig. 7. In the anterior half of the esophagus, increases in length ranged from 9 to 18% (median = 15% for eight determinations on different preparations). Corresponding changes in diameter were <2% and either positive or negative depending on the preparation. In the posterior half, length changes were considerably less than in the anterior half (Fig. 7), and changes in diameter were on the order of the precision of measurement (~ 1%).

When the contraction phase was prolonged, the esophageal dimensions approached different values than during normal repetitive pumping. In the example shown in

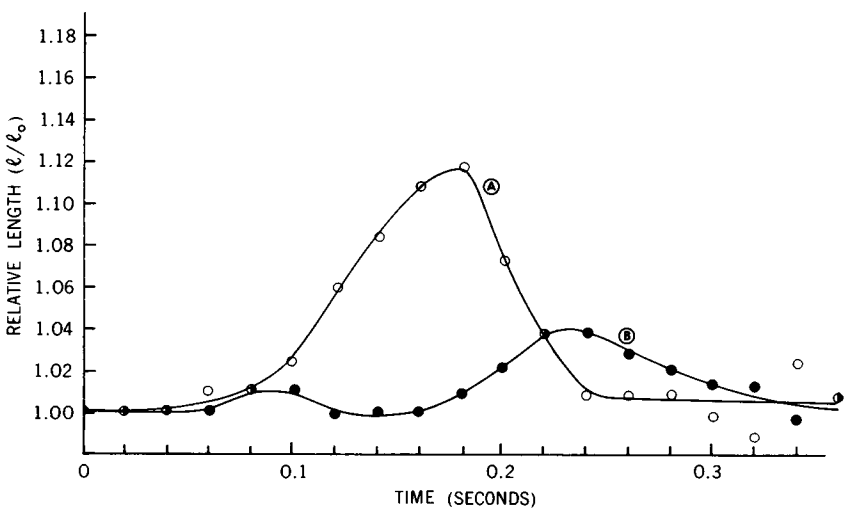

FIGURE 7 Length changes during one cycle of repetitive pumping. Curve A (○), anterior half of esophagus. Curve B (●), posterior half. The small early fluctuation observed in the posterior half is probably an error caused by movement of that half during contraction in the anterior.

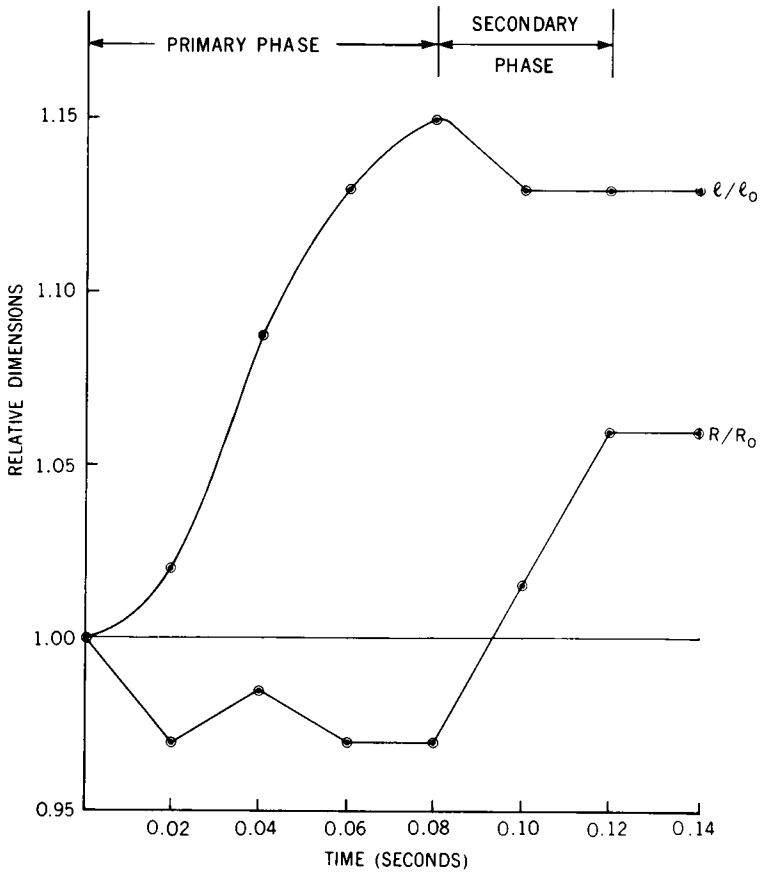

FIGURE 8 Change in dimensions of the anterior half of the esophagus when muscle contraction was initiated but relaxation was delayed. Upper curve, l/l_0. Lower curve, R/R_0. The changes during the primary phase were similar to those observed during the first 0.08 s of a cycle of reptitive pumping. Further contraction occurs during the secondary phase.

Fig. 8, the initial rapid dimensional changes (+15% longitudinal and −2.5% radial) in the first 0.08 s were similar to those observed normally. However, during a secondary contraction phase, there were further dimensional changes in the esophagus, resulting in a further increase in total external volume. The final values of 13% length increase and 6% radial increase were maintained for a time interval equivalent to several normal pumping periods. Over longer periods of time, however, there is a slow exponential decrease in esophageal dimensions until relaxation is initiated.

Changes in Lumen Dimension

The lumen radius attained during contraction can be calculated by Eq. 1*b* knowing only R/R_0 and l/l_0. For prolonged contraction, $R/R_0 = 1.06$ and $l/l_0 = 1.13$. The locus of this point on the graph (Fig. 4) is above the upper line, indicating that the lumen opening attained is greater than the maximum circular configuration obtainable

with no circumferential stretching of the lumen membrane. Eq. 1*b* gives a value of $r/R_0 = 0.49$, 11% greater. Thus, some stretching must occur in the case of prolonged contraction.

During normal pumping, the maximum length strain attained by the anterior half, $l/l_0 = 1.15$, is the maximum in the average of all the local strains in that half because contraction is not uniform. The radial strain was negligible ($R/R_0 = 1.00$). The locus of this point on the graph (Fig. 4) near the lower line indicates that the average opening is approximately equivalent to that for the maximum triangular configuration attainable without stretching. Eq. 1*b* gives $r/R_0 = 0.36$. Thus, the local maxima must be greater than this, but circumferential stretching need not occur until the maximum circular configuration is exceeded.

Changes in Dimension Induced by Osmotic Stress

To obtain a value for the ratio of elastic constants, it was necessary to measure the relative dimensional changes in response to internal pressure while the myofilaments were relaxed. This was done by exposing the isolated esophagus to sucrose solutions ranging from 300 to 80 mosM. Photographs were taken of the initial size in artificial perienteric fluid and after 4 min in each solution. The esophagus enlarges both radially

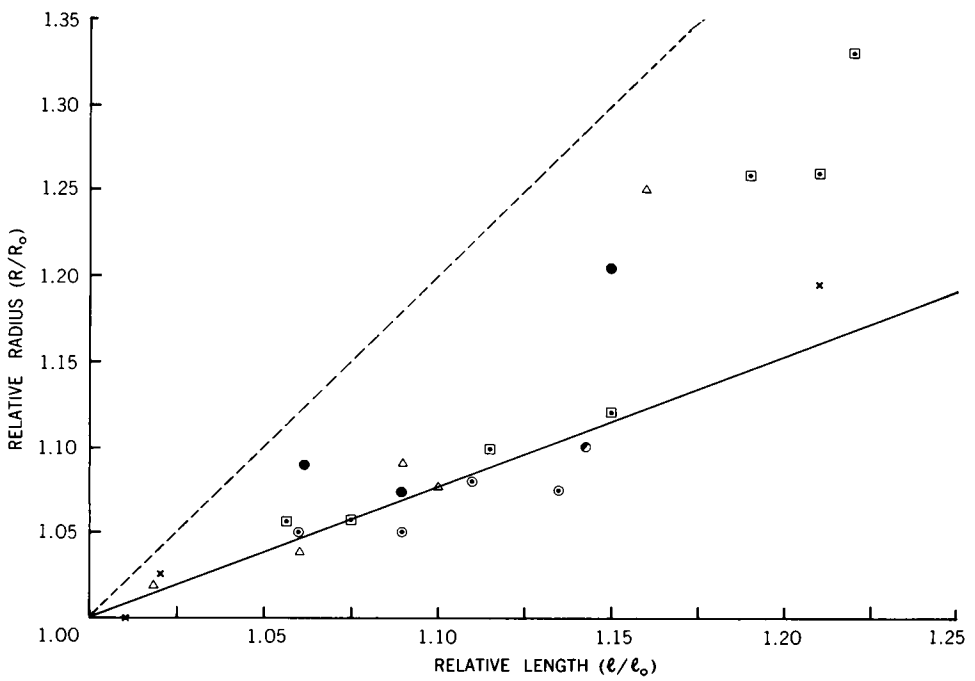

FIGURE 9 Relative radius (R/R_0) as a function of relative length (l/l_0) for six esophagi subjected to osmotically induced pressure stresses. Each symbol represents a different preparation, some at room temperature, others at 37°C. The dotted line, slope = 2.0, is the strain-strain curve expected for an isotropic membrane. The solid line, slope = $\epsilon_\psi/\epsilon_l = 0.73$, was fitted by regression analysis of the data, omitting points for which $R/R_0 > 1.15$.

and longitudinally (Fig. 9), and below $R/R_0 > 1.15$ appears to obey the linear relationship $R/R_0 = 2(E_l/E_\psi)(l/l_0)$ predicted for a pressure-stressed esophagus. The slope in this region is considerably lower than that expected for an isotropic membrane (dotted line in Fig. 9). The ratio of the elastic constants $E_\psi/E_l = 2.74$. The deviation from the solid line at the higher strains can be attributed to inelastic stretching of the esophagus.

Propagation Velocity Measurements

Averaged propagation velocities of the depolarization and repolarization phases of the normal action potential $\bar{v}_C$ and $\bar{v}_R$, respectively, were measured from recordings from four external electrodes, choosing electrode pairs for which it was clear that the pacemaker sites were not located between the electrodes. To examine the difference between $\bar{v}_C$ and $\bar{v}_R$, data were obtained from electrode pairs for which both $\bar{v}_C$ and $\bar{v}_R$ could be measured from the same action potential. The mean values, ± SEM ($\bar{v}_C = 4.0 \pm 0.20$ and $\bar{v}_R = 5.8 \pm 0.23$ cm/s) were obtained from 35 measurements on 20 preparations. The difference between $\bar{v}_C$ and $\bar{v}_R$ is highly significant ($P < 0.001$) as estimated by Student's t test. Because $\bar{v}_R$ is greater than $\bar{v}_C$, the duration of the action potential sequence is greatest near the pacemaker site and is progressively less as it is conducted posteriorly.

The velocity as a function of position along the esophagus was determined by comparing velocities measured from adjacent electrodes in the anterior or posterior regions. The mean value for v_C was the same in both regions (3.8 ± 0.29 cm/s, 18 measurements) and was not significantly different from the value obtained in the above estimation. However, $\bar{v}_R$ was less in the anterior region (5.0 ± 0.37 cm/s) than in the posterior region (6.3 ± 0.32 cm/s). The probability of this difference occurring by chance was < 0.02 (15 measurements).

Pressure Changes Within Esophagus during Pumping

The depth of the indentation caused by an insect pin, Fig. 10 A, would be expected to be a complicated function of the myofilament force, the elastic properties of the membrane, and the shape of the indentation as well as P_i. The elastic properties and the shape of the indentation would not, however, be expected to change significantly during muscle contraction and relaxation, and therefore the depth changes recorded in Fig. 10 B (trace 1) and C (traces 1 and 3) should reflect primarily changes in P_i, longitudinal stress, σ_l, and myofilament force per unit area, F_R. During contraction, the increased P_i and σ_l should decrease the indentation depth, and the increased F_R should have the opposite effect. Because the indentation depth is observed to decrease (in Fig. 10, negative indentation increases), it is evident that P_i plus σ_l have a greater effect on indentation depth than F_R and because increased σ_l is caused by increased P_i, one can therefore regard decreasing indentation depth as an index of increasing P_i.

The relationship between electrical activity and pressure changes within the esophagus during contraction sequences is shown in Fig. 10 B and C. Membrane depolarization initiates a pressure increase, and membrane repolarization was followed by a rapid

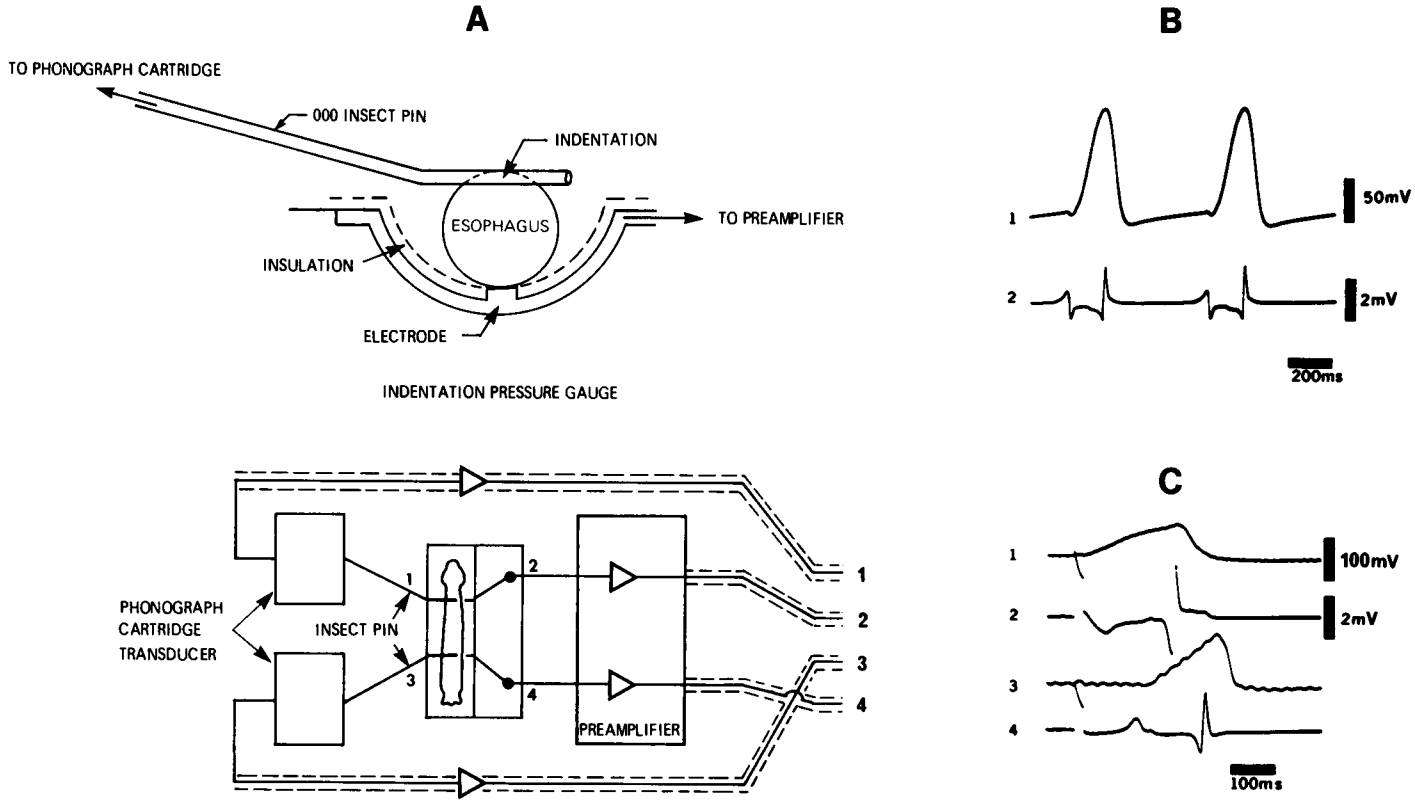

FIGURE 10 Simultaneous recording of electrical activity and changes in esophagus internal pressure. (A) Diagrams of apparatus. The indentation gauges rest on top of the esophagus at anterior and posterior positions. External recording electrodes are located beneath the esophagus at the same positions. (B) Negative indentation (trace 1) and electrical activity (trace 2) recorded at the same position during spontaneous repetitive pumping. Pressure increased after depolarization of the membrane and decreased after repolarization. (C) Negative indentation and electrical activity recorded at anterior (traces 1 and 2) and posterior (traces 3 and 4) positions as in diagram. Depolarization was triggered by electrical stimulation at the anterior end; the first upward (positive) deflection is a stimulus artifact.

decrease in pressure. The latency between electrical and pressure events (about 20 ms) is the same for contraction and relaxation, and is not dependent on position along the esophagus (Fig. 10 C). Thus the values $\bar{v}_C$ and $\bar{v}_R$ determined from electrical recordings are good estimates of the velocity at which the contraction and relaxation phases move along the esophagus.

By recording simultaneously at two points (Fig. 10 C), it was shown that depolarization at either point causes only a localized pressure increase. No pressure increase or decrease was detected at the posterior transducer until pressure changes were initiated at that point by depolarization or repolarization, respectively. Therefore, equalization of pressure must spread more slowly along the esophagus than the action potential.

The Pumping Action

The above data provide good evidence that the pumping action is peristaltic; however, the details of fluid movement can be described further. For this purpose several attempts at reconstructing the pumping action were made, the most satisfactory of which is illustrated in Fig. 2. We incorporated the observations that muscle contraction at each point caused a local increase in cytoplasmic pressure, a local increase in length, and a localized opening of the lumen. The time of initiation of contraction and relaxation at each point was calculated from the measured velocities of the contraction and relaxation waves, and the duration of the action potential at the pacemaker region (which determines the latency between contraction and relaxation). The velocities used were the average values measured on the anterior and posterior regions. The action potential duration assumed for the pacemaker region, 0.14 s, is within the range (0.1–0.2 s) measured at the anterior end. In the reconstruction, the pacemaker was assumed to lie at the anterior tip of the esophagus.

The lumen was allowed to open to the diameter attained during prolonged contraction, $r/R_0 = 0.49$. The time required for the muscle to relax at any point was chosen to be half that required for contraction, as indicated by the pressure measurements, Fig. 10. The time for contraction, 0.12 s, was chosen so that the maximum volume attained by the anterior half of the lumen was approximately the same as the observed value; that for the reconstructed lumen (Fig. 2 at $t = 0.16$ s) turned out to be 0.25 mm^3, slightly greater than $V_L = V - V_0 = 0.22$ mm^3 calculated from the observed maximum and resting dimensions of the anterior half. Agreement between the maximum r/R_0 (corresponding to the average cross-sectional area in the anterior half) from the measurements and from Fig. 2 ($t = 0.16$ s) was better: 0.36 in both cases. As an additional check on the accuracy of Fig. 2, the volume per gulp calculated from the figure ($t = 0.20$ s), 0.34 mm^3, agrees with that measured directly in vivo by Mapes (8), 0.33 ± 0.02 mm^3.

Test of Equation for Equilibrium Contraction

The measured ratio of strains, ϵ_ψ/ϵ_l, can be compared with that predicted by Eq. 7, in the case of the exposed esophagus. In this case, $P_e = 0$, therefore P_i cancels. Substituting the measured values of x/R and E_l/E_ψ, Eq. 7 reduces to $\epsilon_\psi/\epsilon_l = 0.41\ V/V_0$.

TABLE I

VALUES OF THE RATIO OF STRAINS

Case	$\frac{V}{V_0} = \frac{l}{l_0}\left(\frac{R}{R_0}\right)^2$	ϵ_ψ/ϵ_l	
		Predicted*	Actual‡
Closed lumen	1.00	0.41	—
Normal contraction	1.15	0.47	0 ± 0.10
Prolonged contraction	1.27	0.52	0.46 ± 0.10

*According to Eq. 7 (equilibrium contraction assumed).

‡Calculated from $\epsilon_\psi = \Delta R/R$ and $\epsilon_l = \Delta l/l$ using measurements on the anterior half. The value for the closed lumen, with $\Delta R = \Delta l = 0$, is indeterminate.

Values of ϵ_ψ/ϵ_l predicted for three cases are listed in Table I along with the measured values. Only in the case of prolonged contraction do the values agree within error. Because it is reasonable to expect that the forces would be at equilibrium by the time of the secondary phase of prolonged contraction, the model (at least that part derived for equilibrium contraction) appears to be supported.

Case of Normal Pumping, Exposed Preparation

The value of ϵ_ψ/ϵ_l attained during normal contraction is significantly lower than that predicted assuming equilibrium contraction (Table I). Therefore, it appears that the equilibrium assumption does not hold in this case, meaning that the forces at lumen membrane must be unbalanced. Because we cannot measure F_R or P_i, we cannot use the more general Eq. 6 to predict ϵ_ψ/ϵ_l, however, we can deduce that its form is reasonable, as follows. Because the measured $\epsilon_\psi/\epsilon_l = 0$, the bracketed term in Eq. 6 must equal zero and, because $P_e = 0$, therefore $F_R \simeq P_i$. Thus, the equation predicts that the forces at the outer membrane are balanced, and there should be negligible acceleration of the outer membrane, as observed. From geometrical considerations similar to those used in deriving Eq. 2, $F_r > F_R$, which leads to the prediction that $F_r > P_i$. Such an imbalance in force per unit area at the lumen membrane is reasonable for a rapidly contracting esophagus, therefore Eq. 6 is useful for predicting relationships even though its exact form cannot be tested. The imbalance should cause acceleration of the lumen membrane and adjacent fluids. A negative pressure should develop in the lumen during the fluid ingestion stage of the pumping cycle, especially if passage becomes restricted as at $t = 0.16$ in the reconstruction (Fig. 2). This will partially rebalance the forces.

Case of Normal Pumping, in vivo

In this case P_e is equal to the pseudocelomic pressure, 16–225 mm Hg (mean = 70 mm Hg) higher than atmospheric pressure (1). Consequently, the bracketed term in Eq. 6, equal to zero in the excised preparation, should be less than zero, and a negative ϵ_ψ/ϵ_l is predicted for normal pumping in vivo.

DISCUSSION

The proposed physical model should be regarded only as a good first approximation to the real behavior of the esophagus. In using the linearized membrane theory of shells, we neglected possible coupling between circumferential and longitudinal elastic forces, and time-dependent elastic behavior common in viscoelastic material such as muscle. As a first approximation, however, the physical model leads to interesting predictions, testable by experiment, and will probably need only further refinement.

Also the proposed pumping sequence cannot be completely accurate because of some assumptions made in its reconstruction, and because it is based on measurements made under conditions unavoidably different from normal. Excision of the esophagus and the use of artificial perienteric fluid may have affected the electrical measurements. Particularly, because any connections to the nervous system would have been cut, the pacemaker location and frequency are uncertain. However, because there is substantial agreement with Mapes' observations on the fluid ingestion by intact *Ascaris* (8), we believe the pumping sequence is sufficiently accurate to form the basis for future refinements. Additionally, the exercise of reconstruction brought out the importance of each parameter to the efficient operation of the pump.

General Predictions of Physical Model

The model predicts that the dimensional changes possible for the esophagus depend, to a first approximation, only on the relationship of myofilament force per unit area F_R, and esophageal pressure (P_i) (see Eq. 6). When $F_R < P_i - P_e$, as during equilibrium contraction, there will occur a positive, radial strain. When $F_R \simeq P_i - P_e$, the radial strain will be zero, and when $F_R > P_i - P_e$, the radial strain will be negative. The radial strain is observed to be approximately zero during normal sequential pumping, and consequently one must assume that inertial forces and/or negative intraluminal pressure must develop during rapid contraction to counter-balance the high F_r at the lumen membrane. Negative intraluminal pressure has been observed during contraction of a ligated esophagus (13) and would be necessary in the normal esophagus if ingestion of fluid is to occur. An obvious extension of this work and a further test of the model would be a comparison of the ϵ_ψ/ϵ_l measured experimentally at different P_l with values predicted by the model. Varying the viscosity of the ingested fluid should also have an effect.

Work, Efficiency, and the Ratio of Strains

The useful work done by the esophagus is primarily raising the pressure of the lumen contents and injecting it into the gut. Interestingly, this work is not done directly by muscle contraction, but by energy stored in the elastic membrane and released during muscle relaxation. In addition, work of moving fluid along the lumen (overcoming the viscosity of the fluid) would use up a small amount of energy during both muscle contraction and relaxation.

Work is also done during the contraction phase in displacing pseudocelomic fluid equal in volume to the lumen contents. The enlargement of the outer dimensions of

the esophagus will raise the pressure of the pseudocelom and cause some movement of the pseudocelomic fluid. However, viscous energy losses appear to be minimized by the small ϵ_ψ/ϵ_l observed for the anterior esophagus. Pseudocelomic fluid displaced during radial expansion at the anterior region of the esophagus would have to flow posteriorly through relatively long, narrow, and obstructed paths between the esophagus and body wall, whereas displacement of pseudocelomic fluid by a longitudinally expanding anterior region occurs at the posterior end of the esophagus. Not only would longitudinal expansion result in a much shorter and less hindered flow, but also during relaxation, the lumen contents flowing into the flaccid intestine would simply take the place of the pseudocelom volume displaced at the posterior end. Thus, a small value for ϵ_ψ/ϵ_l is probably a requirement for efficient pumping in vivo and a more stringent one for the anterior region of the esophagus than for the posterior region.

Location of Elastic Components

In the model, all elastic components were assumed to be located in the outer membrane, and for the model to exhibit the same dimensional changes as the *Ascaris* esophagus, the outer membrane must be anisotropic with the ratio of the elastic constants, $E_\psi/E_l = 2.74$. Whatever the real location of the elastic elements, their action would have to be equivalent, imparting to the esophagus a greater resistance to expansion in the circumferential or radial direction than in the longitudinal direction. The most likely location of both longitudinal and circumferential elements is the thin external coating (or basal lamina) that surrounds nematode esophagi. The elastic components are similarly located in an external layer in other pressure-stressed biological systems (17), notably in the external cuticle of *Ascaris* (1). The basal lamina of the esophagus, however, does not resemble the layered, cross-helical fiber layer structure found in *Ascaris* cuticle; rather it is probably composed of a less structured mat of fine filaments. Other esophageal structures that may contribute to elasticity are the radial and longitudinal fibers and longitudinal "plates" observed by other authors (2, 18, 19) in the marginal cells (wedge-shaped cells located between the lumen apex and the external membrane). However, if the radial fibers contributed significantly to the resistance of the esophagus to circumferential strain, the circular cross section of the external membrane would become noticeably distorted during contraction.

Shape of Lumen

Mapes' discussion of the shape of the open lumen cross section (8) was based on the assumption that the esophagus expanded radially, not longitudinally, so that many of his arguments for a triangular shape are incorrect. For example, myofilaments inserted near the apex of the lumen should not increase in length during lumen opening, as he proposes, but should decrease in length if the radius of the outer membrane remains constant. The changes in external dimensions found in this study indicate that after prolonged contraction, the lumen must open to a cross-sectional area larger than the maximum circular configuration with no circumferential stretching. This strongly sug-

gests that the configuration of the fully open lumen is circular, and that strain of the lumen membrane is circumferential as well as longitudinal.

The shape of the lumen during rapid myofilament contraction could be more triangular than circular (Fig. 5, sector 3, dashed line) because the inertial forces due to radial acceleration of lumen membrane and adjacent fluids would be greater near the center than near the apices of the triradiate cross section. In addition, near the center, where the speed of contraction is large, the dynamic force exerted by the myofilaments will be less because myofilament force decreases with contraction velocity (20). The exact shape of the lumen at any time should be a complicated function of the flexibility of the lumen lining, the angle of insertion of each myofilament, the tension developed by each myofilament (which depends on speed of shortening and length of the myofilament), the inertia of the lumen lining and adjacent fluids, and the difference in pressure across the lumen wall. Also important may be the amount of tension in the filaments that connect the apices of the lumen to the outer membrane.

One conclusion from the exercise of reconstructing the pumping sequence is that the maximum opening of the lumen during normal pumping probably is the same as the stretched, full-circular opening achieved during prolonged contraction. Otherwise the maximum lumen volume attained, the volume per gulp, and the maximum in the ratio r/R_0 (corresponding to average cross-sectional area) would be lower than observed.

The Pumping Sequence

Mapes (8) first proposed a peristaltic action to explain the periodic uptake of fluid he observed. Our more direct observations of pumping action (Δl, Δr, $\bar{v}_C$, $\bar{v}_R$, and the changes in P_i) also support a peristaltic mechanism and lead to an improved reconstruction of the pumping action (Fig. 2). Our observations do not support the proposal by del Castillo and Morales (13) that the esophagus acts as a diaphragm pump. The action of such a pump requires that the lumen become open over its full length. We observe this only in the case of prolonged contraction, which in our experience usually occurs when the muscle is in poor condition. During normal pumping, the lumen closes at the anterior end before it opens at the posterior end.

It became evident during the reconstruction of the pumping sequence that the operation of the esophagus is critically dependent on the interrelationships among v_C, the latency between contraction and relaxation, and the rates of contraction and relaxation at each point. Should the v_C be too small, latency too short, contraction rate too slow, or relaxation rate too fast, a small volume would be ingested per pump and the esophagus would operate at low capacity. For example, increasing the time for contraction from 0.12 to 0.14 s causes a 40% reduction in total volume per gulp. Should the velocity be too large, latency too long, or relaxation rate too slow, the whole lumen could become open at once. This undoubtedly occurs during prolonged contraction when the latency is unusually long. Mapes observed that pumping was inefficient when the cycle was initiated at higher than normal frequencies in *Ascaris* (8) and at lower than normal frequencies in *Panagrellus* (11).

At a given point along the esophagus, depending on the time required for contraction and the latency between the contraction and relaxation waves at that point, the lumen may normally remain fully open for a brief period, remain fully open only instantaneously, or may relax before full opening is attained. These three cases occur in the reconstructed pumping sequence, Fig. 2. The first case occurs in the anterior, the second in the middle, and the third in the posterior. This is a consequence of the fact that $v_R > v_C$ (therefore the latency would decrease along the esophagus) and that the time for contraction was assumed to be constant along the esophagus. This may also explain why, in Fig. 10, the rise in pressure observed in the anterior is prolonged compared with that observed in the posterior.

Other Nematode Esophagi

Peristaltic motion is probably the only method by which the one-part cylindrical esophagus found in *Ascaris* can pump efficiently. Peristaltic motion would not be necessary, however, in esophagi having two bulbar regions because this configuration could operate successfully as a two-stage pump. Apparently, however, peristalsis does take place in certain regions of the more complex esophagi, as for example by the corpus and isthmus of *Panagrellus* (11) and the isthmus of *Rhabditis* (10).

The mechanics of lumen opening via myofilament contraction are probably very similar regardless of esophageal complexity, because even bulbar regions have a triradiate lumen with approximately radially oriented myofilaments. The model predicts that changes in external dimension would be expected to result from myofilament shortening and lumen opening. Though they are primarily longitudinal in *Ascaris*, there is no theoretical restriction to prohibit radial changes in other esophagi. In fact, radial changes could be advantageous in some cases. However, as discussed above, considerations of efficiency would favor a small radial change in large esophagi such as that of *Ascaris*.

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