Electrical Properties of the Body Wall of *Hydra*

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**ABSTRACT** If the hydrostatic pressure in the enteron of *Hydra* is made more than 2-4 mm of water greater than the outside pressure, the animal becomes distended, indicating that the normal enteron pressure is less than this. Positive enteron pressure attenuates the spontaneous, negative-going electrical spikes across the body wall, which are called contraction pulses (CP's) because of their relation to column contraction. Pressure has little effect on the transepithelial resting potential. The low frequency electrical impedance of the column is nonlinear. The impedance tends to increase as the transepithelial potential is made more negative. The nonlinearity has both initial and delayed components. The dc impedance of the column near the resting potential averages 100 kohms (approximately 5 kohms-cm²). The phase between transepithelial potential and imposed sinusoidal current approaches $-90^\circ$ with increasing current frequency. Bode plots of the column impedance and the phase lag indicate that the column has three or more time constants. CP's show several unusual features: (a) their amplitude and frequency are essentially independent of the transepithelial potential when the latter is altered by imposed current; (b) there is practically no change in column impedance during CP firing; (c) when the transepithelial potential is clamped at zero, CP's continue to appear spontaneously as current spikes. These features are consistent with the hypothesis that the CP-generating membrane forms but a small fraction of the total transverse impedance of the column.

**INTRODUCTION**

Coelenterates (phylum Cnidaria) are "composed essentially of two epithelia with cement or some kind of connective tissue in between" (Hyman, 1940). The outer epithelial layer is termed the ectoderm, the inner layer the endoderm. The two epithelia surround a single body cavity, the gut or enteron, which typically opens to the outside only at the mouth. Although coelenterates are structurally rather simple animals, the epithelia of which they are composed are rather complex. This is to be expected since the properties of the two epithelia alone must account for the animal's ability to locomote, to
grow, to capture and digest prey, to control water and ion content of its cells, and to reproduce.

In Hydra and other hydroid polyps the two epithelia are each one cell in thickness and are separated by a thin, acellular mesoglea. The principal structural cells of both epithelia have expanded, contractile bases which lie against and are partially imbedded in the mesoglea. These cells are termed epithelio-muscular cells. The muscular processes in the ectoderm are longitudinal, those in the endoderm are circularly oriented. Nerve cells form a diffuse net running among the contractile bases of the ectodermal cells and are sparsely distributed among the endodermal cells (Semal-van Gansen, 1952; Davis et al., 1968).

It has recently been shown that Hydra has a resting potential; the fluid in the body cavity is typically 15–40 mv positive with respect to the outer bathing solution (Josephson and Macklin, 1967). Superimposed on this resting potential are spontaneous, rather slow spikes which frequently occur in bursts. The spikes are depolarizing and frequently overshoot the zero potential level so that the inside of the column becomes transiently negative. Before their transepithelial orientation was known, the spikes of Hydra had been the subject of several investigations and their pattern of occurrence and behavioral correlates have been described in some detail (Passano and McCullough, 1964; Rushforth, 1967; Josephson, 1967). The spikes have been termed contraction pulses (CP’s) because they precede and are probably causally related to contraction of the longitudinal musculature of the column. CP’s are conducted up or down the column at about 5 cm/sec.

Epithelia capable of producing propagated electrical pulses appear to be widespread in hydrozoans (Mackie, 1965; Mackie and Passano, 1968), but have not yet been described for other coelenterate classes or in higher phyla. The experiments described in this paper are part of a program to investigate the physiological properties of the interesting and complex epithelia of hydroids. In these experiments we have restricted our attention to the electrical properties of the two epithelial layers of hydra, both at rest and during CP firing.

MATERIALS AND GENERAL METHODS

The animals used were Hydra oligactis, a species which is moderately large, grows well in culture, and has a relatively firm body which makes it easier to handle than some other hydra species. The animals were raised in an artificial culture solution containing $1.5 \times 10^{-3}$ m CaCl$_2$, $1.2 \times 10^{-3}$ m NaHCO$_3$, and $1.2 \times 10^{-4}$ m Na$_4$EDTA in distilled water (Loomis and Lenhoff, 1956). The animals in culture were fed daily with newly hatched Artemia, but all animals used in experiments were starved for 24–36 hr before being used. The animals were grown and all experiments were done at 20° to 23°C.

The contractility of hydra’s column presents some difficulties for recording trans-
epithelial potentials. Microelectrodes inserted into the enteron through the body wall were not satisfactory since the column pulled off the electrodes or tore against them during contraction. Attempts to tie the column to a cannula inserted through the mouth were also unsuccessful. The column always pulled apart at the ligature. The holder illustrated in Fig. 1 with which the hydra is held on an oral cannula by slight suction obviated these difficulties. The holder consists of two concentric glass tubes joined with epoxy cement so that the tip of the inner tube protrudes slightly from the orifice of the outer one. The inner tube is about 0.4 mm in external diameter at the tip and the radial distance between the inner and outer tubes is about 0.15 mm. The inner tube passes through the wall of the outer tube and then expands to form a small cup. The inner tube and cup are allowed to fill by capillarity with culture solution before a hydra is placed on the holder. To mount a hydra on the holder the animal is grasped lightly by the tentacles and its mouth is pulled gently over the protruding end of the inner tube. The animal is then held in place by slight suction applied to the outer tube. The suction is created by withdrawing the plunger of a syringe attached to the outer tube by flexible tubing. Transepithelial potentials were recorded between the fluid in the cup, which is in electrical contact with the inside of the animal, and the outer bathing solution using electrodes consisting of a chlorided silver wire in a glass tube containing 2 M KCl in 1% agar. The recording electrodes were about 0.5 mm in internal diameter at the tip. Potentials were amplified with high impedance amplifiers and displayed on an oscilloscope or penwriter. In this paper the transepithelial potential will be described as positive when the interior of the animal is positive with respect to the external solution and positive current will designate current which is outward through the body wall. Other techniques used are described in appropriate sections of the text.

The tentacles of a hydra may be damaged when it is being mounted on the holder, but otherwise the animal appears to be uninjured. Column contractions and contraction pulses occur spontaneously in animals mounted on the holders just as in unmounted animals. When an animal is put on the holder the condition of the column is probably affected in three ways: (1) the mouth is held open and the tissue about the mouth is somewhat compressed about the holder. This may affect the pattern of spontaneous CP's since it has been found that the frequency and pattern of spontaneous CP's are altered by mechanical stimulation or injury; in injured animals there are more CP bursts and fewer single CP's than is true of undamaged ones (N. Rushforth, personal communication); (2) the fluid inside a hydra is normally hyperosmotic to the surrounding medium (B. Schmidt-Nielsen, personal communication). When an animal is on the holder the ionic concentration in its gut is probably altered by diffusion of water and ions between the gut and the culture solution in the holder. The effects of changes in internal ion concentrations on transepithelial potentials have not yet been examined. Exposing the gut to culture medium cannot be very damaging to the animal for hydra can survive extended yawning periods during which the mouth is open and there is free diffusion between the outside medium and the gut (Steinbach, 1963); (3) since the hydra's mouth is held open by the holder, the animal cannot control its internal hydrostatic pressure and the transepithelial pressure becomes determined by (a) the difference in fluid levels between the inner tube of the holder and the external solution, and (b) the capillary rise of fluid in the inner
tube. The effects of transepithelial pressure were evaluated by placing an animal in a holder in which capillary rise of fluid in the inner tube had reached equilibrium. Transepithelial potentials were then recorded while the transepithelial pressure was altered by raising or lowering the level of the solution surrounding the holder and animal. The hydrostatic pressure across the body wall was measured as the difference between the height of the external fluid level and its original equilibrium level. A displacement which resulted in an increase in the enteron pressure with respect to that

**Figure 1.** The holder used in measuring the electrical potential across the body wall of hydra.
outside was considered positive. The cross-sectional area of the inner cup of the holder was sufficiently large so that the fluid level in the cup was nearly unchanged by fluid movements between the cup and the enteron of the animal. The pressure

![Graph A](image1.png)

**Figure 2.** The effect of hydrostatic pressure difference across the body wall on the transepithelial resting potential (A) and on CP amplitude (B).

The range tested extended from $-4$ mm H$_2$O to $+6$ mm H$_2$O in 2 mm steps. The animals were left at each test pressure for 5 min and were returned to zero transepithelial pressure for 5 min between each test period. The pressures were presented in random order except that $+6$ mm H$_2$O was always the last pressure used. Animals became greatly distended at this pressure and only slowly recovered when the pressure was reduced.
The resting transepithelial potential was found to be essentially independent of the hydrostatic pressure across the body wall in the pressure range tested (Fig. 2 A). The CP’s, on the other hand, became considerably smaller when the hydrostatic pressure in the enteron was made greater than that outside (Fig. 2 B). Further, some animals became distended at +2 mm H2O internal pressure and all were notably distended at +4 mm H2O. Since positive enteron pressure seemed to be deleterious, the holder was arranged so that there was no pressure difference across the body wall in the experiments described below.

The fact that 2-4 mm H2O causes column distension indicates that the normal pressure across the body wall is certainly less than 4 mm H2O. Thus the hydrostatic pressure across the body wall of hydra is apparently smaller than the hydrostatic pressure across the body wall of sea anemones which has been variously measured as 0.2-3 cm H2O at rest and 0.6-15 cm H2O during contraction (Chapman, 1949; Batham and Pantin, 1950; Trueman, 1966).

In most of the experiments described below, electrical current was passed through the body wall using, as a current source, an electrode introduced into the enteron through the inner tube of the holder (Fig. 3). The first current electrodes used were glass capillaries filled with 20 mM NaCl or 2 M KCl in agar. These electrodes were not satisfactory. The introduction of the KCl-filled electrode into the enteron led to reduction in the transepithelial potential, probably due to salt diffusion from the electrode. Further, with salt bridge current electrodes filled with either NaCl or KCl the column impedance transiently decreased following each CP (Fig. 4 A). This impedance change was rather slow and it had nearly the same time course as the mechanical contraction of the column. This impedance change is probably a direct result of the change in body form during contraction. The enteron of the relaxed hydra is a thin channel filled, presumably, with a dilute salt solution. Thus, it is to be
expected that the enteron has considerable longitudinal resistance and that the inside of the animal will not be isopotential when the current is passed through the body wall from a point source such as the salt bridge electrodes used. When the animal contracts, the enteron becomes shorter and wider and approaches isopotentiality. Contraction of the column decreases the longitudinal resistance of the enteron between the current source and distant parts of the column, thus decreasing the input impedance of the animal. That this is the correct explanation of a slow impedance change following CP's and that the impedance decrease is not due to a decrease in the specific impedance of the body wall is shown by the fact that the impedance change is reduced or eliminated if the animal is physically constrained from shortening following CP's (Fig. 4 B, C).

![Figure 4. Impedance changes during column contraction when a salt bridge (point source) was used as a current electrode. Sinusoidal current at 0.2 µamp and 10 Hz was passed through the column. The width of the base line is proportional to the column input impedance. The impedance decrease following CP's, seen best in A, has about the same time course as column contraction. Records B and C are from the same animal. In B, the animal contracted freely. In C, the base of the column was gently sucked into the opening of a fixed capillary tube so that the column was held at rest length. Note the absence of impedance changes in C.](image)

The problem of nonisopotentiality was eliminated by using as a current electrode a long axial wire rather than a salt bridge. The axial electrode consisted of a length of 50 µ silver wire mounted by wax in a capillary tube. The wire protruded from the capillary by approximately 1.6 mm, all of which was in the enteron during current-passing experiments. Before the wire was chlorided, its distal end was melted in a flame so that it formed a small ball. This reduced but did not eliminate the problem of having the electrode pierce the body wall during column contractions. The wire electrodes were apparently effective in maintaining isopotentiality, for little or no slow impedance change associated with contraction was seen when they were used. Axial wire electrodes were used in all the current experiments described below. The long wire electrodes did, however, introduce one difficulty. When the animal contracted, its base came up against the electrode and all too frequently the electrode pierced the column or the animal pulled its mouth from the holder. Most of the experiments were terminated by the animal's pulling free from the holder or puncturing its column with the current electrode when it contracted.
RESULTS

I. Electrical Properties of the Column at Rest

IMPEDANCE NONLINEARITIES

AC impedance analysis was the principal technique we wished to apply in analyzing the electrical properties of the column at rest. This, as usually applied, demands that the system be linear; i.e., that the impedance be independent of the amplitude of the voltage or current used to measure it. We tested the electrical linearity of the column using triangular wave currents with a peak-to-peak amplitude of 1.2 μamp, and with various slopes ranging from 0.48 to 4.8 μamp/sec.

Results to be described below indicate that the passive electrical properties of the body wall can be approximated by a circuit containing one resistor (R) and a parallel capacitor (C). If the body wall has linear electrical properties, then the voltage response (V) of this circuit to an imposed current (I) ramp starting at time t = 0 is given by the following (e.g. Close, 1966)

\[
dV/dI = R[1 - e^{-t/RC}]
\]

Thus, the slope of an I vs. V plot will exponentially approach the value R, the time constant of the approach being RC, the time constant of the circuit. Within about five time constants of the triangular wave peaks, the slope of the plot should be within 1% of the slope ±R. The average time constant of the body wall of hydra is 4.1 msec. Therefore after about 20 msec the voltage/current plot should be a straight line with constant slope. That this is not the case is shown in Fig. 5. The V-I plots recorded were not straight, but rather concave downward indicating that the column impedance increases as the transepithelial potential is made more negative. In addition, the V-I plots frequently showed hysteresis with the voltage in the positive-going segment of the triangular current wave following a different trajectory from the negative-going segment. This was especially true in the negative current region of the V-I plots where often the positive-going segment of the curve lay below the negative-going portion.

The response of the column to current steps indicates that there are both initial and delayed components in the impedance nonlinearities. The impedance measured soon after the onset of a current step shows the same kind of nonlinearity seen with ramp current, the impedance becomes greater as the transepithelial potential is made more negative (Fig. 6). This initial non-

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1 For the more general case, the impedance is given by equation 4; however, since, (a) the time constants are small compared to the ramp slope and, (b) the first time constant dominates the ramp response, equation 1 is adequate for the discussion here.
linearity is augmented by a delayed component which develops during the course of long current pulses. With negative current the impedance increases throughout the pulse; with positive current it often decreases. The delayed impedance changes are greater with negative than with positive current, and are greater the larger the current pulse amplitude. Outward current causes a decrease and inward current an increase in resistance, suggesting an analogy between the nonlinearities of the hydra column and delayed rectification of, for example, the squid axon. The parallel cannot be carried very far, however, for outward current depolarizes the squid axon while it increases the usually positive resting potential of hydra.

Figure 5. The transepithelial potential during imposed triangular current. The current slope was 0.48 μamp/sec. The broken line is drawn to be tangent to the curve at the zero current level.

AC IMPEDANCE ANALYSIS

The passive electrical properties of the column were further characterized by determining the electrical impedance with sinusoidal current of varying frequencies. Although the previous results indicate that the current-voltage relation is nonlinear, the departure from linearity is not very great over small current ranges, especially near zero current. We, therefore, used current of 0.2–0.4 μamp peak-to-peak amplitude, a range in which there is not an appreciable departure from linearity.

The experimental setup is shown in Fig. 3. Several sets of impedance measurements were made with each animal, each set covering a frequency range of 0.1 Hz to greater than 10 KHz in steps of equal logarithmic interval, each frequency being 1.6 times greater than the preceding one. Following this, the animal was blown off the holder by increasing the pressure in the outer tube and a similar set of impedance measurements was made at the same current strengths and frequencies used when the animal was in place. The latter
measurements were used to characterize the impedance of the electrodes, holder, and the solution surrounding the holder. The current and voltage were displayed on the horizontal and vertical axes of an oscilloscope and the resulting Lissajous figures were photographed. The maximum voltage excursion ($V_m$) and the width of the Lissajous figures along the voltage axis at the zero current line (A) were measured from these photographs. These measurements and the peak-to-peak current amplitude ($I_m$) were used to calculate the impedance and phase angle. The impedance is given by $V_m / I_m$ and the phase angle ($\phi$) by $\sin^{-1} A / V_m$. Since the measured impedances are complex numbers, the impedance of the animal alone could not be obtained by directly subtracting the impedance of the apparatus from that of the animal plus the apparatus. To determine the impedance of the animal alone, the measured impedances were separated into in phase (real) and 90° out of phase (imaginary) components. The real and imaginary components are given by $V_m / I_m \cos \phi$ and $V_m / I_m \sin \phi$, respectively. The real and imaginary impedances of the apparatus without the animal were subtracted from those of the apparatus and the animal to give the real impedance ($Z_{re}$) and the imaginary impedance ($Z_{im}$) of the animal alone. This procedure assumes that the impedance of the apparatus without the animal can be treated as being in series with the animal with no appreciable effect from parallel impedance elements.
Over most of the frequency range tested, the apparatus alone behaved as a small, constant resistance so the correction for the impedance of the apparatus generally affected only the real part of the animal impedance. In the 12 experiments resulting in Table I the impedance of the apparatus alone at low and middle frequencies averaged 2.2 kohms (range = 1.2 to 3.0 kohms), and in most cases there was no phase shift to frequencies less than 10 KHz. The complex impedance of the animal alone (Z) and its phase angle are given by the following relations

\[
Z = \sqrt{Z_{re}^2 + Z_{im}^2}
\]

\[
\phi = \tan^{-1}\left(\frac{Z_{im}}{Z_{re}}\right)
\]

**TABLE I**

**SUMMARY OF RESULTS OF AC IMPEDANCE MEASUREMENTS OF THE HYDRA BODY WALL FOR 12 ANIMALS**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting potential, mv</td>
<td>22 ± 1.3</td>
</tr>
<tr>
<td>Time constant, msec</td>
<td>4.1 ± 0.5</td>
</tr>
<tr>
<td>R, kohms</td>
<td>100 ± 9</td>
</tr>
<tr>
<td>C, μF</td>
<td>0.041 ± 0.003</td>
</tr>
<tr>
<td>Calculated surface area, cm²</td>
<td>0.052 ± 0.004</td>
</tr>
<tr>
<td>R, kohm-cm²</td>
<td>5.1 ± 0.5</td>
</tr>
<tr>
<td>C, μF/cm²</td>
<td>0.81 ± 0.05</td>
</tr>
</tbody>
</table>

All values are given as means plus or minus standard errors and were calculated from graphical fits to the data using a one-time constant transfer function. The mean resting potential does not include one animal which had a resting potential of -27 mv. If this animal is included then the resting potential is 18 ± 4.2 mv.

The impedance values for each animal were plotted as a function of frequency on Bode plots (log impedance and phase vs. log frequency, Fig. 7). Multiple sets of measurements on the same animal gave essentially the same Bode plot. The plots for animals were similar with low frequency points falling about constant impedance lines and high frequency points falling about a line with a slope of approximately 45° (i.e. one decade change in impedance per decade change in frequency). The corresponding phase angle plot shows a decrease to -90°. A Bode plot of this shape may be represented by the following general impedance transfer function (D’Azzo and Houpis, 1966)

\[
Z(s) = K \left[ \frac{(1 + sT_2) \cdots (1 + sT_{n-1})}{(1 + sT_1)(1 + sT_2) \cdots (1 + sT_n)} \right]; n = \{1, 3, 5, \ldots\}
\]

where K is the low frequency impedance asymptote, s is the Laplace transform function, and T is a characteristic time constant. As a first approximation.
Figure 7. Bode plots of the column impedance. The points shown are for three consecutive runs for a single animal. The solid lines in A and B are the theoretical curves for a one-time constant transfer function. The upper broken lines in A indicate the high and low frequency asymptotes whose intersection gives the time constant of the transfer function. The lower broken line in A is the impedance of the apparatus without the animal in place. The experimental points have been corrected for the impedance of the apparatus. This correction introduces increasingly large errors as the animal's impedance becomes small as compared to that of the apparatus and the points above about 1.6 KHz are increasingly inaccurate.
we assumed the simplest case and fit the data with a single time constant function

\[ Z(s) = \frac{K}{1 + sT}. \]  

(5)

Here \( 1/T \) may be interpreted as the break frequency or intersection of the high and low frequency asymptotes as shown in Fig. 7. The minimal electrical circuit satisfying this transfer function contains one capacitor (\( C \)) and one resistor (\( R \)) in parallel (Fig. 7). The circuit component values are given by

\[ R = K; \quad C = 1/TR. \]  

(6)

Two or more sets of impedance measurements were obtained from each of 12 animals. The passive electrical properties of these animals are summarized in Table I. The values were obtained by graphically fitting a one-time constant model to the experimental points on a Bode plot. The dimensions of the animals were measured with an ocular micrometer shortly after they were mounted on the holder and at a time when they had a nearly cylindrical shape. The area calculations are based on the assumption that the animal can be treated as a cylinder or, in the more irregularly shaped cases, as a set of contiguous cylinders. The calculated area measurements are not very reliable but they are probably not wrong by a factor of more than two. Area specific resistance and capacitance values are given in Table I for the one-time constant transfer function.

Occasionally animals were found with very low or even negative resting potentials. One of the animals of Table I had a rather large negative resting potential (−27 mv). Although the polarity of the resting potential was unusual, the column impedance parameters were all within 1 SD for all animals. At present no explanation can be given for the variability in the size and sometimes even the polarity of the resting potential. In all animals, no matter what the polarity of the resting potential, the spikes were negative-going.

II. Electrical Properties of the Body Wall during CP firing

SOME FEATURES OF SPONTANEOUS CONTRACTION PULSES

Several contraction pulses recorded as in Fig. 1, were displayed as phase plane plots \((dV/dt \text{ vs. } V)\), a presentation which is particularly useful in identifying exponentially changing portions of a voltage pattern because such signals are linear in the phase plane with a slope equal to the reciprocal of the exponential time constant (Jenerick, 1963).

Some examples of phase plane plots are shown in Fig. 8. One of the most striking features of the displays is their variability. This is especially true for
the early pulses of a burst. The pulse amplitude often increases during the early portion of a burst and then reaches a nearly constant size with a more or less stable shape on the phase plane plots. The negative-going portion of the spike has a long segment which is nearly linear in the phase plane presentation. The spike return generally contains two approximately linear phases. This segment is typically the most consistent part of the phase plane plots and the portion for which successive pulses best superimpose.

### TABLE II

**SUMMARY OF DATA TAKEN FROM PHASE PLANE PLOTS OF MIDBURST CP’S, ONE FROM EACH OF EIGHT DIFFERENT ANIMALS**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum rate of potential change, negative direction</td>
<td>-2.3 ± 0.1 V/sec</td>
</tr>
<tr>
<td>Maximum rate of potential change, positive direction</td>
<td>+0.60 ± 0.05 V/sec</td>
</tr>
<tr>
<td>Time constant for approximately exponential portion of spike onset, msec</td>
<td>9.8 ± 1.7</td>
</tr>
<tr>
<td>Time constant for last phase of spike return, msec</td>
<td>51 ± 4</td>
</tr>
</tbody>
</table>

Means are followed by standard errors.
Measurements taken from phase plane plots of single midburst pulses from eight animals are summarized in Table II. The longtime constant of the final portion of the spike return is particularly interesting. It is much longer than the time constant of the body wall (or time constants, see Discussion).

![Diagram showing phase plane plots and transpithelial potential](image)

**Figure 9.** Spontaneous CP's occurring while the transepithelial potential is being shifted by imposed triangular current. In the lower trace of A the signal has been passed through a high-pass filter (time constant = 0.1 sec) so that the CP's can be displayed at higher gain. The independence between CP amplitude and transepithelial potential is demonstrated in B which is a plot of data taken from the same animal.

determined in the AC impedance measurements. This indicates that the return phase of the spikes is determined by the time course of the spike-producing mechanism rather than by the passive electrical properties of the body wall.

**CONTRACTION PULSES AND THE BACKGROUND TRANSEPITHELIAL POTENTIAL**

During the experiments in which relatively large current was passed through the body wall to test its electrical linearity, it was noted that CP's appeared
spontaneously even when the transepithelial potential was shifted markedly in either the positive or negative direction. This offered an opportunity to examine the effects of transepithelial potential on the size of spontaneous CP's. It was anticipated that the CP's would become smaller as the transepithelial potential was made more negative, and that the relation between CP size and the background potential could be used to determine an equilibrium potential for the spike-generating mechanism.

The experimental setup used in this series of experiments was that shown in Fig. 3. Triangular current waves of 1.2 μamp peak-to-peak amplitude and 20 sec period were passed through the body wall. An example of CP's occurring on a background of changing transepithelial potential is shown in Fig. 9 A. Surprisingly, the amplitude of the CP's is essentially independent of the transepithelial potential from which they arise. In Fig. 9 B, the amplitude of a number of spontaneous CP's recorded from a single animal is plotted against the transepithelial potential at the CP onset. There is some scatter among the points, reflecting the inherent variability in CP amplitude, but again there is no obvious relation between the background potential and the CP size.

It is also evident in Fig. 9 B that the spontaneous CP's are rather uniformly distributed along the transepithelial potential axis. This indicates that the probability of CP occurrence is essentially independent of the transepithelial potential. Fig. 10 summarizes the frequency of spontaneous CP's as a function of imposed current level. This figure is based on data pooled from the seven animals used in these experiments. It can be seen that there is a slight increase in CP frequency near the end of the positive-going section of the current waveform but otherwise the CP distribution is nearly flat.

In summary, from these experiments we can conclude that the amplitude and probability of occurrence of spontaneous CP's are both nearly independent of imposed current and hence of the transepithelial potential in the range tested.
COLUMN IMPEDANCE DURING CP'S

The fact that the CP amplitude remains constant when the transepithelial potential is shifted by extrinsic current suggests that the column impedance

![Diagram of the bridge circuit](image)

**Figure 11.** Measurement of column impedance changes during CP's. A The bridge used to examine impedance changes. The component values were: $R_1$, 10 Mohms; $R_2$, 0-15 Mohms; $R_3$, 0-160 kohms; $R_4$, 0-50 kohms; $C$, 0.5 $\mu$F. To detect any impedance changes during CP firing, sinusoidal current at 0.8 $\mu$amp and 200 Hz was passed through the column. The four traces of each record are, in order: (1) the potential difference between the two arms of the bridge ($V_a - V_b$) with the bridge nearly balanced; (2) a zero line for (1); (3) the potential between the two arms of the bridge at higher gain and with CP's removed by passing the signal through a second order high-pass filter (time constant = 0.8 msec); (4) the potential across the body wall of the animal alone. The inserts below each set of traces show the bridge imbalance recorded in the high-gain trace (trace 3) which occurred when the value of the parallel resistance, $R_3$, was changed by the amount indicated. The bridge in B was that shown above. In C the series resistance, $R_4$, was removed and the current return electrode was a spiral of chlorided silver wire surrounding the animal.

is little changed during CP firing for it indicates that the IR drop due to the imposed current is independent of and additive to the CP. The column impedance during CP firing was examined directly using a bridge circuit. In the first series of experiments the circuit used was the one shown in Fig. 11 A.
The portion of the bridge paralleling the animal is based on the one-time constant equivalent circuit suggested by the AC impedance analysis, with the addition of a variable series resistor \( R_4 \). The fact that the Bode impedance plot (Fig. 7 A) does not approach a horizontal asymptote indicates that there are no purely resistive elements in series with the other column impedance elements. The circuit of Fig. 11 A, however, introduces a resistance of 10–20 kohms in series with the animal, namely that of the external solution and the current return electrode. This series resistance was minimized in the AC impedance analysis by measuring the potential differentially across the body wall. Impedance changes during CP's were examined with the bridge activated by sinusoidal current at 100 or 200 Hz. The bridge was initially balanced by adjusting the series resistance, \( R_1 \), so that there was no phase shift at high frequency (500 Hz) between the potentials \( V_a \) and \( V_b \) measured at the bridge midpoints. The parallel resistance, \( R_3 \), was used to similarly eliminate phase shift at low frequency (50 Hz). \( R_2 \) was used as an amplitude control to complete the balance. Even when the bridge was balanced at high and low frequency it was somewhat out of balance at middle frequencies and some readjustment was required to rebalance it at the 100 or 200 Hz used in these experiments. With all eight animals used in this series the bridge remained balanced or went only slightly out of balance during CP firing (Fig. 11 B).

At 200 Hz, the impedance of the external solution and the external electrode, measured without the hydra on the holder, is approximately the same as that of the animal. This relatively large external resistance reduces the sensitivity of the bridge to changes in the animal’s impedance. In a second series of bridge experiments, the salt bridge external electrode was replaced by a coil of chlorided silver wire which encircled the animal. This reduced the external resistance to less than 5 kohms. Also for the second set of measurements the bridge was simplified by removing the series resistance \( R_4 \). The bridge was now balanced by setting the phase shifts in the two arms equal at the test frequency of 200 Hz using \( R_3 \). The bridge amplitude was balanced using \( R_2 \).

An example of the results from this series is shown in Fig. 11 C. Although the bridge should be more sensitive to changes in the animal’s impedance it again stays nearly balanced during the CP's.

From these results it may be concluded that any impedance change during CP firing is small compared to the resting impedance of the column.

VOLTAGE CLAMP OF THE COLUMN

On the basis of the preceding results, it might have been predicted that the response of the column to voltage clamping would also be anomalous. This was indeed found to be the case. When the column was clamped at zero potential spontaneous CP's continued to appear, only now they occurred as current spikes rather than voltage spikes (Fig. 13). The experimental setup used
to voltage clamp the column is illustrated in Fig. 12. A spiral of chlorided silver wire was used as the external electrode to reduce the external resistance. In these experiments the recording electrode was also a chlorided silver wire to minimize the interelectrode potential. The setup of Fig. 12 made it possible to measure (a) the transepithelial potential clamped at zero, (b) the unclamped transepithelial potential, and (c) the alteration in the transepithelial potential caused by imposed sinusoidal current. In these experiments the clamp was turned on and off several times with each animal. Immediately after each clamped period the column impedance was measured with 1 Hz sinusoidal

![Diagram](https://example.com/diagram.png)

**Figure 12.** The circuit used to voltage clamp the body wall at zero potential. The column impedance was determined with the switch in the upper position and the current electrode connected to a generator producing sinusoidal current at 1 Hz. The resting potential and CP's as voltage spikes were recorded with the switch in the middle position (open circuit configuration). In the lower switch position the current electrode was connected to the output of an operational amplifier (Philbrick P25AU), which held the transepithelial potential at zero.

![Graph](https://example.com/graph.png)

**Figure 13.** Spontaneous CP's with the body wall in the open circuit configuration and clamped at zero potential. At the arrow in A the column was clamped at zero potential. B is from the same animal somewhat later. It begins with the column clamped at zero potential and the arrow marks the transition to the open circuit configuration.
current. The current required to hold the transepithelial potential at zero should be directly proportional to the unclamped potential and inversely proportional to the column impedance. However, the column impedance determined from the ratio of unclamped potential to clamped current was consistently greater than that determined with the same animals using AC current (Table III). This difference is not due to capacitive shunting at 1 Hz as can be seen from Fig. 7 A. The difference is probably due to the nonlinear resistance of the column described earlier. The column resistance increases when the transepithelial potential is shifted in the negative direction (Figs. 5 and 6). Voltage clamping to zero shifts the transepithelial potential in the

<p>| TABLE III |</p>
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The impedance given in items 3 and 4 of the table includes the apparatus impedance which is less than 5% of the total impedance. Five or more determinations for each category were made with each animal. Numbers given are means followed by the interanimal standard error.

negative direction and therefore should be expected to result in an increase in the column impedance.

The ratio between unclamped CP potential amplitude and clamped CP current amplitude is about the same as that between unclamped resting potential and clamped current. However, due to the variability in CP amplitude and the small sample size, this result is not as certain as the data given above.

The dimensions of the animals used in the voltage clamp experiments were not measured but it is possible to estimate the current density through the column at zero transepithelial potential using the average column area determined in the AC impedance study (Table I). The average current density determined in this way is 4 μamp/cm².

DISCUSSION

The data from the AC impedance analysis can be fit moderately well by a one-time constant transfer function, but there are several reasons for believing that this is too simple a model for the electrical properties of the body wall.
The most important reason for doubting the adequacy of the one-time constant model is the morphological complexity of the body wall. The body wall is composed of two cell layers with a mesoglea between and a mucous coat on the outside. It would be difficult to reconcile this morphological complexity with so simple a transfer function.

The independence of CP amplitude and imposed current and hence the transepithelial potential, and the very small impedance changes seen during CP's both suggest that there may be multiple impedance elements in series in the body wall. It seems likely that CP's are caused by transient membrane permeability changes with the result that the potential across the active membrane approaches some fixed level (an equilibrium potential) whose value is determined by the prevailing ion concentrations and permeabilities. But the CP amplitude does not change appreciably when the transepithelial potential is displaced over a range as large as 200 mv (Fig. 9 B). This might mean that the CP equilibrium potential is very large, on the order of a volt or more negative, so that the transepithelial potential range examined is just a small fraction of the equilibrium potential, but another explanation seems more likely. We assume that CP's are produced by one of the membrane layers of the body wall. If the spike-generating membrane were in series with a passive impedance, then the voltage across the body wall during imposed current would be distributed across both the passive impedance and the CP-generating membrane. If the CP-generating membrane were only a small part of the total series impedance of the column, then the potential change across the active membrane during imposed current would be only a small fraction of that across the body wall as a whole and the CP amplitude might thus be relatively unaffected. Furthermore, if the active membrane formed only a small fraction of the total series resistance of the column, then even relatively major impedance changes in the active membrane during CP firing would cause only a small relative change in the impedance of the body wall as a whole. This seems a likely explanation for the small impedance changes seen in the column during CP firing.

There is also more direct evidence for the inadequacy of a one-time constant model. The AC bridge based on this model could not be adjusted so that it would be in balance at all frequencies. And although the theoretical curve for a one-time constant transfer function fits the data from the AC impedance study well at high and low frequencies, the fit is not as good at middle frequencies, especially near the break frequency (Fig. 7). With all 12 animals used in the AC analysis, the experimental points fall below the theoretical curve near the one-time constant break frequency.

With these facts in mind, the impedance data were reexamined to see whether the experimental results might be better fit by a model with multiple impedance elements in series. The approach to a −90° phase shift (Fig. 7 B)
with increasing frequency indicates an impedance transfer function for the column of the following form

\[ Z(s) = K \left[ \frac{(1 + sT_1) \cdots (1 + sT_{n-1})}{(1 + sT_1)(1 + sT_2) \cdots (1 + sT_n)} \right] ; n = \{1, 3, 5, \ldots\} \tag{4} \]

The terms of the transfer function, \((1 + sT_i)^{-1}\), are multiplicative and therefore additive on the logarithmic Bode plot. This facilitates graphical analysis.

The method used to obtain transfer functions with multiple time constants from the AC impedance data was a graphical one suggested to us by Dr. L. Ostrander. Templates were constructed for the following transfer functions

\[ Z(s) = (1 - sT) \quad \text{phase lag function} \tag{7} \]

\[ Z(s) = (1 + sT) \quad \text{phase lead function} \tag{8} \]

The phase lag template was moved along a Bode plot of experimental data until it best fit the first experimental points which diverged from the low frequency asymptote. The break frequency of the template in this position gives the first time constant for that set of experimental data. The difference between the template and the higher frequency points was replotted on logarithmic paper and the template-fitting procedure was repeated successively using the phase lead template when the impedance initially increased with increasing frequency and the phase lag template when the impedance initially decreased. The time constants determined in this manner and the low frequency impedance asymptotes were used to calculate impedance transfer functions for the 12 animals used in the AC impedance study. In each case a three-time constant transfer function fit the experimental data quite well (Fig. 14). There was no significant improvement in the fit to the data in the few cases in which a five-time constant transfer function was tried. The values of the three-time constants for the transfer functions best fitting the AC impedance data are summarized in Table IV.

The circuit shown in Fig. 14 is one of four minimal two-port circuits that would give a three-time constant transfer function with a \(-90^\circ\) phase lag at high frequency. It is an attractive model for it indicates that the impedance properties of the column might be due to two membranes in series, each with resistive and capacitive properties. One of the resistances is approximately eight times larger than the other (Table IV). The large resistance might form the large passive impedance suggested to be present in the body wall. It must be emphasized, however, that there are three other minimal circuits and a large number of nonminimal circuits that would also give appropriate three-time constant transfer functions. Further, because of variability in the experimental data, transfer functions with more than three-time constants certainly cannot be ruled out.
Because the transfer functions are tailored to fit the experimental data, too much importance should not be given to the agreement between the two. Nonetheless, it is clear that the impedance data are better fit by a transfer function with three or more time constants than by a single time constant model. This substantiates the conclusion that there are multiple impedance elements in series in the body wall.

**Figure 14A**

**Figure 14B**

**Figure 14.** Fitting the AC impedance data with a three-time constant transfer function. The experimental points are the same ones shown in Fig. 7. In A the points have been fitted with a one-time constant transfer function and, lying somewhat below this and better matching the experimental points, a three-time constant transfer function. Only the three-time constant transfer function is shown in B.
The appearance of CP's as current spikes when the transepithelial potential is clamped at zero is also consistent with the hypothesis that there is a large impedance in series with the CP-generating membrane. Under the clamp conditions the current flow created by activity in the CP-generating membrane will initiate potential drops across passive series impedances in the body wall. When the transepithelial potential is zero, the sum of potential differences across the passive series elements must be equal and opposite to the sum of potential differences across current-generating membranes. With moderate CP currents and a large passive series impedance, the voltage drop across the passive impedance and hence that across the CP-generating membrane will change considerably during a CP even when there is no over-all transepithelial potential. Therefore, a passive series impedance prevents the CP-generating membrane from being voltage-clamped even when the body wall as a whole is.

The circuit of Fig. 15 illustrates this point. It consists of a subcircuit \((E, R_1, R_3)\) representing a membrane producing a variable potential and a passive series impedance \((R_2)\). The CP time course is long compared to the time constants of the body wall and capacitive elements in the circuit have therefore been ignored. A resting potential which is of opposite polarity to that of \(E\) could be added by placing an appropriate battery in series with either \(R_1\) or \(R_2\). \(V_{ae}\) and \(V_{ab}\) are the potentials across the entire circuit and across the subcircuit representing the active membrane.

In the open circuit configuration (switch open)

\[
V_{ae} = V_{ab} = E \left[ \frac{R_3}{R_3 + R_4} \right]
\]

Closing the switch is the equivalent of clamping the circuit at zero potential.
In this configuration

\[ V_{ac} = 0, \text{ and } V_{ab} = E \left[ \frac{R_1 R_2}{R_1 R_2 + R_3 + R_3 R_3} \right] \] (10)

For any given value of \( R_3 \), equations 9 and 10 may be combined to yield

\[ V_{ab\text{ clamped}} = V_{ab\text{ open}} \left[ \frac{R_3 (R_3 + R_3)}{R_1 (R_1 + R_3) + R_3 R_3} \right] \] (11)

If \( R_3 \) is large as compared with \( R_2 \) and \( R_3 \), then the potential across the active membrane is approximately the same in the open and clamped configurations. It has already been suggested that the CP-generating membrane represents

but a small fraction of the transverse impedance of the column. From this it is expected that the potential across the active membrane is little altered by voltage-clamping the body wall and therefore, that spike production by the active membrane should not be greatly changed by the voltage-clamping procedure.

In voltage clamp experiments with squid axon, considerable attention has been given to the problem of reducing impedances which are external to the axon membrane. This is necessary if the potential across the membrane itself is to be adequately controlled (Cole and Moore, 1960). In hydra, the principal impedance in series with the active membrane is within the body wall and so it would be rather difficult to eliminate this series impedance and voltage clamp the active membrane itself.

The nearly independent relation between CP frequency and imposed current through the column is surprising in view of the usual sensitivity of pacemakers to imposed current (Bullock and Horridge, 1965, p. 159). The CP pacemakers are probably in the hypostome region of the animal (Passano and McCullough, 1964), but their exact location is not known. It has been suggested that rhythmic activity in coelenterates generally originates from spontaneously active neurons (Passano, 1963). The nerve cells of hydra are thin and parallel to the body surface. Transverse current through the body wall would be perpendicular to the axes of the nerve fibers and so their membrane...
potentials would not be changed appreciably. This might account for the inability to alter CP activity with imposed currents. On the other hand, the possibility that pacemaker activity is a property of the CP-generating membrane itself cannot be ruled out, since, as has been indicated, the CP-generating membrane may be only slightly depolarized or hyperpolarized by current which significantly changes the over-all transepithelial potential.

The results of this study do not allow any definitive statements to be made about the relation between structural and electrical impedance elements in the body wall, but some reasonable hypotheses can be presented based on these results and those of other workers. The column is essentially two cells in thickness. Septate desmosomes occur between adjacent epithelial cells near the inner and outer surfaces of the column (Wood, 1959; L. Davis, personal communication). Wood suggests that these desmosomes form permeability barriers between the enteron and the intercellular spaces of the column and between the intercellular spaces and the external medium. The mean dc resistance of the body wall of 5 kohms-cm² (Table I) is within the range found for continuous cell membranes (100 ohms-cm² to 25 kohms-cm², Davson, 1964, p. 681). This indicates that there are not any significant continuous channels between the enteron and the external medium and supports Wood's suggestion that the desmosomes are permeability barriers. In fact, because of microscopic irregularities in the surface of the column, the measured column areas are probably too small and the computed area specific resistance of 5 kohms-cm² is an underestimate.

If indeed the septate desmosomes are areas of very high transverse resistance, then there are probably four major impedance elements in series between the enteron and the external medium: (a) the endodermal cell membranes which form the enteron surface; (b) the membranes of endodermal cells on the mesogleal side of the endodermal desmosomes; (c) the ectodermal cell membranes on the mesogleal side of the ectodermal desmosomes; and (d) the membranes on the external surface of ectodermal cells. The impedance contributions of mesoglea and mucous coats may or may not be significant.

Osmotic experiments and chemical analysis indicate that the cells of hydra are hyperosmotic to the medium and have tissue concentrations estimated at 40–80 milliosmols even when the external medium is less than 1 millimolar salt (Lilly, 1955; Steinbach, 1963; Koblick and Yu-Tu, 1967). This suggests that the external cell surfaces of the column are not very permeable to salt ions and that the outer surface membrane of the ectodermal cells is the most significant contributor to the transverse electrical impedance. Ion-accumulating mechanisms in this membrane layer are likely candidates for the genesis of the column resting potential (Macklin, 1967).

CP's are associated with and are probably causally related to contraction of the longitudinal musculature of the ectoderm (Josephson, 1967). Thus, the CP-generating membrane is almost certainly ectodermal. The inner mem-
branes of the ectodermal epitheliomuscular cells, which surround the contractile elements, seem the most probable candidate for the site of CP production. The inner portion of these cells is extremely irregular in shape with long contractile processes (Lentz, 1966; Haynes, Burnett, and Davis, 1968). Thus, the inner membrane of these cells is large in area, possibly explaining the small contribution of the CP-generating membrane to the total column impedance.

The experiments described above were begun to characterize the mechanisms of electrogenesis in the hydra column, especially the mechanisms resulting in the production of the large, spontaneous voltage pulses, the CP's. The principal result of the study is the hypothesis that the CP-generating membrane forms only a small part of the transverse impedance of the column. Unfortunately, this means that the CP-generating membrane cannot be adequately studied by measuring potentials across or passing current through the body wall as a whole, since the presence of large series impedance elements masks the properties of the CP-generating membrane itself.

The mechanism by which CP's are propagated along the column is not known. It is possible that epithelial cells are triggered to produce electrical spikes by a wave of activity conducted in the nerve net of the column. However, conduction in nerve-free epithelia has been demonstrated in hydrozoans (Mackie, 1965) so it seems likely that the CP conduction is due to properties of the epithelial cells themselves. Septate desmosomes have been implicated as pathways of low electrical resistance between adjacent cells (Loewenstein and Kanno, 1964). If CP conduction is a feature of the epithelial cells, then it is likely that the CP-generating membrane is electrically excitable and that propagation of the CP's is due to electrical current flow between adjacent cells through the septate desmosomes joining them. This suggests that a large electrical impedance transversely in series with and essentially insulating the CP-generating membrane is a necessary adaptive feature of the animals, for otherwise a hydra could not open its mouth without depolarizing the column which would trigger CP's and column contraction.

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Electrical Properties of Hydra Body Wall


