Electrical Transmission at the Nexus between Smooth Muscle Cells

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ABSTRACT The hypothesis that nexuses between cells are responsible for the core conductor properties of tissues was tested using smooth muscle preparations from the taenia coli of guinea pigs. Action potentials recorded from small diameter preparations across a sucrose gap change from monophasic to diphasic when a shunt resistor is connected across the gap. This indicates that transmission between smooth muscle cells is electrical, because the resistor only allows current to flow. Nexal fusion of cell membranes occurs especially where one cell sends a large bulbous projection into a neighbor. Hypertonic solutions rupture the nexuses between smooth muscle cells. Hypertonicity also increases the resistance of a bundle across the sucrose gap and blocks propagation of action potentials. Thus the structural and functional changes in smooth muscle due to hypertonicity correlate with the hypothesis.

There is controversy as to whether or not action potentials propagate electrically between smooth muscle cells (Prosser and Sperelakis, 1956; Barr, 1961; Barr, 1963; Dewey, 1965; Sperelakis and Tarr, 1965). The possibility of obtaining direct evidence concerning electrical transmission between cells is provided by the sucrose gap technique (Stämpfli, 1954). The portion of a preparation in the gap exposed to the superfluent sucrose solution does not exhibit an action potential and the high resistance of the sucrose solution in the gap blocks local circuit currents and hence propagation. The experimental question is whether a resistor connected between the end compartments to give an "artificial extracellular space" will allow enough current flow for excitation to be transmitted past the inactive region in the sucrose gap. If such tissue-generated current were sufficient for propagation, it would be strong evidence in favor of the hypothesis of electrical transmission between cells.

In this regard it is critical to define the morphological link which allows current flow between cell interiors. Recent electron microscopic studies (Dewey and Barr, 1962; Dewey and Barr, 1964; Oosaki and Ishii, 1964; Evans and Evans, 1964; Taxi, 1965) show regions of fusion between the membranes

of neighboring smooth muscle cells. These nexuses have often been invoked as sites involved with electrical transmission between smooth muscle cells.

For discussion purposes we will follow the usage that propagation means spread of action potentials through a tissue without regard to mechanism. Conduction refers to propagation by excitatory local circuits along a continuous plasma membrane as along a single cell or syncytium. Transmission refers to propagation or at least the spread of excitatory or inhibitory influences between cells without regard to mechanism. The working hypothesis used in this paper is that nexuses are sites of connection between cell interiors such that local circuits involving the plasma membranes of two cells are possible. This is taken as the basis for electrical transmission between cells. Currently there are only a few data (Nagai and Prosser, 1963) to indicate whether there is an appreciable time required for conduction along individual cells or that propagation is saltatory. Although many investigators since Bozler (1941, 1948) have assumed that either conduction or electrical transmission occurs, there have been others who have considered chemical transmission between smooth muscle cells more likely. We believe that the present data make the latter view untenable.

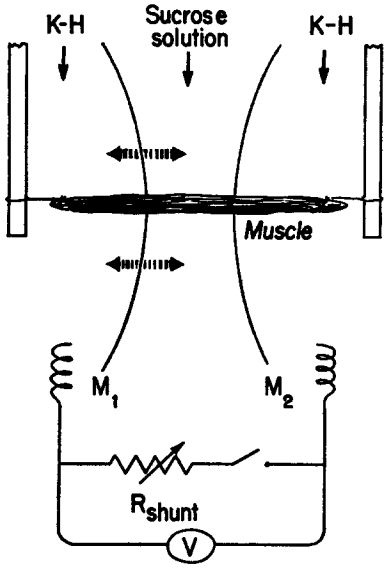


FIGURE 1. A schematic diagram of sucrose gap. Superfluent sucrose solution washes away extracellular ions in the gap region between the rubber membranes M_1 and M_2.

METHODS

Electrical Recording

SUCROSE GAP RECORDING Preparations of taenia coli of the guinea pig about 200 μ in diameter and 1 cm long were used. The electrical and mechanical recordings were essentially as previously described (Barr et al., 1965). See Fig. 1. The physiological saline was Krebs-Henseleit (K-H) solution (Krebs and Henseleit, 1932).

EXTRACELLULAR RECORDING IN OIL Strips of taenia coli of guinea pig were dissected free of the underlying circular layer. Strips (approximately 2 cm in length and 0.5 mm in diameter) were mounted in Plexiglas chambers and the mechanical activity sensed with a Grass FT03 tension transducer. The electrodes were either fine platinum wires or cotton wicks. The wicks worked better. Both electrical and mechanical activity were recorded at 22° to 25°C on a Grass polygraph. The solutions used were either K-H solution or modifications of it as noted.

Electron Microscopy

1% sodium permanganate (MnO_4^-) fixatives were used (Dewey and Barr, 1964; Barr et al., 1965). The osmolarities were determined on an Advance osmometer and varied by addition of solid sucrose or NaCl to match the experimental situation. All specimens of taenia coli were dehydrated through a graded series of ethanols and embedded in Epon 812. Sections were obtained on a LKB Ultrotome and mounted on carbon-coated Formvar films. Some sections were stained in uranyl acetate and lead hydroxide. An Hitachi HU-11A electron microscope was used. Tissues were incubated at 37°C in K-H solution for 1 hr. They were then transferred to K-H solutions to which were added either nothing or various amounts of sucrose, NaCl, glycerol, or urea as noted.

RESULTS

Electrical Transmission

Action potentials of taenia coli recorded across the sucrose gap were considerably smaller than those obtainable with microelectrodes. The largest action potentials recorded were about 42 mv, less than two-thirds of those obtained with microelectrodes. This deficiency did not seem to be relieved by making bundles of smaller diameter. It was probably not a matter of inadequate washing out of ions from the extracellular space of the segment of bundle in the sucrose gap. The increased duration of the recorded action potentials indicates asynchrony was involved.

At room temperatures preparations were often spontaneously active on only one side of the sucrose gap. Therefore, since the action potentials stopped at the sucrose solution they appeared monophasic. When a relatively large resistance was connected as a shunt across the recording electrodes, the size of the action potentials was reduced. However, their form was not noticeably changed. When the shunting resistance was decreased the recorded action potentials became biphasic in an all-or-none fashion. Since the excitability of smooth muscle cells varies in time, it is not surprising to find that some action potentials of a series were monophasic while others were biphasic when a given resistance was put across the gap.

Records from this type of experiment are shown in Fig. 2. Results shown in the upper record are from an experiment with no shunt resistor across the gap.

Only monophasic action potentials are recorded (upper trace). Tensions from the end segments are recorded on the middle and lower traces and contractions appear on only one side. The end segments are mechanically isolated from each other, presumably by the rubber membranes. The lower record of Fig. 2 is from an experiment in which a 30 kΩ shunting resistor was connected across the sucrose gap. Since the shunt resistor reduces the magnitude of the observed action potential, greater amplification must be used. This increases the apparent noise level. The first action potential of each series is biphasic

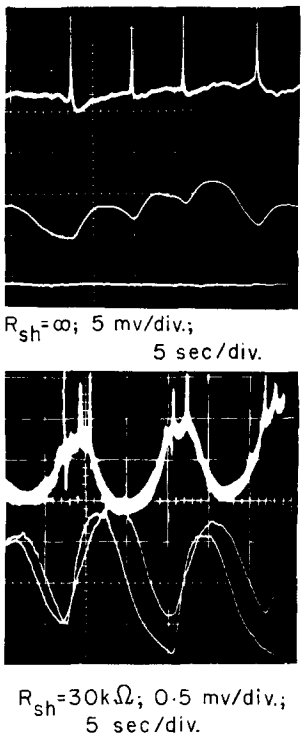


FIGURE 2. Sucrose gap recordings of electrical transmission of spontaneous electrical activity in the presence of a shunting resistor across the sucrose gap. Upper record. In a control experiment four monophasic action potentials (upper trace) trigger contractions (middle trace) on one side only. There is no tension developed by the segment on the other side of the gap (lower trace). Lower record. When a shunting resistor is connected across the gap, the upper trace, at necessarily higher gain due to the shunt effect, shows diphasic propagating action potentials. The two lower traces show synchronous mechanical activity. Note that the shunting resistor also changes the pattern of spontaneous discharge.

(upper trace). This is accompanied almost simultaneously by contractions of the segments on either side of the gap (middle and lower traces). The recorded voltage appears to fluctuate in a nearly sinusoidal mode. This may be a mechanical artifact due to changes in the interfaces of the solutions. However, it is possible that greater synchrony of the pacemaker potentials than of the action potentials could account for this.

To avoid misinterpretations of the experiment due to rhythmic activity, a second kind of experiment was performed. Many preparations were inactive on both sides of the gap. This is due partly to the relatively low temperature (22° to 25°C) and partly to the smallness of the preparations (100–200 μ). Though inactive, they were excitable and responsive to acetylcholine, carba-

chol, or KCl solutions injected into the superfluent K-H solution. Larger preparations were almost always spontaneously active. In the upper record of Fig. 3 the upper trace shows the sucrose gap voltage when 10^{-7} w/v acetylcholine was injected into the flowing K-H solution. Monophasic action potentials accompany the rising phase of the transient depolarizations. The lower trace shows that the tension of the segment not exposed to acetylcholine remains constant. The lower record shows the result of repeating the experiment with a 5 kΩ resistor connected across the sucrose gap. The second action potential evoked by the acetylcholine is biphasic and the segment of the muscle not exposed to acetylcholine contracts.

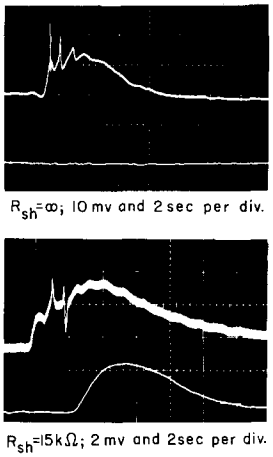
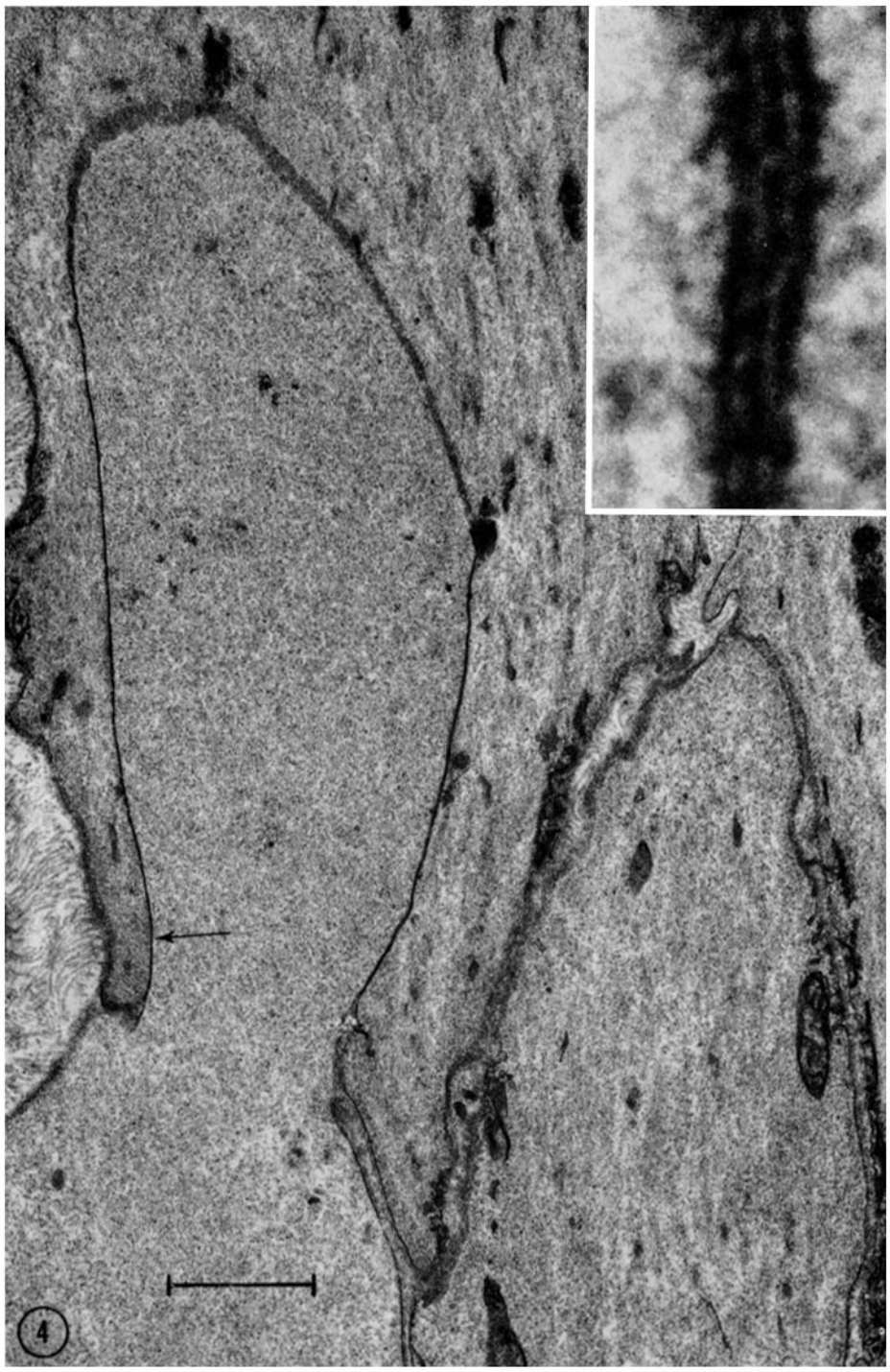


FIGURE 3. Sucrose gap recording as in Fig. 2 of electrical transmission of action potentials elicited by acetylcholine. Upper record. In a control experiment Ach was added to the superfluent K-H solution causing a transient depolarization and monophasic action potentials (upper trace); no tension is developed by the segment of the muscle unstimulated by acetylcholine (lower trace). Lower record. However, when a shunt resistor is connected across the gap, a diphasic action potential appears (upper trace) and a twitch-like tension response is elicited from the segment of the muscle unstimulated by acetylcholine (lower trace).

Thus both action potentials occurring spontaneously and those evoked by the transient presence of acetylcholine propagate past the inactive region of muscle in a sucrose gap when a resistor is connected across the gap. Moreover, the transmitted action potentials seem to spread over the entire segment. This is indicated by the full-sized contraction triggered by the transmitted electrical activity. This means that the interiors of the cells must be connected by pathways of low enough resistance to allow electrical transmission between cells in vivo with a large safety factor.

Morphologic Intercellular Relationships

Smooth muscle cells of the taenia coli of guinea pig are long tapering spindles about 150–250 μ long and 4–7 μ wide. The cells are joined together by nexuses (Dewey and Barr, 1962). The actual areas of membranes involved in the fusion at nexuses can be very large, especially where one cell sends a bulbous projection into a neighboring cell (Figs. 4 and 5). This is the usual site of nexal fusion in taenia coli although simple plaque-like nexuses are sometimes seen. The larger bulbous projections are usually observed at places where cells are con-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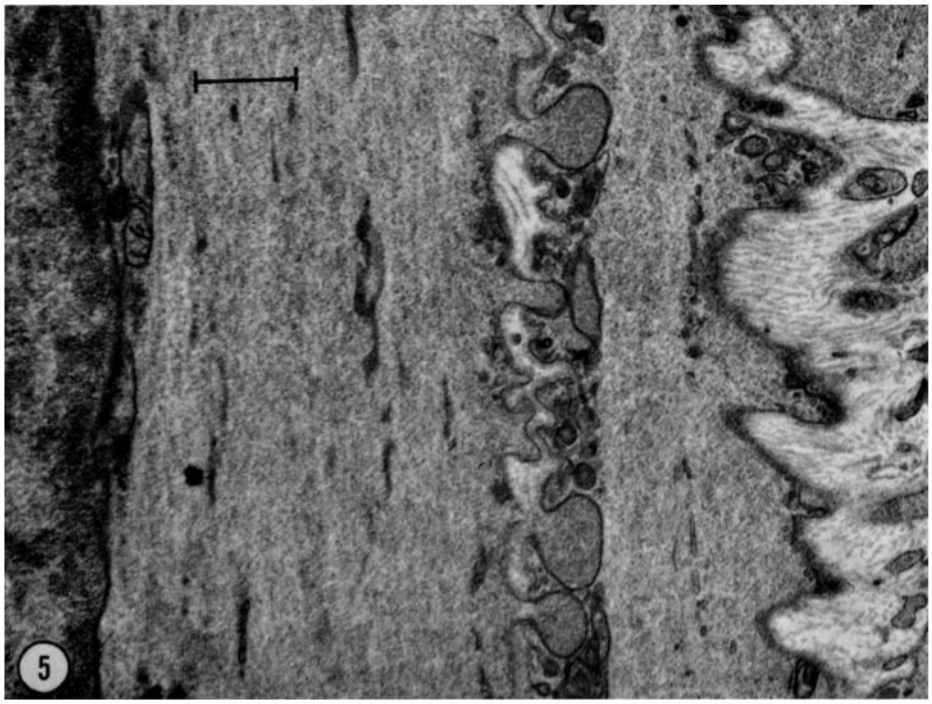


FIGURE 5. Numbers of small nexuses occur between the midregion of one cell and an adjacent cell. The size of nexuses seen in this smooth muscle ranges between those illustrated here and in Fig. 4. The length of the calibration line indicates 0.5 μ. Same specimen as in Fig. 4. × 27,000.

nected end-to-end (Fig. 4). There are smaller but more numerous ones where cells are connected side-to-side (Fig. 5). Unfortunately, because of problems of fixation and lack of controls the in vivo number, extent, and geometry of nexuses are unknown.

The interconnecting cells are organized into bundles (or fascicles) usually smaller than 100 μ. Many collagen but no elastic fibers are present in the extracellular space of these bundles. Within these bundles there are only muscle cells, a few small axons, and their accompanying Schwann cells. The

FIGURE 4 (opposite). In taenia coli large bulbous projections from one cell into another characteristically occur near the ends of cells. As shown here the entire surface between adjacent plasma membranes appears to be involved in a nexal fusion (inset). The preparation was incubated for 2 hr in K-H solution. The length of the calibration line indicates 1 μ. × 20,000.

The inset at a higher magnification, shows the region indicated by the arrow. The nexus consists of three equivalent dark lines and two lighter regions. The thickness of the nexus is 145 A. This is less than the total thickness of two membranes juxtaposed. × 680,000.

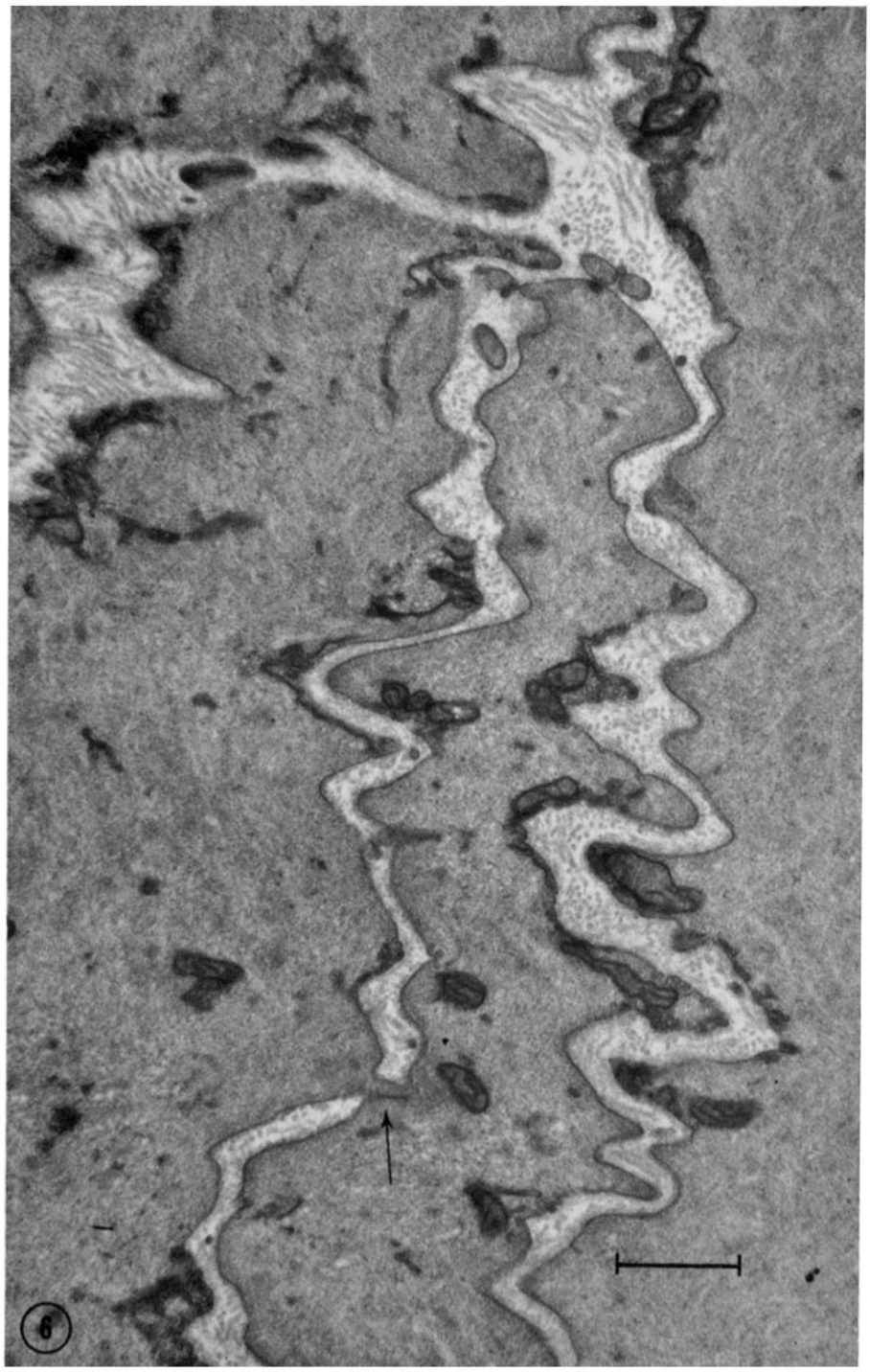


FIGURE 6. This electron micrograph illustrates the effect of incubating taenia coli for 1 hr in K-H solution made hypertonic (630 milliosmolar) by addition of sucrose. Nexuses do not occur in such preparations. Occasional small remaining projections (arrow) may indicate the position of a nexus prior to hypertonic treatment. The length of the calibration line indicates 0.5 μ. × 34,000.

bundles themselves are interconnected by branches across intervening regions of fibroelastic connective tissue.

Structure of the Nexus

A range of structures similar to the nexus has been described but it is not known whether or not these structures represent the same in vivo structure. (For discussion see Martin and Veale, 1967; Rosenblueth, 1965; and Coggeshall, 1965.) In order to define the nexus operationally, let it be the structure underlying the electron microscopic image of three dark lines of uniform thickness separated by similar light lines which result from the fusion of the outer surfaces of cellular membranes, as seen in permanganate-fixed specimens. The nexal image in taenia coli is 125–150 A in width (Figs. 4 and 6). The image of an individual cell membrane is 75–90 A thick. In any given electron micrograph the difference between the thickness of the nexal region and the thicknesses of the constituent membranes is always the thickness of one dark line. If there were no loss of thickness the membranes could be simply abutting and such a structure might not be functionally equivalent to the nexus at all.

Muscles contract strongly when fixed (Dewey and Barr, 1964; Winegrad, 1965). They shorten if unrestrained. If held isometrically a major portion of the tension is due to the true contractile process but with an unknown component due to syneresis or some other similar denaturing process. It was found that tying strips of taenia coli to glass rods before incubating and fixing resulted in more nexuses being present in the specimens. Incubation after the trauma of dissection is necessary for the tissues to recover their normal electrical and mechanical function. It is not known how these empirical findings are related. Nonetheless they emphasize the lability of the nexus. Nexuses were observed in taenia coli and other smooth muscle preparations fixed in permanganate but not in those fixed by OsO_4 or by gluteraldehyde followed by OsO_4 despite the fact that the same techniques preserve the nexus in other tissues (Farquhar and Palade, 1963; Dewey and Barr, 1964). Two observations are pertinent to this peculiarity of smooth muscle. First, smooth muscle plasma membranes fixed by OsO_4 appear asymmetric. Second, there are no desmosomes or other structures to protect the nexuses mechanically.

The Effect of Hypertonic Solutions on the Nexus

Earlier studies on cardiac muscles (Barr et al., 1965) indicated that nexuses are unstable in hypertonic solutions. The same is true for the nexuses of taenia coli. They separate leaving the images of the plasma membranes in electron micrographs unaltered. The specimens illustrated here were incubated for 1 hr in K-H solution to allow recovery from the dissection and then further incubated in either hypertonic sucrose solutions, K-H solutions made hyper-

tonic by addition of sucrose (Fig. 6), or K-H solutions made hypertonic by addition of NaCl (Fig. 8). The tonicities of the fixative solutions were not so important but were routinely adjusted with NaCl to match the osmolarities of the experimental incubation solutions. The results of all three treatments are similar. Total osmolarities up to 960 milliosmolar were used. Treatment with solutions of more than twice normal tonicity results in the appearance of large

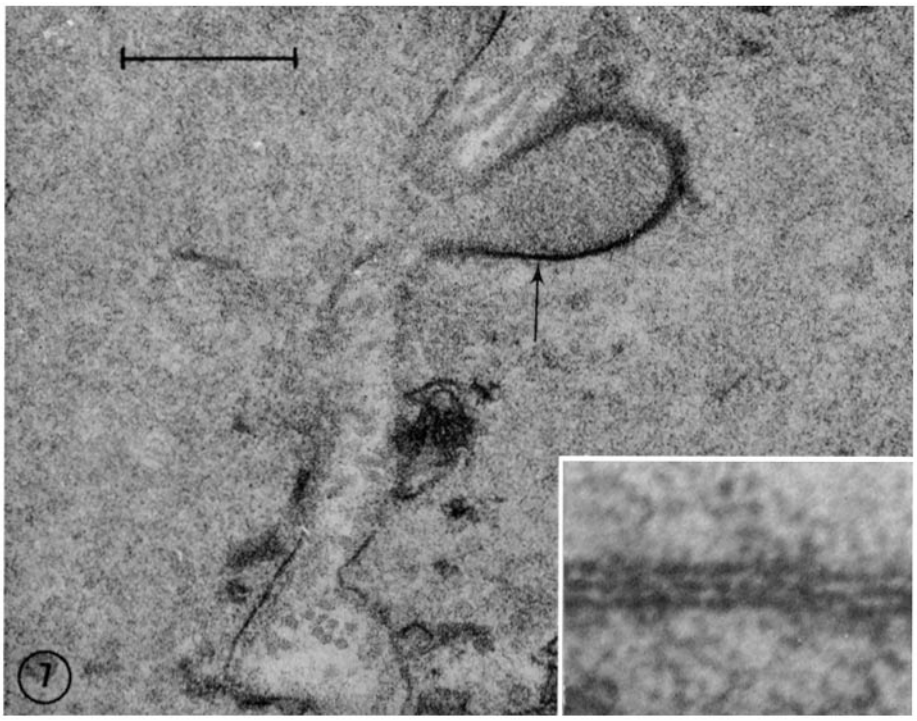


FIGURE 7. This electron micrograph illustrates a nexus from a specimen treated in the same way as in Fig. 6 except that in addition it was allowed to recover for 1 hr in normal K-H solution. Specimens like this look the same as those never exposed to hypertonic solutions. The length of the calibration line indicates 0.5 μ. × 46,000.

The inset, at a higher magnification, shows the region indicated by the arrow. The nexus is 150 A thick. × 400,000.

intercellular spaces and shrinkage of the cells. The nexuses all seem to be destroyed by such treatment. However, the interiors of large specimens were not thoroughly examined because the quality of fixation is poor there and the electrophysiological studies were performed on small preparations.

Recovery from the Effects of Hypertonicity

The effects of hypertonicity can be reversed completely by reincubating the preparations in normal K-H solution following their exposure to the hyper-

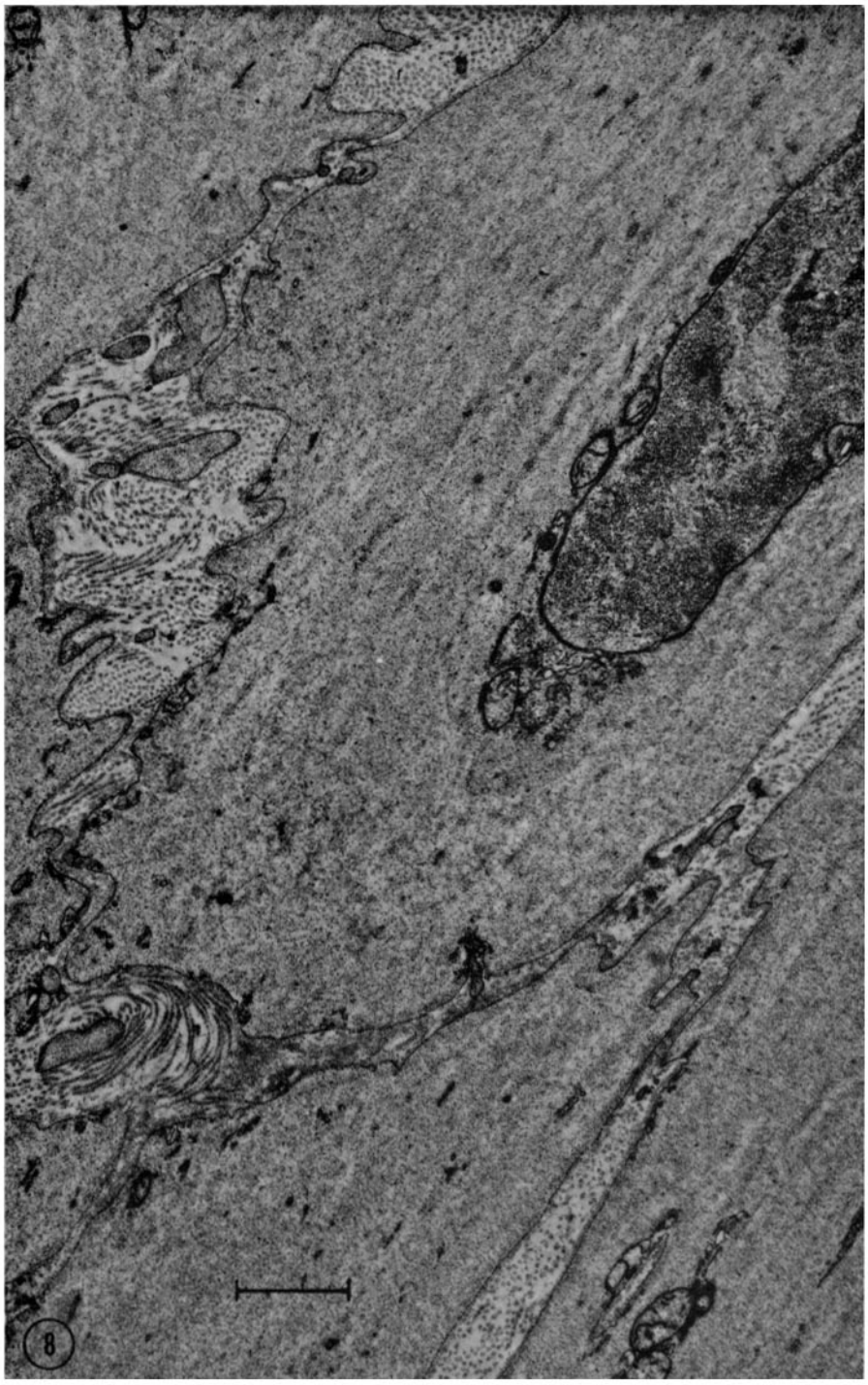
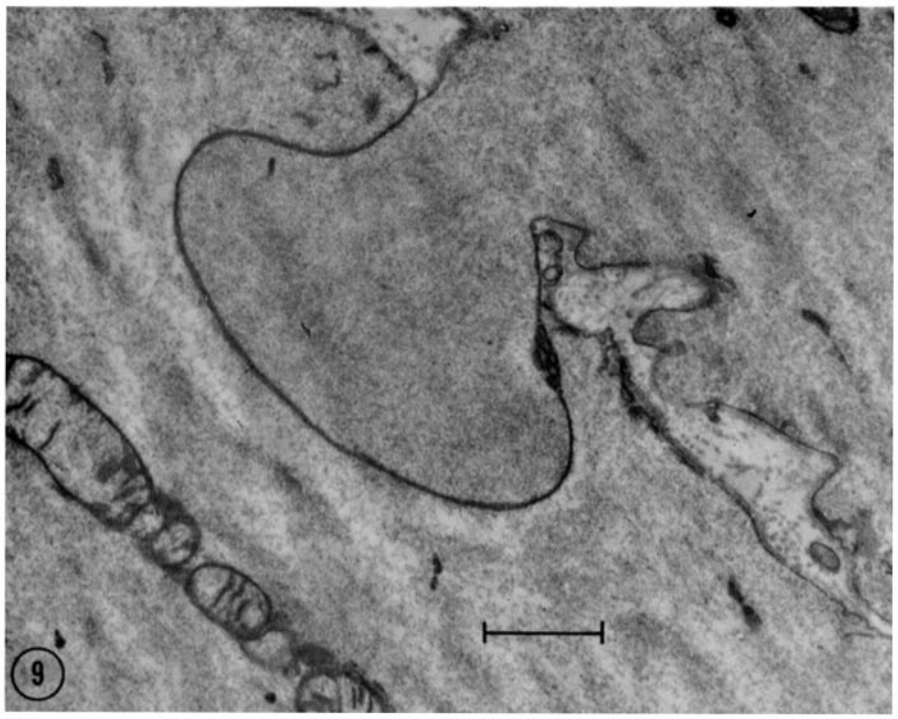


FIGURE 8. The incubation of preparations of taenia coli for 1 hr in K-H solution made hypertonic (630 milliosmolar) by addition of NaCl also ruptures the nexuses. There are, however, many residual projections as is illustrated by this electron micrograph. The length of the calibration line indicates 0.5 μ. $\times$ 31,000.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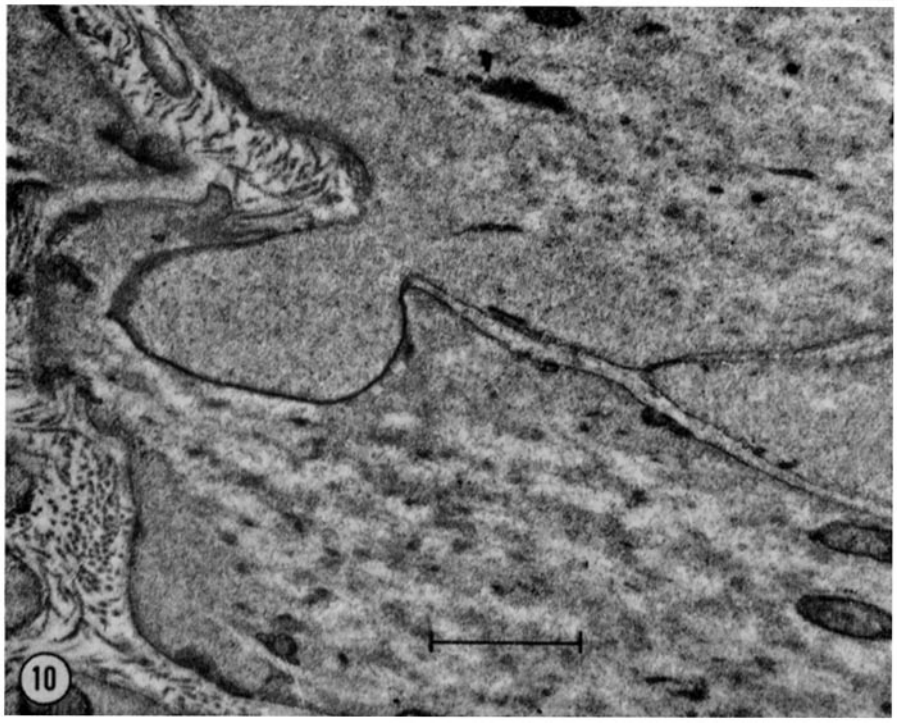
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tonic solutions. Fixation of preparations following the incubation regimes (*a*) 1 hr each in K-H, K-H plus sucrose, K-H (Fig. 7); or (*b*) 1 hr each in K-H, K-H plus NaCl, K-H (Fig. 9), results in specimens which are morphologically similar to control specimens.

Lack of Effect of Solutions Made Hyperosmotic with Urea or Glycerol

Since the ionic strength of the above solutions varied considerably and independently of the occurrence of nexuses in the specimens, it would seem that ionic strength is not very important for the stability of the nexus. Evidence which bears on the question of whether it is activity of the solvent water or the shrinkage of the cells which ruptures the nexus was obtained by incubating preparations in solutions made hyperosmotic by the addition of urea or glycerol. Short-term effects were quite variable but gave support to the idea that the nexuses were transiently broken and then reformed. Incubations in these solutions for longer than 15 min leave nexuses between muscle cells intact (Fig. 10). This is true for solutions made up to 960 milliosmolar by adding permeant urea or glycerol to K-H solution.

Increased Longitudinal Resistance Due to Hypertonicity

If the nexuses between cells are the connecting structures which conduct current between cell interiors, then the breaking of the nexuses by hypertonic solutions should cause an increase in the longitudinal resistance. The resistance to current flow through the sucrose gap, transgap resistance (R_g), was measured because it is largely a function of the longitudinal resistance of the muscle segment in the gap. The current used in different experiments varied from 1 to 5 namp. A rather wide gap, 9 mm, was used in these experiments to minimize the importance of other resistances; for example, the effective membrane resistance of the end segments.

The data from eight experiments are tabulated in Table I. Switching from isotonic to hypertonic sucrose solution in the gap causes an increase in the transgap resistance and a decrease in the action potential observable across the gap. These functional changes caused by hypertonicity are at least partially reversible.

FIGURE 9. This electron micrograph illustrates a bulbous nexus from a specimen treated in the same way as in Fig. 8 except that in addition it was allowed to recover for 1 hr in normal K-H solution. This tissue is not distinguishable from those not exposed to hypertonic solutions. The length of the calibration line indicates 1 μ. × 16,000.

FIGURE 10. As is illustrated in this electron micrograph, hypertonicity not hyperosmolarity is responsible for rupturing the nexuses between muscle cells. This preparation was incubated for 1 hr in K-H solution made hyperosmotic (630 milliosmolar) by the addition of glycerol. The nexuses have a normal conformity. The length of the calibration line indicates 1 μ. × 20,000.

TABLE I

EFFECTS OF HYPERTONIC SUCROSE ON GAP RESISTANCE AND OBSERVED ACTION POTENTIAL AMPLITUDE

Date	R_g (iso)	Hypertonic			Recovery		
		$\frac{R_g \text{ (hyp)}}{R_g \text{ (iso)}}$	$\frac{AP_{obs} \text{ (iso)}}{AP_{obs} \text{ (hyp)}}$	$\frac{\text{"}R_i\text{"(hyp)}}{\text{"}R_i\text{"(iso)}}$	$\frac{R_g \text{ (iso recov)}}{R_g \text{ (iso)}}$	$\frac{AP \text{ (iso)}}{AP \text{ (iso rec)}}$	$\frac{\text{"}R_i\text{" (hyp)}}{\text{" }R_i\text{" (iso)}}$
	MΩ						
19-12-66	3.3	1.6	20	32	1.4	0.7	1.0
20-12-66	0.43	$1.2\bar{3}$*	36	44	—	—	—
4-1-67	0.8	1.7	$9.\bar{4}$	16	1.1	1.1	1.2
5-1-67	1.0	1.8	$18.\bar{3}$	33	1.2	$0.7\bar{5}$	0.9
18-1-67	5.8	1.17	$5.\bar{6}$	6.6	1.1	1.0	1.1
19-1-67	4.0	2.0	5.0	10	1.5	—	—
20-1-67	7.5	$1.4\bar{3}$	$1.7\bar{5}$	2.5	$1.1\bar{6}$	$0.9\bar{5}$	1.1
14-3-67	9.3	$1.1\bar{1}$	$5.\bar{0}$	5.6	$1.0\bar{2}$	$1.1\bar{1}$	1.1

* Bar over numeral indicates estimation of the last significant figure.

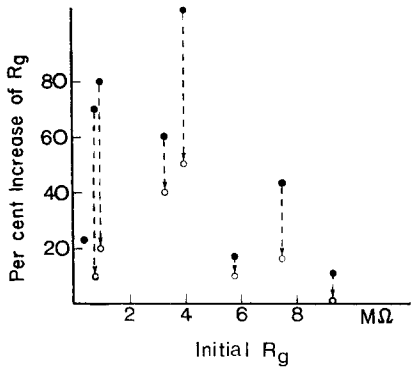

FIGURE 11. The increase in transgap resistance (R_g) when hypertonic sucrose replaces isotonic sucrose, is plotted (filled circles) against the initial transgap resistance in isotonic sucrose. The change in resistance is less as the initial resistance is greater. The correlation coefficient (Pierson product moment) for this relationship is −0.58. If the 0.43 MΩ initial R_g point is deleted the correlation coefficient is −0.74. The persistent increase in transgap resistance is plotted (open circles) against the initial transgap resistance when isotonic sucrose is used again in the gap. In all cases measured the transgap resistance returned toward normal values indicating some degree of recovery.

The transgap resistance increases more when the initial values are lower (Fig. 11). This is reasonable since the transgap resistance (R_g) comprises the longitudinal resistance (R_i) and the extracellular resistance (R_{suc}) in parallel:

$$1/R_g = 1/R_i + 1/R_{suc}. \quad (1)$$

Transgap resistance is more nearly proportional to longitudinal resistance, as longitudinal resistance is progressively smaller than the extracellular resist-

ance across the gap. Therefore, changes in the longitudinal resistance would be more strongly reflected in the transgap resistance.

The attenuation of the observed action potentials caused by switching to a hypertonic sucrose solution is much greater for lower initial transgap resistances than for the higher ones (Fig. 12). The opposite is predicted from Equation (2) which describes the simple equivalent circuit for the sucrose gap previously used to analyze results from similar experiments on frog atrial

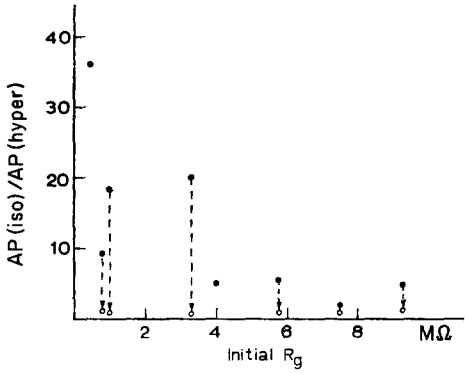

FIGURE 12. The ratios by which the amplitudes of the observed transgap action potentials are attenuated due to hypertonic sucrose (960 milliosmolar) in the gap are plotted (filled circles) against the original transgap resistance. The effect is less as the original transgap resistance is less. The correlation coefficient (Pierson product moment) for this relationship is -0.44. If the 0.43 MΩ initial R_g point is deleted the correlation coefficient is -0.66. The ratios of the amplitudes of the observed action potentials when isotonic sucrose is in the gap before and after using hypertonic sucrose are plotted (open circles) against the initial resistances. In all cases the ratios came back to near one, indicating recovery.

bundles (Barr et al., 1965). Thus it can be seen from

$$AP_{obs} = AP_{true} \times R_{suc}/(R_{suc} + R_i) \quad (2)$$

that as the internal resistance (R_i) is less, the observed action potential (AP_{obs}) is closer to the true one (AP_{true}). Percentage changes in the internal resistance should therefore be less important. Even though the simple formula derivable from Equations (1) and (2) (Barr et al., 1965)

$$R_i(\text{hyp})/R_i(\text{iso}) = [AP_{obs}\ (\text{iso})/AP_{obs}\ (\text{hyp})]\ [R_g\ (\text{hyp})/R_g\ (\text{iso})] \quad (3)$$

is, therefore, not accurate for the experiments described here, calculations from it probably set an upper limit to the percentage resistance change in hypertonic sucrose. It further provides a mixed measure of the ability of the tissue to recover from treatment with hypertonic solution. Comparison of the fifth

and last columns of Table I shows that recovery is quite complete as far as this parameter is concerned.

Propagation Block in Hypertonic Solutions

Bundles up to 500 μ in diameter were exposed to physiological solutions of various tonicities. Solutions made 640 milliosmolar or more by addition of sucrose or NaCl to K-H solution always blocked propagation (Figs. 13 and 14). Solutions made as much as 960 milliosmolar by adding permeant urea or glycerol to K-H solutions did not block propagation; indeed, these solutions

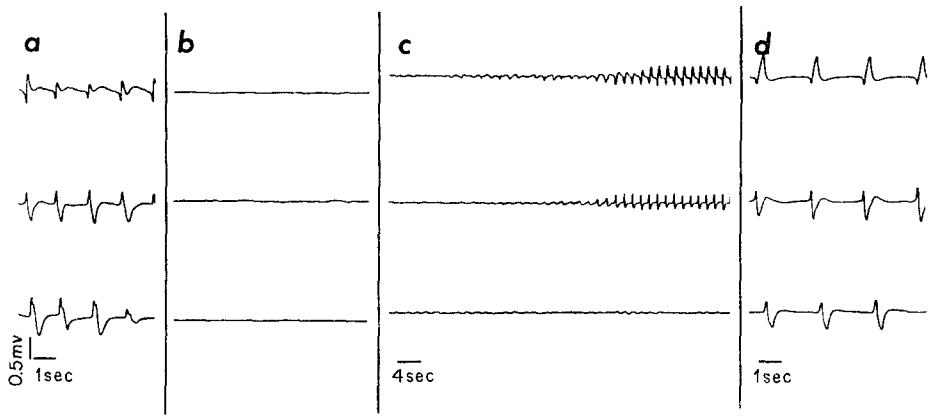

FIGURE 13. Electrical recordings from three pairs of external electrodes. (a) Control recording showing pacemaker between first and second pairs. (b) After 10 min of exposure to solution made hypertonic (640 milliosmolar) by addition of NaCl. (*c*) After 3 min recovery from (b) in a solution isotonic, but made 960 milliosmolar by addition of glycerol to K-H solution. Propagation between the second and third pairs of electrodes is still blocked. (d) After 2 min more in the recovery solution propagation is normal.

allowed recovery of bundles previously exposed to hypertonic solutions (Figs. 13 d and 14 e). Spontaneous activity localized to only part of the bundles was often seen as the bundles recovered (Fig. 13 c).

Gradation of the hypertonicity of the bathing solutions might be expected to gradually decrease the velocity of propagation. However, graded effects between solutions were seldom observed. In any given experiment the test solution either blocked or did not block in an all-or-none way. Nevertheless, graded effects in time were usually seen. Typically, propagation would slow before failing. It was also common to find that solutions 1.5 and 1.75 times normal tonicities caused a block between regions so that action potentials would fail to spread throughout the bundles. Finally asynchronous electrical activity was often seen. These phenomena are illustrated in Fig. 14 as stages in time after exposure to twice normal tonicity. When the bundles became in-

active in hypertonic solutions, propagated action potentials or contractions were not observed in response to electrical stimuli several times normal threshold values. Whether some cells were excited by the electrical stimuli was not determined but small contractions could be elicited with very large stimuli. However, if some cells were excited their activity did not spread.

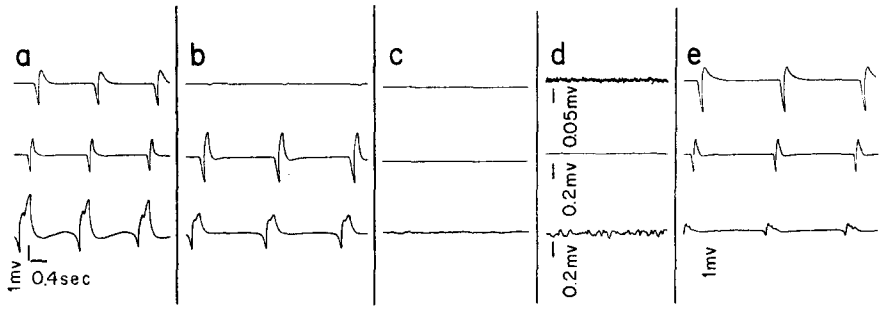

FIGURE 14. Electrical recordings from three pairs of external electrodes. (a) Control recording showing propagation between pairs at an average velocity of 2.5 cm/sec. (b) After 20 min exposure to solution made slightly hypertonic (477 milliosmolar) by addition of sucrose to K-H solution. Note block between the second and third electrode pairs. (c) After 5 min exposure to solution made 640 milliosmolar by addition of sucrose. (d) Same as (c) but at higher gains to show asynchronous activity in the region of former pacemaker (lower trace) and level of electrical noise (upper trace). (e) 15 min after replacement of solution used in (c) with solution made 640 milliosmolar by addition of urea.

DISCUSSION

The action potentials recorded with the sucrose gap are smaller than the true maximum difference in membrane potentials for at least two reasons. The first reason is inherent in all extracellular techniques and is due simply to the fact that the true difference in membrane potentials is divided across the resistance of the extracellular fluid between the electrodes (the sucrose gap resistance) and the effective total resistance of the tissue (Equation 2). Since the observation of voltage is made across the gap, the greater its resistance the better. However, the observed potentials were not made larger either by making smaller diameter bundles or by increasing the width of the gap. Both these should have increased the relative resistance of the sucrose gap because of better washing out of the extracellular space. Thus it would seem that this consideration does not provide the reason for values reported here being so low.

Another reason for observing only attenuated action potentials is the absence of synchronous excitation of all the muscle membrane in one K-H pool. That many cells in the boundary region between the sucrose and K-H solutions are probably inactive or have small action potentials is important in this

regard. The diffusion potential between the sucrose and the saline tends to hyperpolarize cells at the boundary and the external sodium ion concentration is graded to zero. Both these factors influence the observed voltage. Although these causes of difficulty have been minimized by rapid flow rates of solutions at the boundaries and the use of rubber membranes (Berger, 1963), they certainly have not yet been entirely eliminated. Recordings from other muscles do not seem to suffer from the same artifacts (Barr and Berger, 1964).

The observed electrical transmission past the sucrose gap, despite the above factors and the disadvantageous geometry, indicates that propagation occurs in this tissue with a large safety factor. In the transmission experiments the artificial extracellular current pathway (the resistor), the extra longitudinal resistance of the inactive region in the gap, and shunting effects of the abnormal state of the cell at the boundary, all tend to decrease the excitatory current far below the in vivo values.

Irrespective of its exact geometry in vivo the electrotonic spread of current ahead of the action potential is sufficient to allow electrical transmission between the cells of this smooth muscle. It is an electrical syncytium.

The experiments involving hypertonic solutions indicate that the electrical syncytial properties of smooth muscle depend on the existence of nexuses between the cells. The agency by which the nexuses were ruptured is not so important in this regard as the fact that when they were ruptured the cells were electrically separated and propagation was blocked. In smooth muscle there seems little chance that this is a secondary effect due to destruction of some other structure or trapped pockets of extracellular fluid between cells. The only structure between the cells seems to be the nexus with no evidence for trapped extracellular regions. The rupture of the nexuses seems to be strictly mechanical due to shrinkage of the cells. The chemical activity of the solvent water, the osmolarity, seems to be relatively unimportant to the stability of the nexuses.

The resistance of the internal pathway is the sum of the myoplasmic and nexal resistances. Their relative contributions vary from tissue to tissue and probably from one physiological state to another. Since nexuses separate similar solutions there can be only a small diffusion potential across them. Nexal membranes are exposed on both sides to solutions of low calcium and high potassium concentration. These factors probably contribute to their low specific resistance (Woodbury and Gordon, 1965; Weidmann, 1966;Loewenstein, 1966). In smooth muscle from the cat intestine Nagai and Prosser (1963) found low resistance but no rectification. However, Furshpan and Potter (1959) found a considerable rectification at the lateral giant to motor synapse in the crayfish.

The blocking of propagation and the decrease of excitability caused by hypertonic solutions reported here are contrary to Tomita's report (1966) that

excitability and propagation velocity were unchanged by twice normal tonicity. Tomita also quoted unpublished observations of Schoenberg as an indication that the occurrence of nexuses was not noticeably reduced in their preparations. He apparently used the whole taenia coli at 37°C while the results reported here were collected on smaller bundles at room temperatures. Since the rupture of nexuses seems to be mechanical, perhaps in the depths of a larger preparation they are not ruptured when the tissue shrinks. In one sense Tomita's report corroborates the present findings. The most important aspect of this experimental study is that when the nexuses are broken, propagation is blocked and this correlates with increased internal resistance. On that basis, since the nexuses were not broken by Tomita's procedure, one would expect to see what he observed; i.e., no change in propagation velocity. Our results should not be explained on the basis that cessation of spontaneous activity was due to hyperpolarizing effects of the hypertonic solutions. Propagation blockade almost always was demonstrable before the cessation of spontaneity. This occurred over a large range of times; i.e., up to half an hour. In this, the present data agree more with Holman's (1957) than with Tomita's (1966) findings.

In any case the present results confirm those of Johansen and Ljung (1967) who found disruption of synchrony of the ends of a portal vein preparation when a center segment was exposed to hypertonic solution. They are also in accordance with the findings of Melton (1956) that rat uterine smooth muscle became electrically inexcitable or showed only nonpropagating responses in the absence of adequate amounts of estrogen therapy to castrate rats and those of Bergman (1968) who found that castrate rat uterine smooth muscle cells have very few nexuses between them. The results reported here concerning smooth muscle are also in agreement with the similar studies on cardiac muscles which indicate that nexuses are broken by hypertonic solutions (Woodbury and Crill, 1961; Barr et al., 1965) and that this electrotonically decouples the cells (Woodbury and Crill, 1961; Barr et al., 1965) and causes decreased propagation velocity and blockade (Sperelakis et al., 1960; Barr and Evans, unpublished results).

In complementary studies on different cardiac muscles it was shown that cells were electrotonically decoupled when nexuses were broken but desmosomes were still intact (Barr et al., 1965) and cells remained electrotonically coupled when the desmosomes were destroyed but the nexuses were still intact (Dreifuss et al., 1966).

Nexuses also occur between the cells of the frog skin. Hypertonic solutions on the outside apparently break the side-to-side nexuses between the surface cells which seal the skin; the transskin resistance goes down. On the other hand hypertonic solutions on the inside break the series nexuses in the stratum

spinosum which increases the skin resistance. Both treatments decrease the short-circuit current (Ussing, 1966; Barr and Dewey, unpublished results).

The withdrawal of calcium from various epithelia breaks the cells apart (Sedar and Forte, 1964; Hays et al., 1965), makes the layer leaky (Hays et al., 1965), and electrotonically uncouples neighboring cells (Nakas et al., 1966). However, the desmosomes are also separated by this treatment and the nexuses may not separate at all but stay intact adhering to one of the separated cells and leaving a hole in the other (Muir, 1967). Calcium depletion has also been used to electrotonically decouple nerve cells of the leech but the effect on the junction is irreversible (Penn and Loewenstein, 1966).

In summary, the nexus serves as a connection or link between the interiors of cells in smooth and cardiac muscle and probably in many other tissues. The cells remain as discrete protoplasmic masses surrounded by plasma membranes but the nexuses allow the tissue to function as an electrical syncytium. How permeable nexal membranes are to larger chemical entities remains to be seen.

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