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LOCALIZATION OF CATION INTERACTIONS IN THE SMOOTH MUSCLE OF THE GUINEA-PIG TAENIA COLI

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SUMMARY

1. An electron microscopic method has been developed and used to study cation binding sites in smooth muscle.

2. Uranyl cations normally bind to the external surface of the plasma membrane. However, uranyl also binds to the inner surface when it is accessible. Similar binding has been observed in skeletal muscle, nerve and red blood cells.

3. Uranyl binds electrostatically, and the binding can be competitively reversed by other cations. By a quantitative procedure the relative affinities $Ca^{2+} \simeq Mg^{2+} \gg K^+ > Na^+$ for the membrane sites have been determined. This sequence is in agreement with previous values determined analytically.

4. The results support a counter-cation hypothesis for the plasma membrane surfaces of the taenia coli, and may explain features of the electrical activity in smooth muscle.

INTRODUCTION

The study of ion contents and ion movements in smooth muscle has provided evidence that ions are not simply distributed between an extracellular and an intracellular space (Buck & Goodford, 1966; Somlyo & Somlyo, 1968). It has been postulated that a number of cellular regions or compartments may exist, one of which may be fixed anionic sites on the smooth muscle cell membrane (Goodford, 1970). Goodford (1966, 1967) and Sparrow (1969) have presented evidence that there is a competition between Mg^{2+}, Ca^{2+}, Na^+ and K^+ for superficial negative charges in the smooth muscle cells of taenia coli. Cation competition for similar superficial sites has also been described in cardiac and skeletal muscle (Wilbrandt & Koller, 1948; Niedergerke & Lüttgau, 1957; Schaechtelin, 1961).

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The exact location of the superficial anionic sites has not yet been established in the smooth muscle of guinea-pig taenia coli, and at least three structures could be involved; i.e. the extracellular collagen fibres, the mucopolysaccharide basement membrane, or the surface of the plasma membrane itself. The present study was designed to localize such sites electronmicroscopically, by interacting them with heavy metal (electron dense) cations.

A preliminary demonstration and communication has been made to the Physiological Society (Wolowyk, 1971; Goodford & Wolowyk, 1971).

METHODS

Solutions

The compositions of the solutions used are given in Table 1. The pH was maintained by equilibration with mixtures of oxygen and carbon dioxide, and was frequently monitored. In some experiments these solutions also contained heavy metal salts. Glutaraldehyde was supplied by TAAB as a 25 % solution.

Procedure

White male guinea-pigs weighing between 300 and 450 g were stunned and bled, and the abdomen was opened. Eight to ten pieces of taenia coli 20 mm long were dissected from each animal, and immersed in oxygenated solution A at room temperature. After tying a cotton thread and attaching a glass bead weighing 1·5 g to each muscle, they were suspended in solution A at 37° C and left for 30 min to equilibrate. In some experiments 2 × 20 mm segments of the guinea-pig psoas muscle were used as skeletal muscle samples.

All tissues were fixed for 4 hr in glutaraldehyde solution B at 4° C. In some experiments the initial temperature of the fixative solution was 37° C for 5 min before cooling to 4° C. After fixation the tissues were given five 15 min rinses with solution C; four at 4° C and a fifth rinse warmed to 20° C. At this point most experimental tissues were transferred to 0·5–2 % magnesium uranyl acetate, or 2 % uranyl acetate plus $NaHCO_3$, at pH 5·9–6·1 for 15 min. They were then rinsed with isosmotic sucrose (300 mM) solutions containing varying concentrations of Ca^{2+}, Mg^{2+}, Na^+ or K^+, dehydrated and embedded.

A 'sodium-free' variation on the above standard method was to transfer samples to solution D for 15 min at 37° C after the initial equilibration in solution A. Solutions B and C were then replaced by E and F for the subsequent fixation and rinsing procedures. Both normal and 'sodium-free' tissues were then treated with sodium or potassium precipitating agents instead of the uranyl solutions. 2 % solutions of potassium pyroantimonate or chloroplatinic acid were used, and the method of Zadunaisky (1966) was also applied.

All tissues were finally dehydrated with a graded 2–100 % ethanol-water series. During the dehydration stage the tissues were carefully trimmed and cut into 1 × 2 mm blocks. After dehydration these tissue blocks were infiltrated with propylene oxide and embedded in Araldite (CIBA Ltd). Thin sections were cut, collected on 400 mesh copper grids, and examined in a Philips 200 electron microscope.

TABLE 1. The composition of physiological saline and fixative solutions. The O_2/CO_2 ratio was adjusted to maintain the indicated pH

Soln.	Concentration (mM) of							% Glutaraldehyde	pH at		
	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	Glucose		37° C	20° C	4° C
A	137	5·96	1·19	2·38	135	15·5	11·9	—	7·4	—	—
B	137	5·96	1·19	2·38	142	7·75	11·9	2·5	7·1	—	6·6
C	137	5·96	1·19	2·38	142	7·75	11·9	—	—	6·8	6·5
D	—	143	1·19	2·38	135	15·5	11·9	—	7·4	—	—
E	—	143	1·19	2·38	142	7·75	11·9	2·5	—	—	6·6
F	—	143	1·19	2·38	142	7·75	11·9	—	—	6·8	6·5

RESULTS

Pl. 1*a* is an electron micrograph of the smooth muscle of guinea-pig taenia coli prepared by a conventional electron microscopic method which differs from the procedure described above. The difference is the additional inclusion of osmium tetroxide fixation before dehydration, and the further post-staining of the sections with uranyl acetate and lead citrate. The structural detail of the tissue in Pl. 1*a* is made visible by the electron density imparted by the heavy metals osmium, uranium and lead. However, for the present study the latter treatments were avoided in order to keep background electron density to a minimum. The smooth muscle cells then appear only as faint shapes, devoid of any ultrastructural detail (Pl. 1*b*), although nuclei can occasionally be distinguished.

Effect of solutions containing heavy metal cations

The object of this series of experiments was to try and make visible the superficial anionic regions in the smooth muscle by exposing them to heavy metal cations. Using tissues which had been fixed with glutaraldehyde only as controls, experimental tissues were prepared for electron microscopy by a similar method with the additional inclusion of heavy metal salts (uranyl acetate, magnesium uranyl acetate, $BaCl_2$, $LaCl_3$, CsCl, $InCl_3$ and $[Co(NH_3)_6]Cl_3$) at various stages of the preparative procedure. When any one of these salts was included in solution A, B or C no specific electron dense deposits were observed in the tissue, although irregular deposits of lanthanum, or precipitated crystals of the uranyl salts, were occasionally seen in the extracellular space. The results of later experiments show that the high concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} in solutions A, B and C would be sufficient to prevent UO_2^{2+} from staining the superficial anionic sites under these conditions.

When tissues were transferred to isosmotic solutions of the heavy metal salts for 15 min just before dehydration, no staining was seen except when 0·5–2% magnesium uranyl acetate or 2% uranyl acetate $+NaHCO_3$, at pH 5·9–6·1 were used. However, with these uranyl ions a specific electron dense staining of the cells was regularly observed.

Uranyl binding with smooth muscle cells

The uranyl cation was bound primarily to the membrane surface of smooth muscle cells (Pl. 2). This electron dense staining was distributed evenly over both the smooth and the vesiculated surfaces of the plasma membrane (Pl. 3*a*). Similar membrane-specific staining was observed in nerve, red blood cells and skeletal muscle treated with uranyl (Pl. 3*b*, *c* and *d*). In skeletal muscle the uranyl binding was extended into the cells

along the transverse tubular system (Pl. 3*d*). These results suggest that the uranyl binds to all the outer surfaces of the plasma membrane which are exposed to the bathing solution.

In the great majority of cases the uranyl staining was only seen on the outer cell surface, but very occasionally a cell was observed with electron dense stain inside the cell as well (Pl. 2). Uranyl has penetrated this cell and further interacted with other cellular structures such as the outer mitochondrial membrane. Pl. 4*a* is a higher magnification of such a cell showing uranyl staining at both the outer surface and the inner surface of the cell membrane. These surface layers of uranyl were approximately 2·5 nm thick, and were separated by a lighter gap some 3·0 nm wide. The rarity of such cells has led us to believe that this phenomenon represents a damaged cell, and not one of the 'dark' cells described by Gansler (1960, 1961) which usually occur in larger numbers in glutaraldehyde-osmium fixed smooth muscle.

Uranyl appears to have high affinity for membrane sites. However, in one smooth muscle sample, uranyl was observed not only at the cell membrane surfaces but also on the basement membrane and collagen fibres in the extracellular space (Pl. 4*b*). This binding was localized to a small area of this particular tissue sample and it must be emphasized that such an effect was only observed once in two years work. This result leads us to conclude that collagen and basement membrane material can bind uranyl, but that the affinity for the membrane sites is normally higher under our conditions.

Experimental conditions required for optimal uranyl binding

The membrane staining was dependent on the pH of the uranyl solution and the best results were obtained in the 5·9–6·1 pH range. The pH of a 1 or 2 % aqueous magnesium uranyl acetate solution falls within these limits and such solutions were therefore used for most experiments. Using a 1 % aqueous solution of uranyl acetate, which has a pH of 4·2, no membrane staining was seen unless the pH of the solution was increased to 5·9–6·1 by the addition of $NaHCO_3$. Lowering or raising the pH of magnesium uranyl acetate solutions below 5·9 or above 6·1 by the addition of acetic acid or magnesium acetate resulted in a less effective to complete loss of membrane staining.

The critical pH dependence for uranyl binding to the membrane may be related to uranyl complex formation. In aqueous solutions uranyl ions can form polynuclear complexes with the general formula UO_2 $((OH)_2UO_2)_n^{2+}$ (Ahrland, Hietanen & Sillen̄, 1954). Above pH 5 the value of n increases with increasing pH (Jain, Jain & Vaid, 1967), and at pH values above 6·5 this value becomes so large that the uranyl solutions begin to precipitate.

Such complex formation does not occur with the other heavy metal cations which were studied in the preliminary experiments, and this may explain why they could not be visualized at the cell membrane surfaces. However the actual pH dependence for uranyl binding may also be dependent on the pK of the membrane ligands with which the uranyl interacts, and the present method would not detect metal cations exchanging at anionic groups whose pK appreciably exceeds 6.

Glutaraldehyde fixation does not destroy cellular osmotic activity (Bone & Denton, 1971) and the tissues were therefore dehydrated slowly beginning with an isosmotic alcohol solution to minimize cell shrinkage. A 5 min exposure to fixative solution at 37° C before cold fixation was also useful in obtaining good structural preservation. However, these refinements were not necessary to obtain good uranyl staining, but simply improved the morphological appearance of the cells.

Effects of sodium and potassium precipitating agents

Since uranyl salts are used for the determination of sodium (Vogel, 1951), experiments were carried out to test the possibility that the membrane uranyl staining might in fact be due to precipitation of the uranyl salt with sodium associated with the cell membrane. When tissues were exposed to solutions of pyroantimonate or chloroplatinate (sodium and potassium precipitating agents) instead of uranyl, no membrane-specific staining was observed although generalized electron dense precipitates were seen in the extracellular and intracellular spaces. Furthermore, tissues treated with Na^+-free solutions D, E and F before, during and after fixation still bound uranyl at the membrane surface, even though they had not been exposed to Na^+ for the previous $5\frac{1}{2}$ hr.

Reversal of uranyl binding by other cations

Previous studies on counter-cation interactions in smooth muscle have shown that the cation binding to the superficial sites is competitive with respect to Na^+, K^+, Ca^{2+} and Mg^{2+} (Goodford, 1970). The present experiments have demonstrated the location of uranyl binding sites, but it is still necessary to establish if the uranyl ions do in fact bind electrostatically and competitively with the smooth muscle membrane surfaces.

Repeated rinsing of uranyl treated tissues with isosmotic sucrose solutions did not remove the uranyl from the plasma membrane. However, when such tissues were rinsed with an isosmotic sodium chloride solution before dehydration, no membrane staining was seen. This is compatible with the concept that the sodium ions compete with uranyl ions for the anionic sites, thereby driving the uranyl off the membrane. If this inter-

pretation be correct it should be possible to obtain different degrees of membrane staining by varying the concentration of cation in the rinse, and the next experiments showed that this was indeed the case.

The quantitative estimation of membrane staining

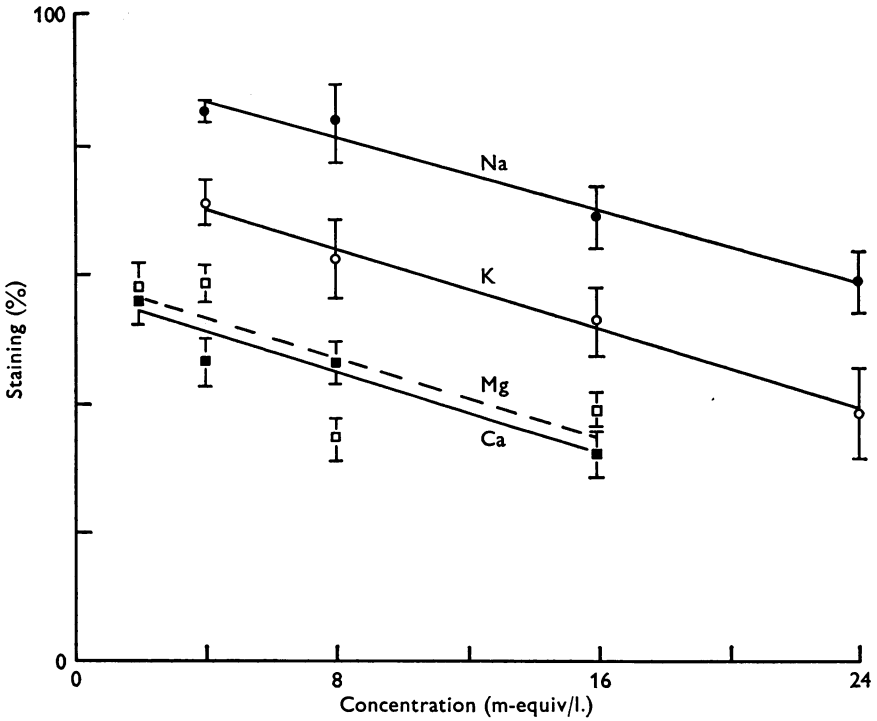
Attempts have been made to quantitate the cation competition experiments by assigning arbitrary numerical values to the different degrees of membrane staining. A five-point scale was used ranging from 0 to 4. The complete lack of uranyl binding to the membrane (Pl. 1*b*) was scored zero; membranes which were just visible in small areas (Pl. 5*a*) were scored the value 1; membranes visible but still patchy in areas (Pl. 5*b*) the value 2; well defined, uniformly stained membranes (Pl. 5*c*) the value 3; and the score for uniform heavy electron dense staining of the membrane (Pl. 5*d*) was 4.

Four photographs were taken at random from the sections of three different embedded specimens (blocks) of each muscle which had been subjected to a specific treatment. The photographs were enlarged seven times to a final magnification of 26,250 on 6×8 inch paper. Each photograph was coded and then evaluated independently by two different observers using double-blind methods. This procedure was adopted as a result of the statistical analysis of a control experiment designed to investigate animals; muscle samples; batches of uranyl acetate; pH fluctuations in the range 5·9–6·1; blocks; observers; grids; photographs and any interactions between these as possible sources of variance. A total of 768 photographs was subjected to analysis of variance, and the estimates of the variance components due to blocks and to photographs were both an order of magnitude greater than the components due to any other sources. An f value of 22·2 was obtained with 288 and 288 degrees of freedom when the photographs component was tested against the appropriate mean square and this was significant at the 0·1 % level.

The scores for the different degrees of membrane staining were carefully but arbitrarily assigned in the above procedures, and the results were calculated on the assumption that they would be normally distributed. An analysis was therefore carried out on the observations to test the validity of this assumption. Some observations showed significant deviations from a normal distribution and the score values 1, 2 and 3 were adjusted in order to minimize these deviations. This provided new assigned values 0, 1·5, 2·3, 2·9 and 4 for the standard degrees of membrane staining, although in practice the final conclusions were little affected whether the original scores or the new assigned values were used.

Cation competition at the smooth muscle cell membrane

Text-fig. 1 shows the results of experiments in which uranyl treated tissues were rinsed by isosmotic sucrose solutions containing varying concentrations of Na^+, K^+, Ca^{2+} or Mg^{2+}. Control tissues were rinsed with isosmotic sucrose only. Increasing the concentration of cation in the rinsing solution invariably caused a gradual decrease in membrane



Text-fig. 1. Observations on the reversal of uranyl-membrane staining by Na^+, K^+, Ca^{2+} or Mg^{2+}. Abscissa: concentration of cation in rinsing solution in m-equiv/l. Ordinate: uranyl-membrane staining expressed as per cent of the control tissues which were rinsed with isosmotic sucrose only. Symbols: ●, ○, ■ and □ represent Na^+, K^+, Ca^{2+} and Mg^{2+} respectively. Each point is the mean of forty-eight observations, with the S.E. limits of the mean. Each set of points was fitted with a straight line by the method of least squares, in order to show the trend of diminishing UO_2^{2+} staining with increasing cation competition.

staining with K^+ being more effective than Na^+. However, the divalent cations Ca^{2+} and Mg^{2+} were still more effective in their ability to displace uranyl from the membrane.

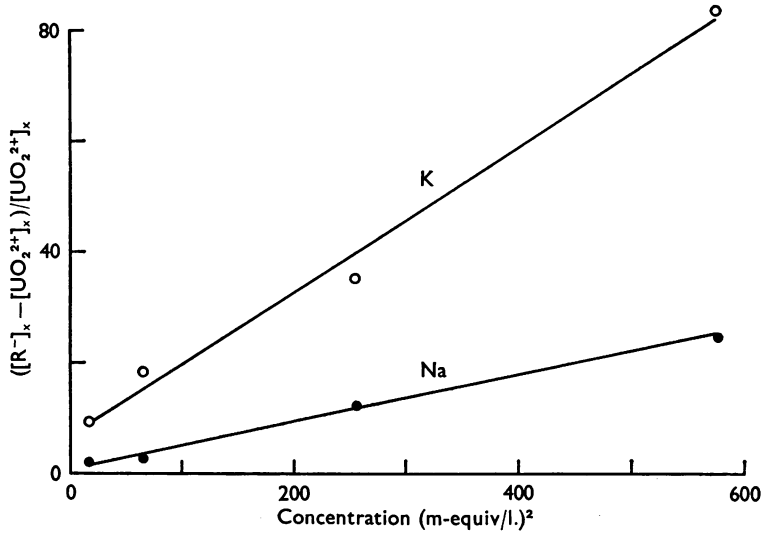
These findings can be interpreted on the hypothesis (Wilbrandt &

Koller, 1948; Niedergerke, 1963*a*, *b*; Goodford, 1968) that there are fixed anionic sites R^- in the membrane, and that these sites can interact with cations stoicheiometrically

$$UO_2^{2+} + 2NaR = 2Na^+ + UO_2R_2. \quad (1)$$

Using the nomenclature of Goodford (1968) in which [ ] indicate concentration; subscripts $_0$ and $_x$ refer respectively to the extracellular space and the region in the cell membrane; and the constants *na*, *k*, *ca*, *mg*, and *u* are specific for each ion, incorporating activity coefficients and distribution coefficients between the phases, it may be shown that

$$[Na^+]_0^2 = \frac{u[UO_2^{2+}]_0}{na^2} \cdot \frac{([R^-]_x - [UO_2^{2+}]_x)^2}{[UO_2^{2+}]_x}. \quad (2)$$



Text-fig. 2. The mean Na^+ and K^+ values in Text-fig. 1 replotted according to eqn. (2). Abscissa: the square of the cation concentration in m-equiv/l. Ordinate: the expression

$$([R^-]_x - [UO_2^{2+}]_x)^2/[UO_2^{2+}]_x.$$

Symbols as Text-fig. 1. The slopes of the least squares best fit straight lines were compared to obtain the selectivity coefficient.

In this equation extracellular concentrations [ ]$_0$ are expressed in equivalents, and concentrations in the region [ ]$_x$ as percentage staining so that $[R^-]_x$ has the value 100.

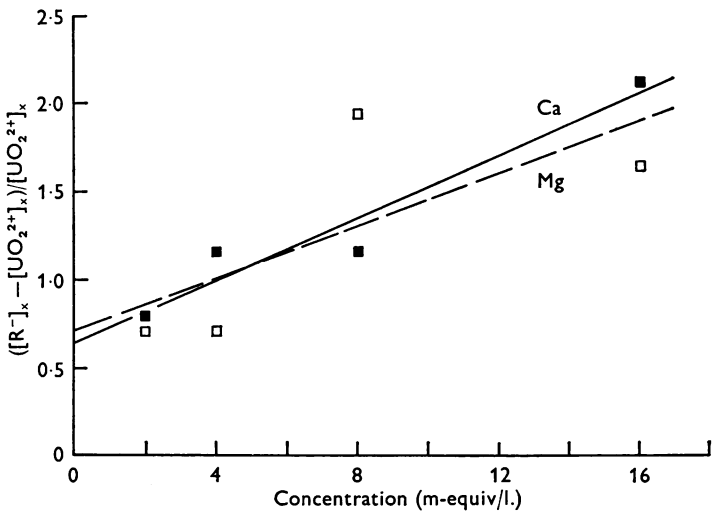
$$\text{For divalent cations: } [Mg^{2+}]_0 = \frac{u[UO_2^{2+}]_0}{mg} \cdot \frac{([R^-]_x - [UO_2^{2+}]_x)}{[UO_2^{2+}]_x} \quad (3)$$

assuming in both eqns. (2) and (3) that free anions such as chloride do not enter the tissue region appreciably. When the results for Na^+ and K^+ are

replotted (Text-fig. 2) according to eqn. (2) the observations fall approximately on to straight lines which do not deviate significantly from the origin. Furthermore, there are no significant deviations from linearity and the observations are therefore compatible with the assumption that the scale of assigned values is linearly related to the actual amount of stain in the membrane. Since the slopes of these lines are in the ratio

$$\frac{k^2}{na^2} = 3{\cdot}04$$

the relative affinity of K^+ for the sites is higher than for Na^+ by a factor of about $\sqrt{3{\cdot}04} = 1{\cdot}74$, which is in reasonable agreement with previous results using analytical methods (Goodford, 1966). When the Ca^{2+} and



Text-fig. 3. The mean Ca^{2+} and Mg^{2+} values in Text-fig. 1, replotted according to eqn. (3). Abscissa: the cation concentration in m-equiv/l. Ordinate: the expression

$$([R^-]_x - [UO_2^{2+}]_x)/[UO_2^{2+}]_x$$

Symbols as Text-fig. 1. The slopes of the least squares best fit straight lines do not differ significantly. See text.

Mg^{2+} observations were analysed in the same way (Text-fig. 3) there was no significant difference between the slopes for these divalent cations, but the present results are not incompatible with the Ca/Mg difference found by Sparrow (1969) because of the relatively large scatter of the Ca^{2+} and Mg^{2+} observations.

Closer inspection of Text-fig. 2 shows that the potassium line has a small positive intercept at zero potassium concentration, and the divalent

cations have more noticeable intercepts in Text-fig. 3. Text-fig. 1 shows that low concentrations (below 2 m-equiv/l.) of calcium or magnesium are already sufficient to drive nearly half the uranyl from the membrane. At higher concentrations, however, this uranyl reversal does not progress as fast as would be expected, assuming interaction at a single array of identical anionic groups.

Rothstein (1961) has shown on the basis of similar findings that there are two types of anionic binding sites on the surface of yeast cells, and it is difficult to avoid the conclusion that there may be two (or more) types in the smooth muscle cell membrane. On this interpretation one array apparently takes up calcium until it is almost saturated at 2 m-equiv/l., while the other had only partly exchanged at eight-times higher calcium levels. The two arrays therefore ensure that the membrane always contains some calcium at normal physiological concentrations. However, a comparison of the vesiculated regions with the flat membrane has yielded no evidence to support the view that one of the arrays might be preferentially associated with vesicles.

DISCUSSION

With the aid of electronmicroscopic methods it has been possible to define more precisely the location of the superficial anionic regions in smooth muscle, by interacting the anionic sites with electron dense uranyl cations. It has been found that uranyl binds primarily to the outer surface of the plasma membrane. The results are compatible with an ionic competition mechanism since the membrane binding can be readily reversed by Na^+, K^+, Ca^{2+} and Mg^{2+}. The uranyl binding, and the reversal by other cations, does not differ significantly between the flat and the vesiculated areas of the smooth muscle cell membrane. Similar uranyl binding has been observed at the cell membranes of skeletal muscle, nerve and red blood cells, and on the inner surface of the plasma membrane whenever uranyl ions have access to the cytoplasm.

In the present study, the tissues were exposed to the fixative action of glutaraldehyde, and the likelihood that this agent may have an effect on the superficial negative sites must be kept in mind. Glutaraldehyde causes cross-linking of protein molecules, primarily by reacting with the amino groups of lysine and with some aromatic amino acids (Hopwood, 1969; Hopwood, Allen & McCabe, 1970). Since the uranyl binding sites on the membrane may be composed of amino as well as carboxyl and phosphate groups (Lombardi, Prenna, Okolicsanyi & Gautier, 1971), the glutaraldehyde fixation could slightly limit the number of sites available for binding. However, the present competition studies are compatible with

previous observations on living muscle, and to that extent they are compatible with the view that fixation has not seriously affected the availability or affinity of the uranyl-binding sites in the taenia.

The binding of uranyl ions to yeast cells has already been studied extensively by Rothstein (1961) and by Rothstein & Meier (1951), who demonstrated cation-competition at the cell surface. They found that 5 mM-Ca^{2+} or Mg^{2+} were able to displace uranyl ions as effectively as 100 mM-Na^+ or K^+, and it may be calculated from their observations that the average area of an anionic binding site on the yeast cell surface is of the order of 1 nm^2. A value of 0·19 nm^2 has been calculated for smooth muscle cells (Goodford, 1967) assuming the cell surface to be smooth, although a somewhat larger area would result if allowance were made for the extra surface area of the vesicles (Goodford, 1970). One may therefore tentatively conclude that the uranyl ion interacts quantitatively like the physiological cations Na^+, K^+, Mg^{2+} and Ca^{2+} in smooth muscle and yeast cells, save that UO_2^{2+} has a higher binding constant. Furthermore, these interactions have now been observed in other cell types, and they may be a not uncommon property of plasma membranes in general.

Rothstein (1962) has presented evidence that there are two different binding sites with different properties on the surface of yeast cells, and the present observations on smooth muscle can be best explained on the same hypothesis. Calcium or magnesium (2 m-equiv/l.) almost completely displaces UO_2^{2+} from some 40 % of the sites on the taenia coli, but further increases in $[Ca^{2+}]_o$ or $[Mg^{2+}]_o$ only produce a marginal extra effect. Low concentrations of potassium or sodium, on the other hand, produce relatively little displacement, and appreciable effects are not observed until $[K^+]_o$ or $[Na^+]_o$ are in the range 10–100 mM. With these monovalent cations the highest concentrations would be needed to produce full displacements, and their behaviour apparently complements that of Ca^{2+} and Mg^{2+} although precise relationships cannot be deduced from the present semiquantitative methods. It is not unreasonable to suggest that at least two arrays of cation binding sites are present on the outer surface of the taenia coli's plasma membrane, and that the sites have affinities for Ca^{2+} and Mg^{2+} which ensure that there is always a significant proportion of these cations in the membrane. Moreover an appreciable quantity of sodium will also be present when the membrane is exposed to normal extracellular concentrations of Na^+, although potassium may be poorly represented on its outer surface.

Sparrow (1969) concluded that there are some 12 milliequivalents of fixed anionic charge on the outer surface of the plasma membrane of 1 kg of taenia coli, but his estimate significantly exceeded Goodford's (1966) earlier figure of 4 m-equiv/kg. However, if there are in fact two arrays of

binding sites as the present findings suggest, then this apparent discrepancy would be explained, since Sparrow's observations were based on the Ca^{+2}:Mg^{2+} competition, whereas Goodford investigated Na^{+} and K^{+}. The calcium and magnesium ions in Sparrow's experiments would have interacted with both arrays of membrane sites, but sodium and potassium were investigated at concentrations which would only react with one array on the present interpretation.

Cereijido & Rotunno (1968) have proposed, on the basis of their observations in frog skin, that 'when an ion from the outside bathing solution reaches the outward facing membrane, the tendency to penetrate the bilipid membrane and to get into the cell is very small compared with the tendency to travel tangentially by jumping from fixed polar group to fixed polar group.' The present findings are consistent with this view for the taenia coli and provide information on the composition of the ionic mixture which would be moving tangentially over the surface of the smooth muscle cells. This concentrated mobile population of cations is well placed to partake in transmembrane movements and could supply many of the ions moving through the membrane during the action potential (Goodford, 1967). Furthermore, the double array of sites binding calcium would ensure that there was always a supply of this ion in the membrane where it might be involved in several different processes. First, it might partake in excitation–contraction mechanisms directly. Secondly, it could migrate to or from the vesiculo-reticular-mitochondrial complex, and thirdly it might compete with other cations thereby changing their distribution and controlling membrane excitability. Thus the flow of calcium over and through the membrane could be associated with the contractile response and contribute significantly to the depolarizing current, while the movement of other membrane ions might be responsible for the characteristic properties of the action potential itself.

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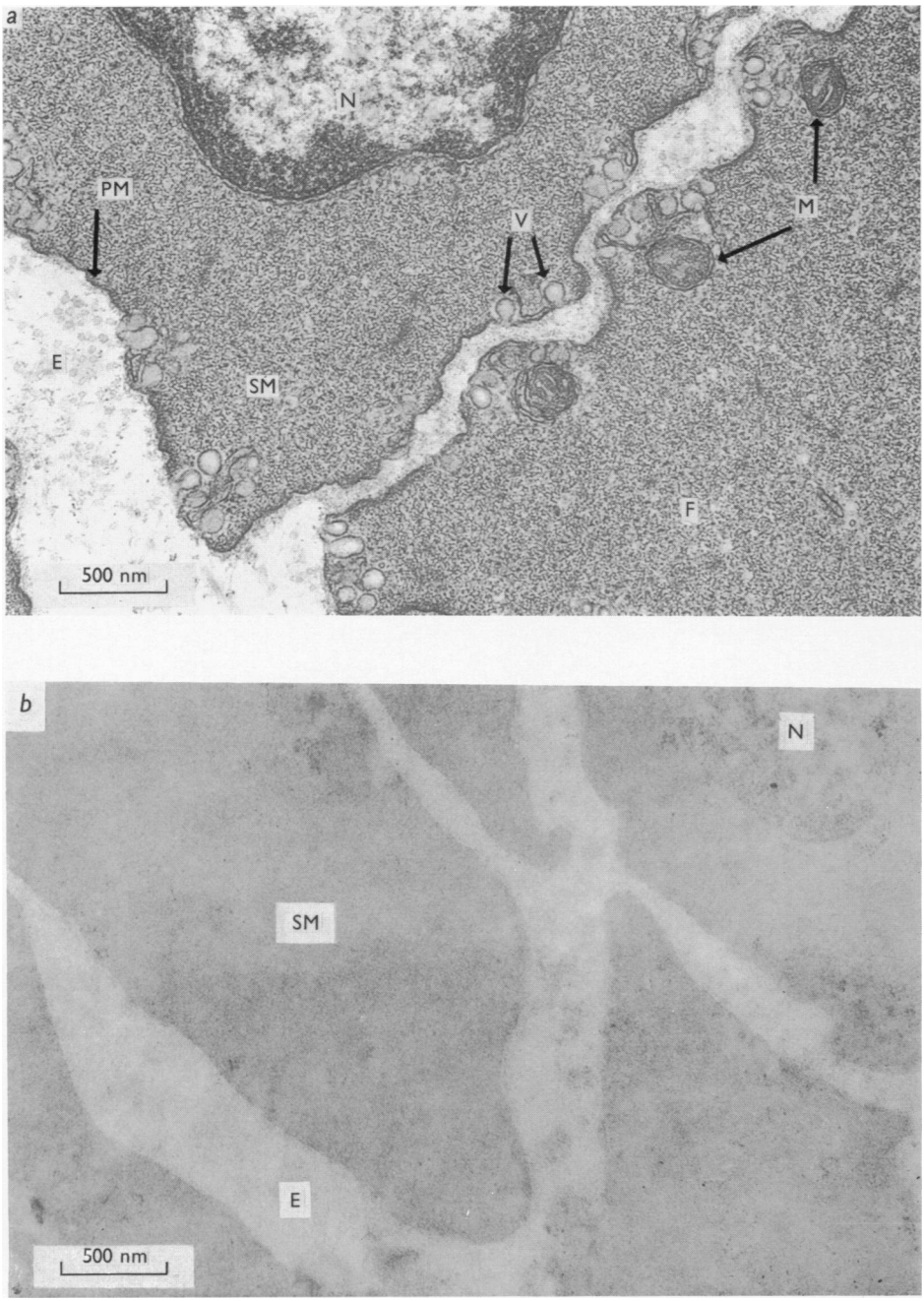
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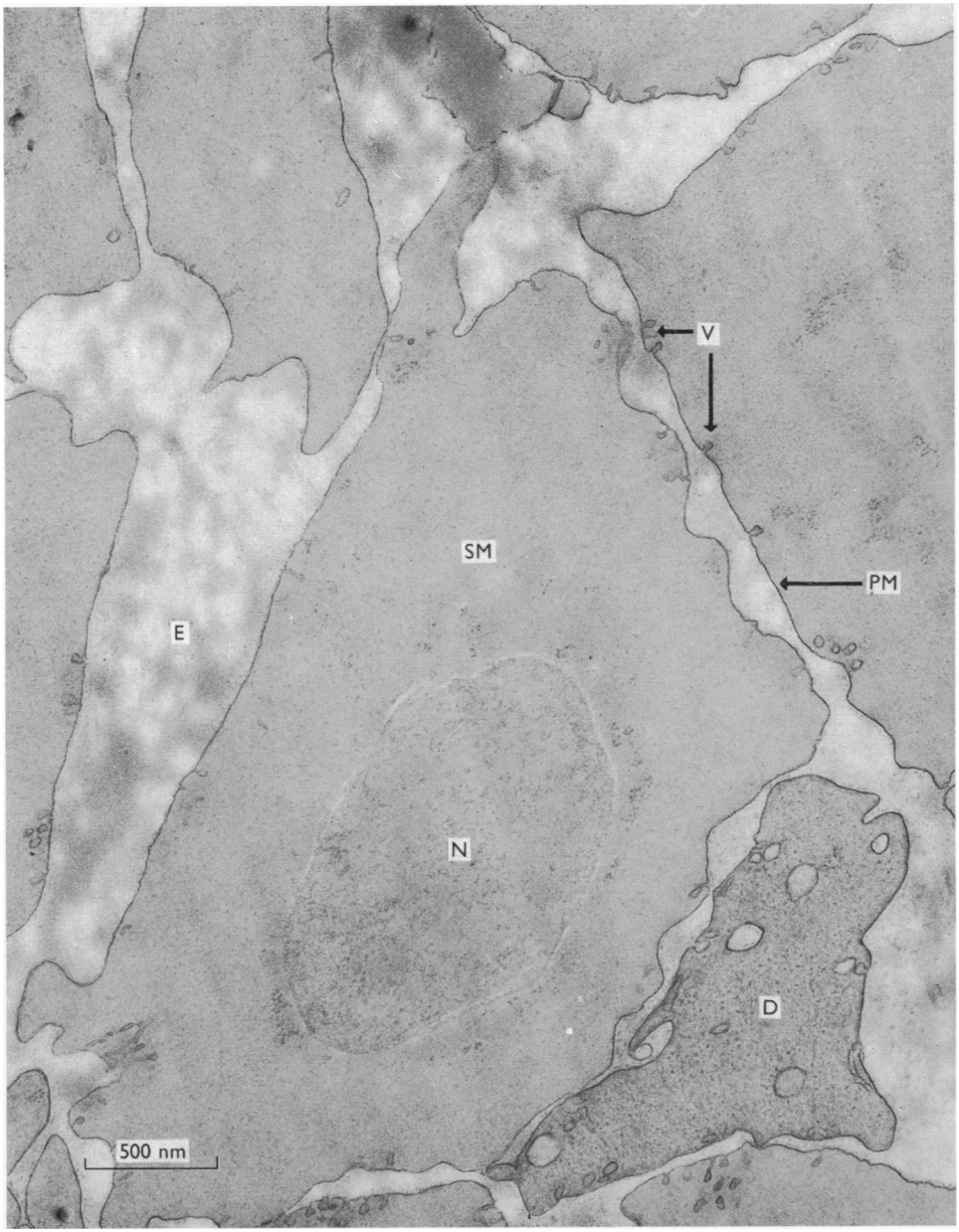
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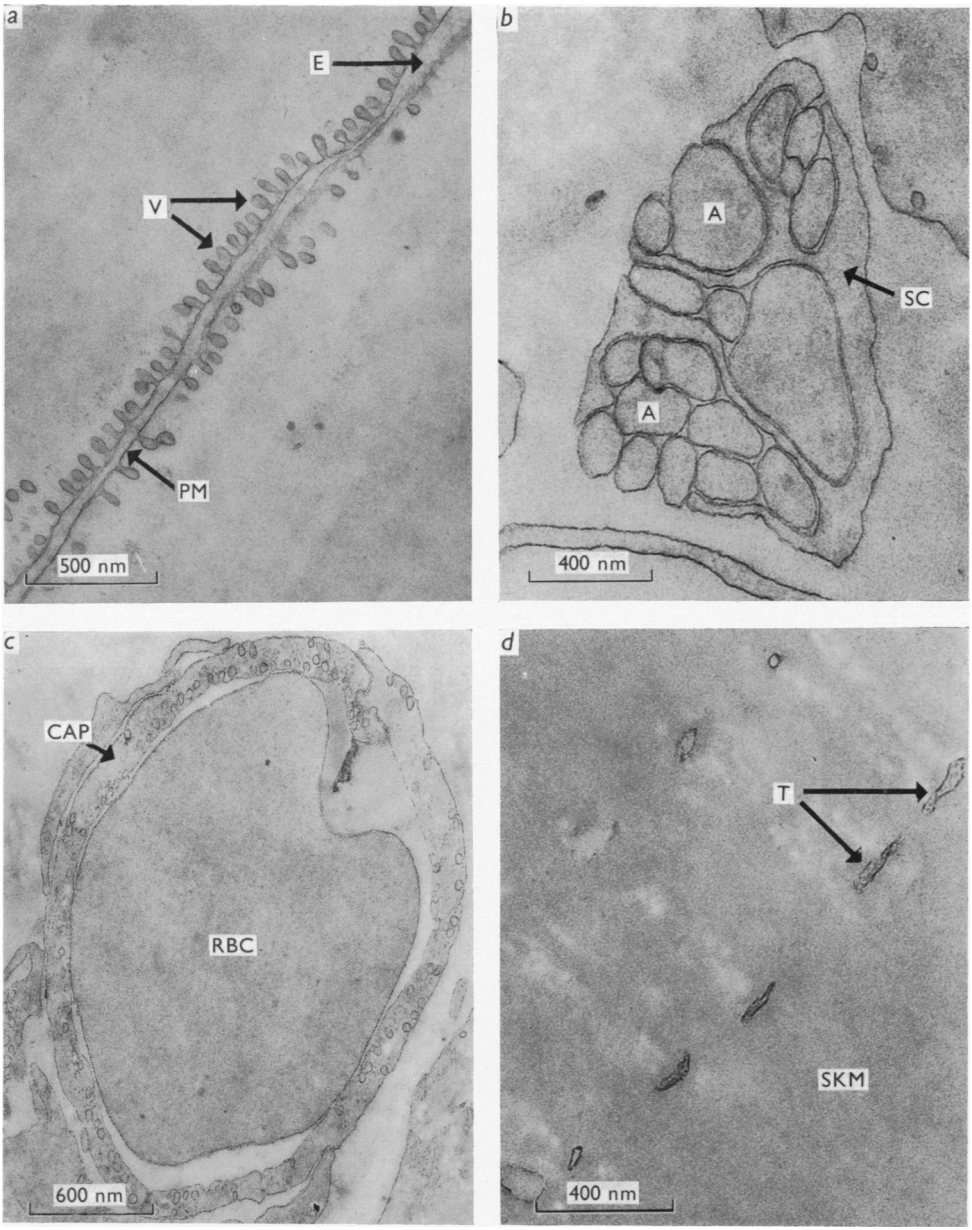
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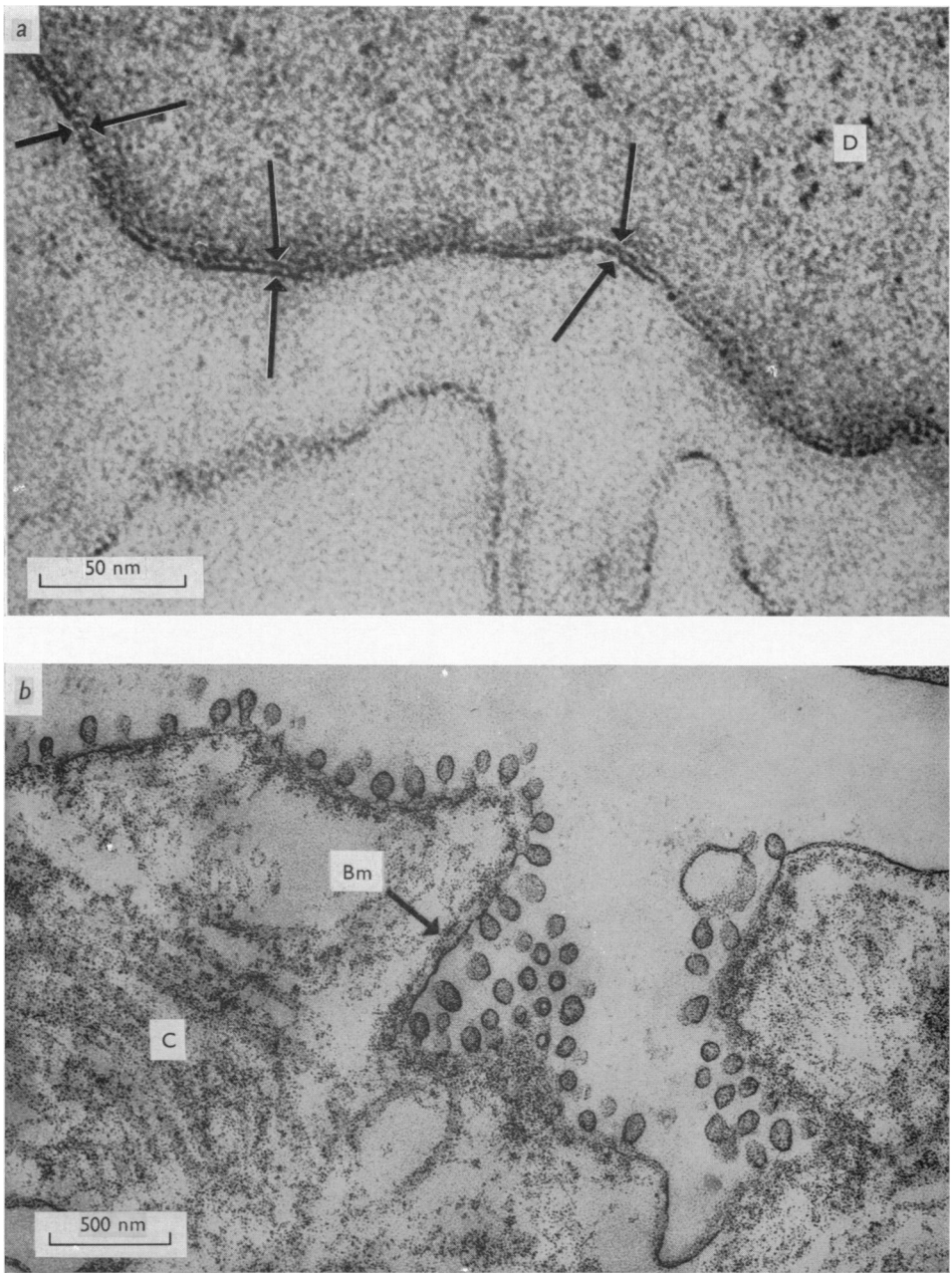
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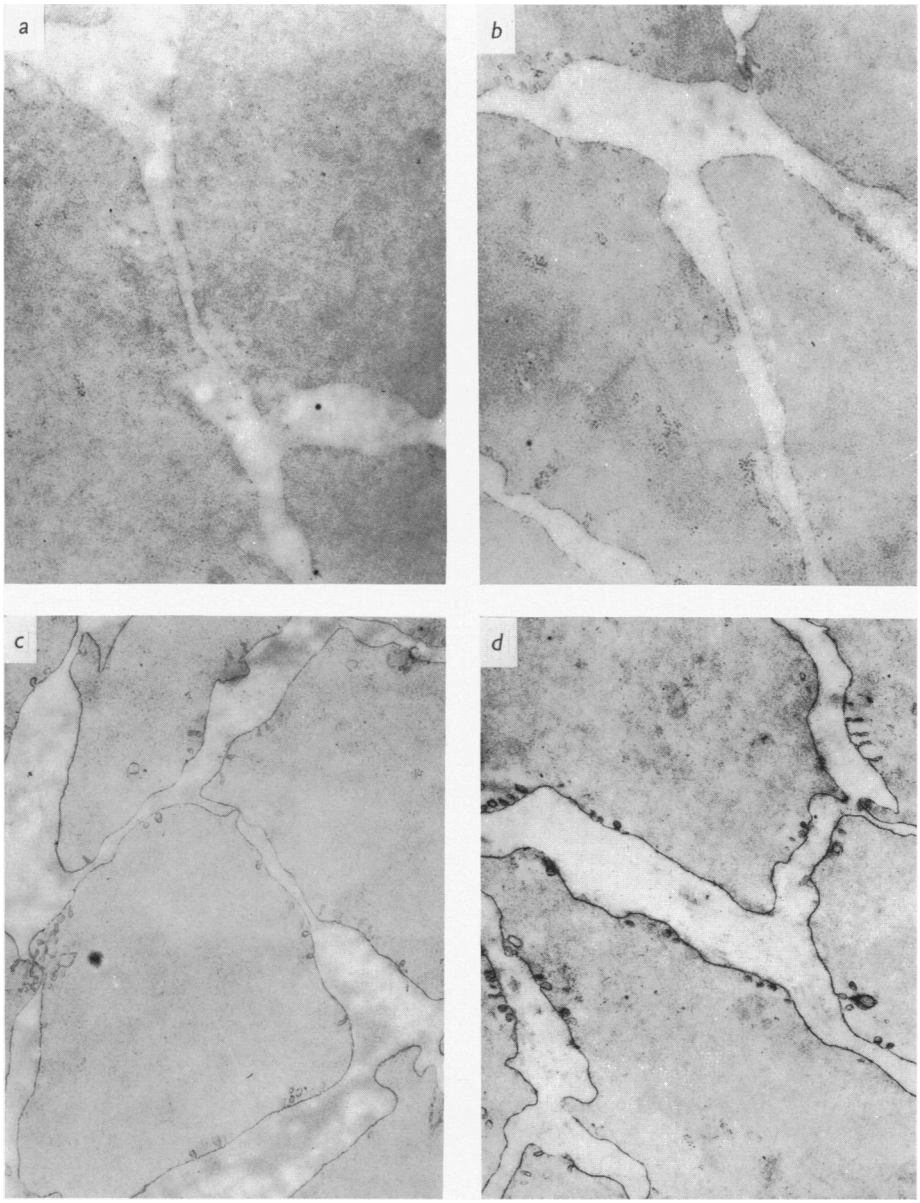
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EXPLANATION OF PLATES

Symbols used in the Plates were: A, nerve axon; Bm, basement membrane; C, collagen; CAP, capillary; D, 'damaged' smooth muscle cell; E, extracellular space; F, myofilaments; M, mitochondria; N, nucleus; PM, plasma membrane; RBC, red blood cell; SC, Schwann cell; SKM, skeletal muscle cell; SM, smooth muscle cell; T, transverse tubules; V, vesicles.

PLATE 1

Transverse sections through guinea-pig taenia coli: *a*, Prepared by glutaraldehyde and OsO_4 fixation, and the section post-stained with lead citrate and uranyl acetate. *b*, Control tissue prepared by glutaraldehyde fixation as described in the methods with no heavy metal stains.

PLATE 2

Transverse section through guinea-pig taenia coli. Glutaraldehyde fixed tissue treated with 1 % magnesium uranyl acetate 15 min before dehydration.

PLATE 3

Glutaraldehyde fixed specimens treated with 1 % magnesium uranyl acetate 15 min before dehydration. *a*, Longitudinal section through guinea-pig taenia coli showing flat and vesiculated plasma membranes. *b*, Nerve bundle. *c*, Capillary and red blood cell. *d*, Longitudinal section through skeletal muscle cell showing stained tubules.

PLATE 4

Abnormal sections through uranyl treated guinea-pig taenia coli. *a*, Two adjacent smooth muscle cells. The outer membrane surface of the lower cell has been stained as usual. However, uranyl ions have anomalously entered the upper cell, and have therefore stained the contractile mechanism and both inner and outer membrane surfaces (arrows). Such a cell may have been damaged out of the plane of section. *b*, A unique observation showing uranyl bound to collagen and basement membrane as well (see text).

PLATE 5

Transverse sections of uranyl treated guinea-pig taenia coli. *a*, Membrane stained to a numerical score of 1. *b*, Score of 2. *c*, Score of 3. *d*, Score of 4.