J. Anat. (1970) **107**, 1, pp. 101–114
With 19 figures
Printed in Great Britain

Some observations on the fine structure of the guinea-pig taenia coli after incubation in hypertonic solution

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(*Received* 9 *July* 1969)

INTRODUCTION

A study of the fine structure of smooth muscle after incubation in hypertonic solution has been undertaken since information on the effect of hypertonicity on cell-to-cell connexions is of interest for the interpretation of electrophysiological observations.

Intercellular junctions in which the plasma membranes of two excitable cells are fused have been reported in many tissues, e.g. nervous tissue (Bennett, Aljure, Nakajima & Pappas, 1963; Dewey & Barr, 1964; Hamilton, 1968), cardiac muscle (Dewey & Barr, 1964; Muir, 1965; Dreifuss, Girardier & Forssmann, 1966), intestinal smooth muscle (Dewey & Barr, 1962, 1964; Oosaki & Ishii, 1964; Bennett & Rogers, 1967) and smooth muscle of chick amnion and constrictor pupillae of cat and rabbit (Evans & Evans, 1964).

When Dewey & Barr (1962), using permanganate-fixed circular muscle of dog intestine, described the special regions where the membranes of two neighbouring cells were fused, they called them 'nexuses'. In excitable tissues, these specialized junctions have been considered to be the sites of low electrical resistance pathways between adjacent cells. It is known that in most visceral smooth muscles excitation is conducted from cell to cell (see reviews by Prosser, 1962; Tomita, 1966; Abe & Tomita, 1968).

Recent investigations of the nexal function, combining electrophysiological techniques with electron microscopy, indicated that exposure to hypertonic solution increased the resistance between the smooth muscle cells (and also cardiac muscle cells), blocked propagation of spontaneous action potentials and ruptured the nexuses (Barr, Dewey & Berger, 1965; Barr, Berger & Dewey, 1968). In contrast, Tomita (1966) reported that the electrical response of the guinea-pig taenia coli to external electrical stimulation in hypertonic solution was essentially the same as that in physiological solution.

The main purpose of the present investigation was therefore to study the nexus with the electron microscope under the same conditions as those used for electrophysiological studies by Tomita (1966). In the course of comparing fresh tissue with tissues incubated in physiological solution and in hypertonic solution, additional information on the fine structure of taenia coli and of the changes produced by hypertonicity has been obtained.

MATERIAL AND METHODS

Young adult guinea-pigs (average weight 350 g) were stunned and bled. A piece of taenia coli 3 cm long was dissected and held at its *in situ* length in a horizontal organ bath through which experimental solution flowed at about 2 ml/min. The temperature of the solution was kept constant at 35 °C by a thermostatically controlled heater immersed in oil surrounding the chamber.

The specimens were incubated for 30 min in Krebs solution aerated with 97 % O_2 and 3 % CO_2 (Kuriyama, 1963) and subsequently for 1 h in a solution made hypertonic by adding 10 g sucrose to 100 ml Krebs solution which was aerated as above (Tomita, 1966). Specimens which had not been incubated, and others incubated in aerated Krebs solution for 1·5 h, were used as controls. The osmolarity, measured with a Heulett Packard Model 302 vapour pressure osmometer, was 620 m-osmole when sucrose was added to Krebs solution, and 292 m-osmole for the normal Krebs solution.

Some of the specimens were fixed in ice-cold 2 % osmium tetroxide buffered with s-collidine at pH 7·4 for 2 h (Bennett & Luft, 1959), whilst others were fixed in ice-cold 2 % potassium permanganate buffered with s-collidine at pH 7·4 for 2 h. Dehydration was carried out in a series of graded acetones and all materials were embedded in Epon 812 (Luft, 1961).

All osmium-fixed specimens were stained with 2 % aqueous solution of uranyl acetate for 1 h before dehydration (Mercer & Birbeck, 1966). Thin sections were cut with glass knives on a Porter–Blum microtome and stained with lead acetate (J. H. Luft, personal communication). They were examined in either a Siemens Elmiskop I or an AEI 6B electron microscope.

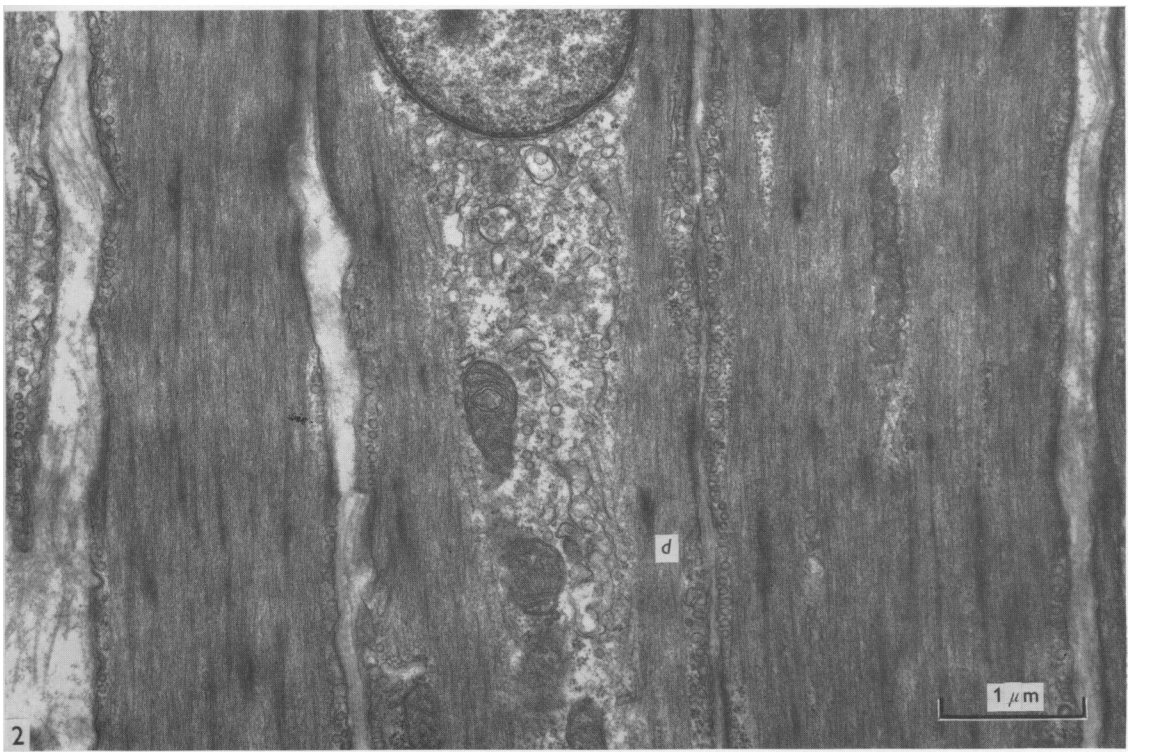
RESULTS

In osmium-fixed taenia coli (Fig. 1), incubated in hypertonic solution, muscle fibres were spindle-shaped with a long region of constant diameter. The plasma and nuclear membranes were smooth and the nuclei were elongated. The mitochondria were usually elongated and orientated along the long axis of the cell. They were located mainly within the sarcoplasm of the polar perinuclear region but some of them were scattered throughout the cytoplasm or close to the cell membrane. The cristae of the mitochondria were generally aligned in sub-parallel fashion and were well preserved. The other common cell organelles, a small Golgi complex and a small amount of the granular endoplasmic reticulum, were found near the nucleus. Ribosomes were also common in the central area of the cell and some were in

Fig. 1. General appearance of a longitudinal section through muscle fibres of the guinea-pig taenia coli after incubation in hypertonic solution, to be compared with the controls in Figs. 2 and 3. The outline of the muscle fibres is relatively smooth and many plasmalemmal vesicles (*pv*) are seen. The contour of the nuclear membrane is smooth. The cristae (*cr*) of mitochondria (*m*) are well preserved. The myofilaments are roughly parallel to the long axis of the fibre but are less clearly defined than in the controls. The dense bodies (*d*) are longer. *N*, nucleus; *G*, Golgi complex; *er*, endoplasmic reticulum; *r*, ribosomes; *bm*, basement membrane; *c*, collagen.

Fig. 2. Muscle fibres of fresh normal control. The dense bodies (*d*) are shorter than those after treatment with hypertonic solution. The myofilaments are clearly seen.


N
pv
c
d
G
er
r
m
cr
bm
1 μm
1


d
1 μm
2

scattered groups throughout the cytoplasm. Each plasma membrane showing a three-layered structure was accompanied by a thin and delicate basement membrane-like material adherent to its outer surface. A number of collagen fibrils were situated loosely in the extracellular space. These features are fundamentally very similar to those of normal smooth muscles either fresh (Fig. 2) or incubated in normal Krebs solution (Fig. 3).

Fig. 3. Muscle fibres after incubation in Krebs solution. The general features are similar to those of freshly dissected normal fibres. Arrows indicate close approach regions.

Cross-sections of muscle fibres incubated in hypertonic solution (Fig. 4) showed that the intervening extracellular spaces between cells seemed to be enlarged in comparison with those of the normal control tissues (Fig. 5). The dense bodies among the myofilaments and dense patches just under the plasma membrane in tissue incubated in hypertonic solution were larger, i.e. wider (Fig. 4) and longer (Fig. 1). The myofilaments of the tissues bathed in hypertonic solution were arranged roughly longitudinally in ill-defined groups along the axis of the fibre and seemed to be more densely packed with dense bodies than those in normal controls, although these dense areas where myofilaments might be aggregated varied from area to area within a cell (Fig. 6).

Although the myofilaments in the smooth muscle cells were randomly distributed and their arrangement could not be demonstrated definitely, the appearance of the

myofilaments in longitudinal sections of the tissues incubated in hypertonic solution (Fig. 7) was not so clear as that of the controls (Fig. 8). Two types of myofilaments, thick and thin, as reported by Nonomura (1968), were clearly distinguished in the two control tissues (Fig. 8), particularly in transverse sections under higher magnification (Fig. 10). In a cross-section of a specimen treated with hypertonic solution (Fig. 9) the two types were less clearly defined. However, some filaments were found of a size approximately intermediate between the thick and thin filaments of the controls. These observations show that treatment with hypertonic solution does not produce a significant contraction of the muscle fibres, but that the sarcoplasm appears more closely packed, as may be expected if the cell had lost water (Brading & Setekleiv, 1968) and muscle proteins were affected in some way.

The smooth contour of the plasma membrane was seen to be interrupted by many small pocketings or invaginations, variously called micropinocytotic vesicles, caveolae intracellulares (Yamada, 1955) or plasmalemmal vesicles (Palade & Bruns, 1968). Usually these were randomly distributed along the inner surface of the plasma membrane (Figs. 11, 13). Their limiting membrane was triple-layered. The three-layered structure of the unit membrane in the plasma membrane and in the plasmalemmal vesicle could always be observed even in the tissue exposed to hypertonic solution (Fig. 12). The staining of specimens with uranyl acetate before dehydration probably contributed to the visualization of this structure as described by Palade & Bruns (1968). After treatment with hypertonic solution, the plasmalemmal vesicles were often found to be more numerous than those of the control tissues and to be more elongated, flask-like and narrow-necked.

In this investigation no intercellular bridges with cytoplasmic continuity, as described by Mark (1956) in rat uterus, Thaemert (1959) in rat pyloric muscle and Silva (1967) in rat uterus, were observed. The present findings are, therefore, similar to the observations in other types of smooth muscle by Caesar, Edwards & Ruska (1957), Bergman (1958), Prosser, Burnstock & Kahn (1960), Rhodin (1962) and Merrillees, Burnstock & Holman (1963), who also found no cytoplasmic continuity between cells.

In several specimens the plasma membranes of two neighbouring cells were separated by a nearly uniform gap of less than 20 nm (Fig. 14). Sometimes cytoplasmic processes from one cell protruded into invaginations of another cell (Fig. 15) and here the separation between the two plasma membranes resembled that in the region of close approach. End to end, side to side and end to side approaches between cells were observed in specimens treated with hypertonic solution and also in fresh tissue and in tissue treated with Krebs solution (Fig. 16). With osmium tetroxide fixation the nexus was not seen, an observation in agreement with that of Dewey & Barr (1962, 1964).

In permanganate-fixed material, much of the fine structure within the smooth muscle fibres was obliterated. However, the membranous components such as the sarcolemma, mitochondria and endoplasmic reticulum remained intact. Plasmalemmal vesicles were seen remarkably clearly in specimens fixed with osmium, but they were not observed in permanganate-fixed taenia coli. Yamauchi & Burnstock (1969) also reported that permanganate fixation abolished the structure of plasmalemmal vesicles in vas deferens of new-born mouse.

In taenia coli treated with hypertonic solution the muscle fibres fixed with permanganate were grouped together as in osmium-fixed material to form muscle bundles. Intercellular close approach and cytoplasmic projections or protrusions into an adjacent cell were still observed (Fig. 17). In the protrusion the opposing

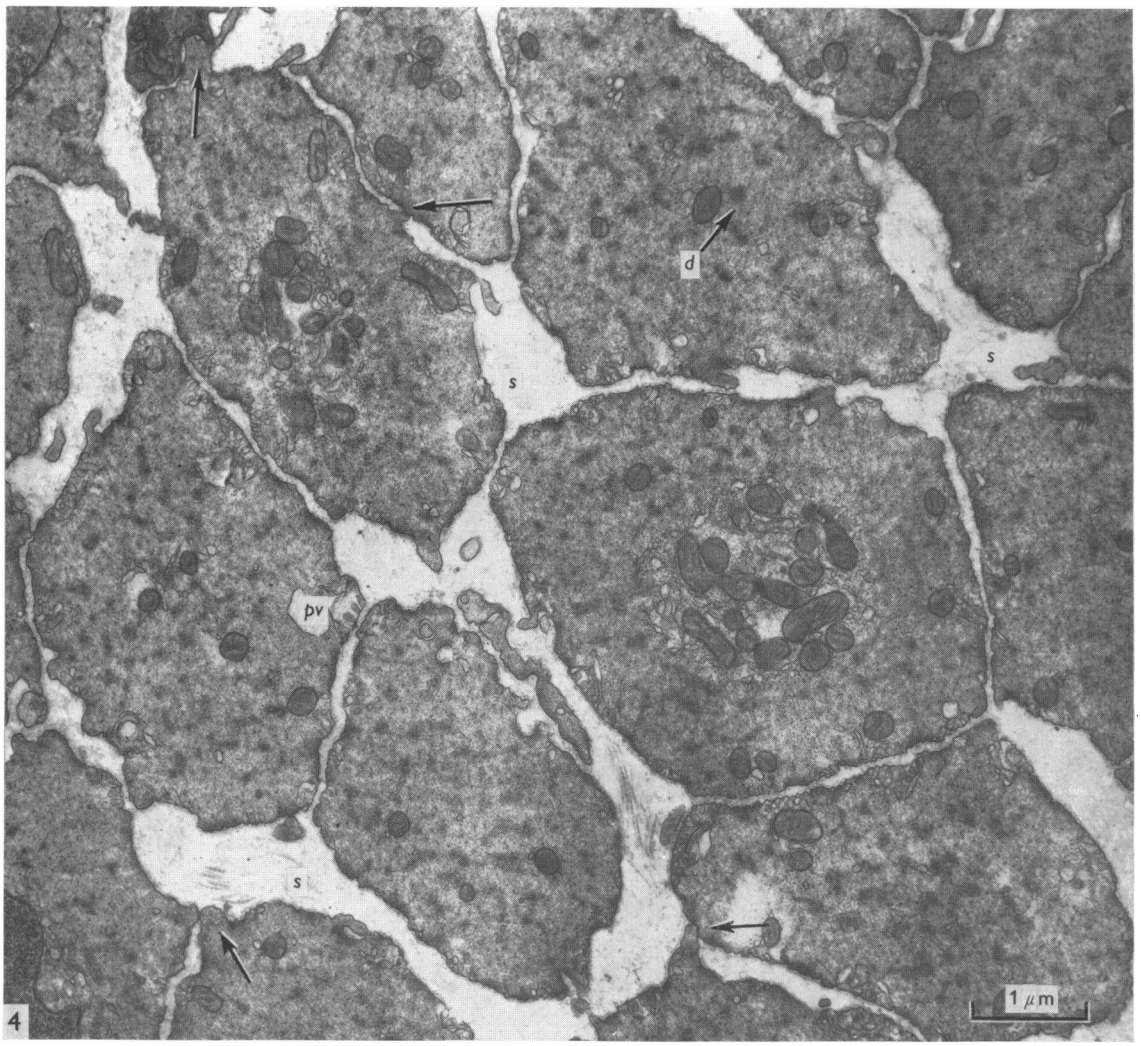


Fig. 4. Cross-section of muscle fibres after incubation in hypertonic solution. Compare with Fig. 5. Extracellular spaces (*s*) are enlarged. Note wider dense bodies (*d*). Close approach regions and cytoplasmic intrusion are still seen (arrows); *pv*, plasmalemmal vesicles.

plasma membrane was very close and seemed to form a nexus. In these special regions of intimate fibre apposition the nexus could be seen under higher magnification (Fig. 18). The width of the nexus was less than 13 nm. The nexal structure was as well preserved as in the control tissue incubated with normal Krebs solution

(Fig. 19). No particular structural differences between the fresh normal tissue and the tissue incubated in Krebs solution could be seen, and the adhesion of cells persisted even after incubation in hypertonic solution.

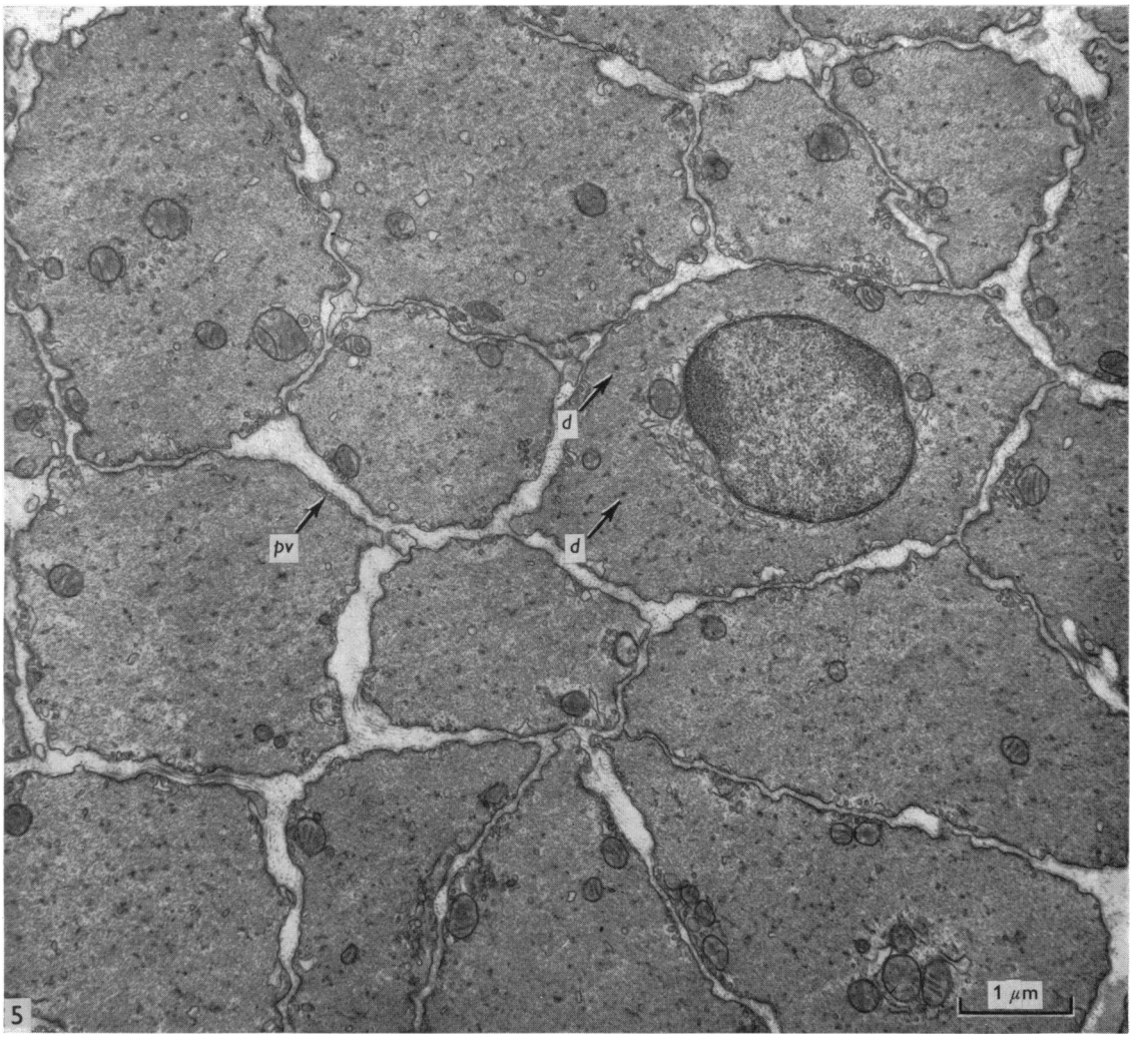


Fig. 5. Cross-section of muscle fibres after incubation in Krebs solution.
d, Dense bodies; *pv*, plasmalemmal vesicles.

DISCUSSION

The observations described above show that the nexal structure of the guinea-pig taenia coli remains intact in hypertonic solution. If it is accepted that the nexus provides the pathway for electrotonic coupling between adjacent cells, the results support the electrophysiological observations obtained by Tomita (1966) on the

same tissue under the same experimental conditions. He found that, in two times hypertonic solution (620 m-osmole), the spike triggered by external stimulation was propagated in both directions along the fibre, and the conduction velocity measured with two microelectrodes was the same as that in isotonic solution.

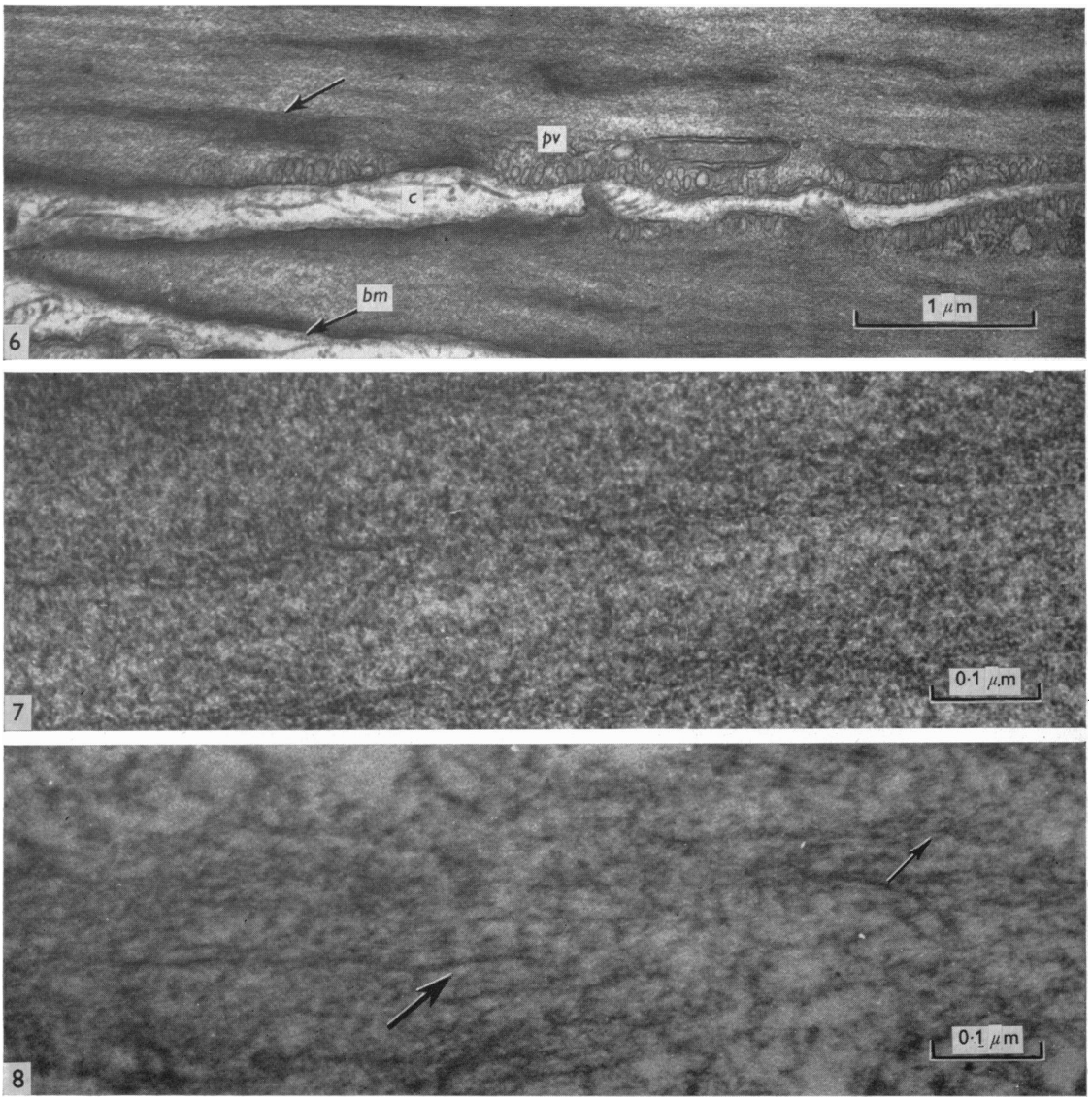
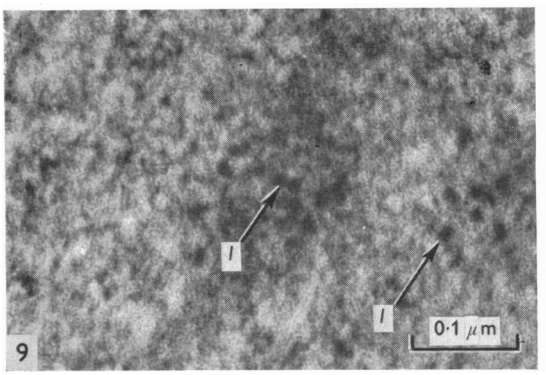
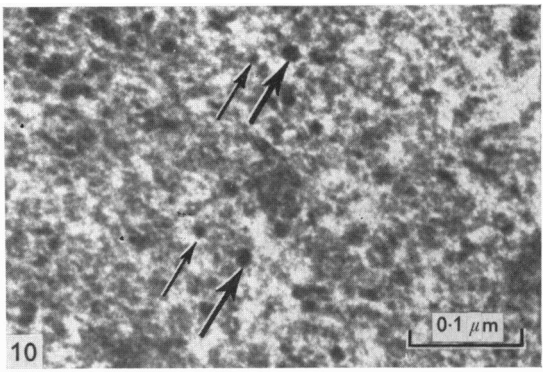


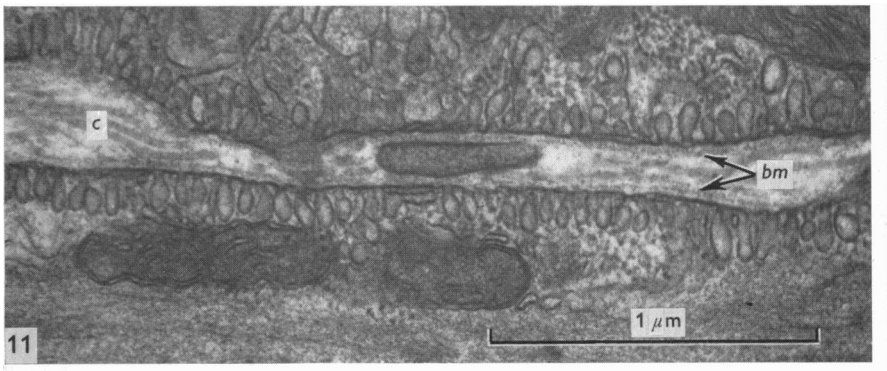
Fig. 6. Longitudinal section of muscle fibres after incubation in hypertonic solution. The myofilaments are not so clearly seen; they are probably aggregated with dense bodies (arrow). Also compare with Fig. 2. *bm*, Basement membrane; *pv*, plasmalemmal vesicles; *c*, collagen.

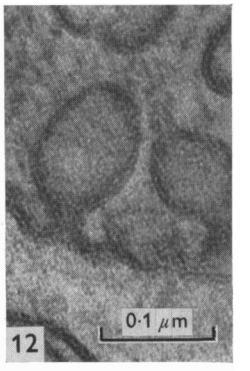
Figs. 7 and 8. Comparison of the appearances of myofilaments after incubation in a hypertonic solution (Fig. 7) and in Krebs solution (Fig. 8) as a control. Thick and thin arrows indicate the two sizes of myofilaments.

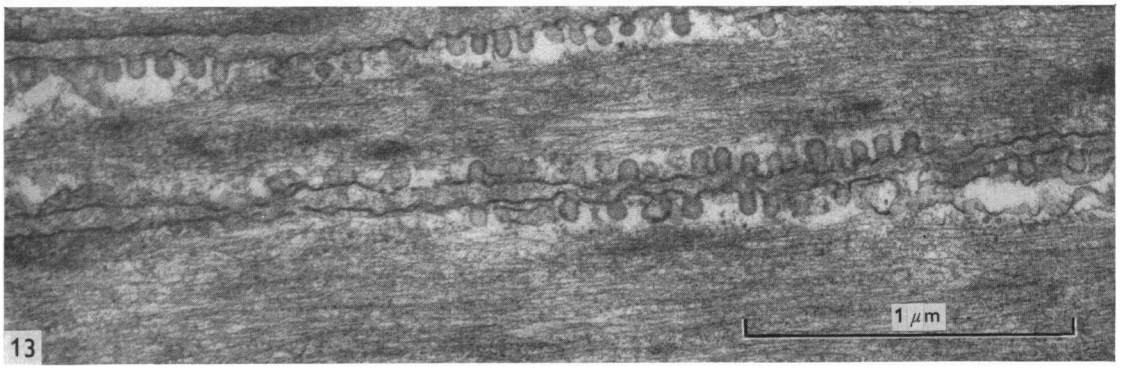
Barr *et al.* (1968) correlated the block of spontaneous spike propagation in hypertonic solution with their finding that the nexus was ruptured. Cessation of spontaneous activity was also observed by Tomita (1966), but it was attributed to the









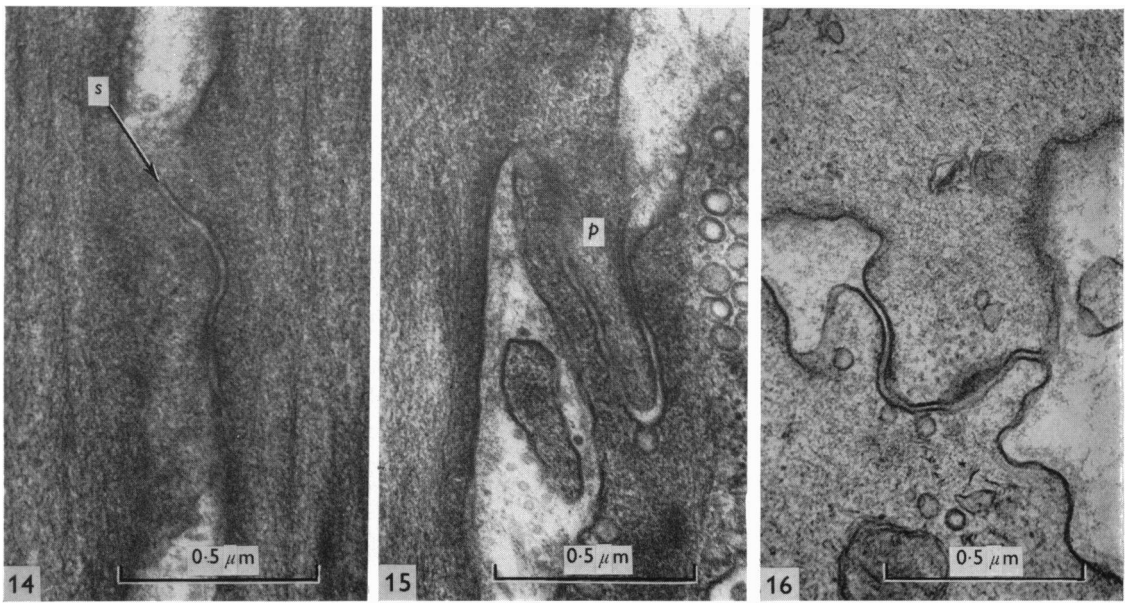


Figs. 9 and 10. Cross-section of a fibre incubated in hypertonic solution (Fig. 9). Some myofilaments of intermediate size are visible (*I*). Compare with cross section of a fibre obtained from fresh tissue in which two types of myofilaments (thick and thin arrows) can be distinguished.

Figs. 11–13. These figures allow the comparison of the number, size and shape of plasmalemmal vesicles, in tissue which has been incubated in hypertonic solution (Figs. 11 and 12) and in fresh normal tissue (Fig. 13). Fig. 12 also shows the triple-layered nature of the plasma membrane and associated vesicles. Myofilaments are clearly visible in Fig. 13. *bm*, basement membrane; *c*, collagen.

hyperpolarization of the cell membrane. This is probably a result of the increased internal potassium concentration brought about by the loss of water (Brading & Setekleiv, 1968).

Even though some of the nexuses may be destroyed by the effect of hypertonicity, it is clear that complete rupture of every nexus in the tissue does not occur. It has also been reported that in rat cardiac muscle exposure to hypertonic media causes



Figs. 14–16. Fibres incubated in hypertonic solution: one muscle fibre approaches another across the narrow intercellular space (*s*), (Fig. 14). A process (*p*) of a muscle cell intrudes into a neighbouring cell (Fig. 15). Compare with fibres incubated in Krebs solution (Fig. 16). Side-to-side type of close approach is shown.

no structural alterations of the nexus, but that disruption of the desmosomes and swelling of the sarcotubular system occurs (Dreifuss *et al.*, 1966). At the time of writing this paper, a paper by Cobb & Bennett (1969) appeared which showed that the nexus in various smooth muscle preparations, including taenia coli, guinea-pig and mouse vas deferens and chick and pigeon gizzard, was a stable structure, affected neither by hypertonicity nor by stretch. The present results confirm these observations.

Several workers have been able to demonstrate the nexus between smooth muscle cells of different tissues after osmium fixation (Evans & Evans, 1964; Holman, Kasby, Suthers & Wilson, 1968; Cobb & Bennett, 1969). However, in the present study the nexus could not be shown in taenia coli fixed with osmium tetroxide. Permanganate seems to be a better fixative for its preservation.

A remarkable change in the fine structure produced by hypertonicity is the increased number and the enlarged and elongated appearance of the plasmalemmal

vesicles. Huxley, Page & Wilkie (1963) reported the occurrence of clusters of plasmalemmal vesicles in frog sartorius muscle under hypertonic conditions. It is not possible to decide on the basis of the present study whether the characteristic shape and the increased numbers of vesicles are due to dilatation of existing vesicles and appearance of new ones, whether they are a manifestation of more vigorous micropinocytosis, or whether merely a result of the decrease in cell volume. According

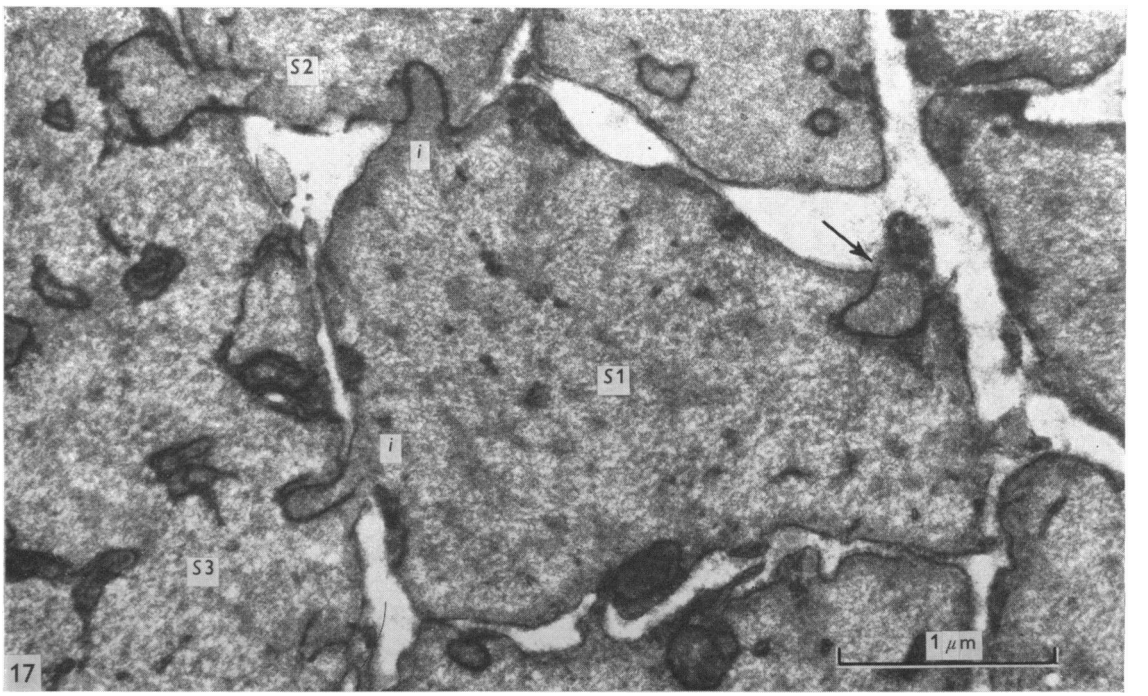
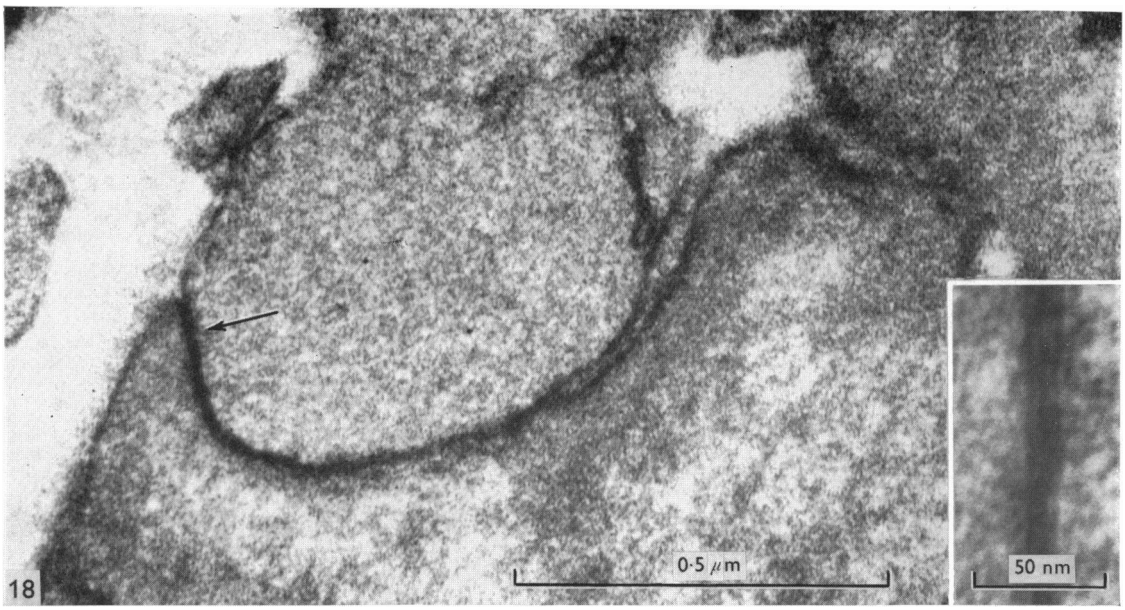


Fig. 17. Cross section of muscle fibres incubated in hypertonic solution. Two intrusions (*i*) of a smooth muscle cell (*S*1) into neighbours (*S*2 and *S*3) are shown. One intrusion from another plane into the muscle cell (arrow). Permanganate-fixed material.

to Palade & Bruns (1968), similar shapes in blood capillaries have been interpreted as representing different stages in the formation and loading of membrane invagination.

In a comparative study of intestinal smooth muscle cells in various stages of contraction (Lane, 1965), the criteria for identifying a contracted muscle fibre have been: the deformity of the nucleus, the invaginations of cell borders, the changes in the alignment of cytoplasmic organelles and the network formation of myofilaments. In the present study, no such structural changes were noticed. Therefore, the smooth muscle cells do not seem to be contracted in hypertonic solution.

Another effect of hypertonicity on the fine structure concerns the myofilaments. In striated muscle treated with hypertonic solution, Huxley *et al.* (1963) found that the protein filaments were packed more closely together than usual and that the individual fibrils could no longer be distinguished. In the present investigation of



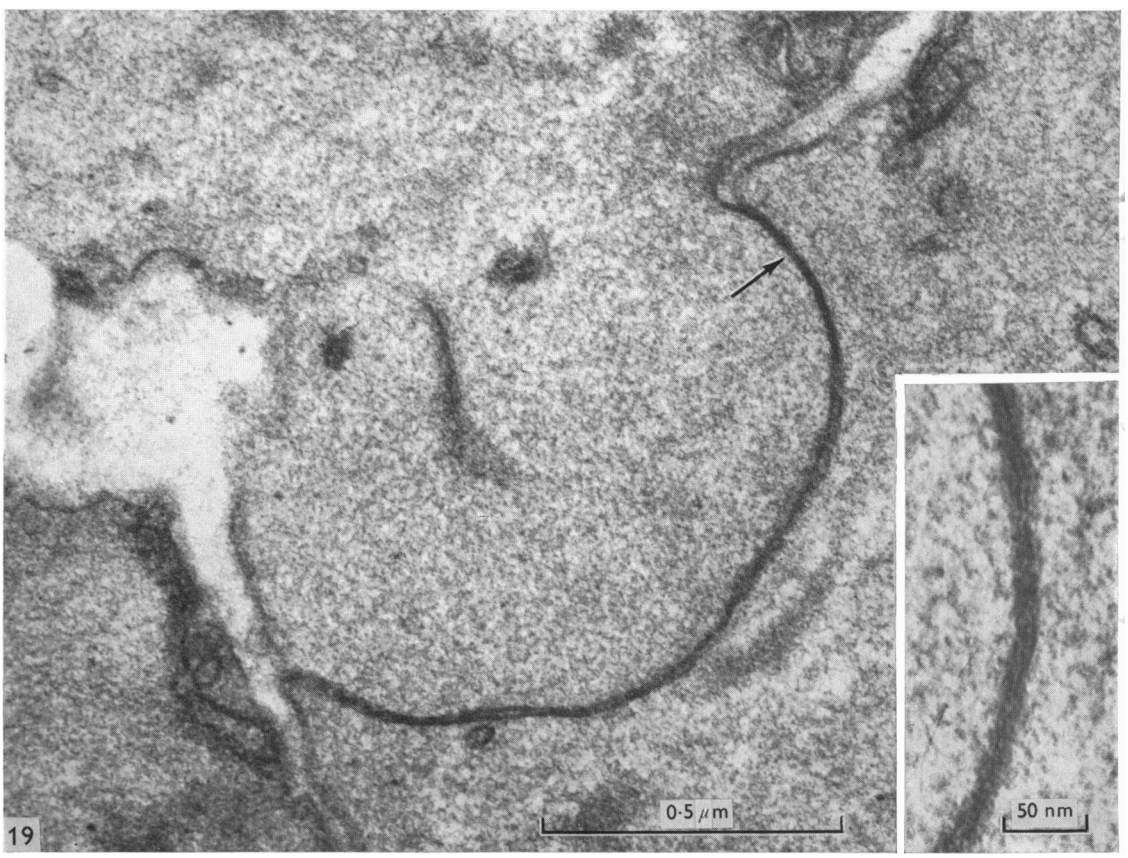


Fig. 18. Higher magnification of a side-to-end type of nexus between smooth muscle cells after treatment with hypertonic solution. The inset shows the region indicated by an arrow. Permanganate-fixed material.

Fig. 19. Higher magnification of a side-to-side type of intrusion in tissue incubated in Krebs solution. The inset shows the region indicated by an arrow. Permanganate-fixed material.

smooth muscle the appearance of the myofilaments was not so clear after exposure to hypertonic solution as it was in normal tissue. Moreover, the dense bodies were wider and longer after treatment with hypertonic solution. It may be that the muscle proteins are modified by the increased intracellular ionic strength, which was observed by Brading & Setekleiv (1968). This might be the reason for the abolition of contractility in hypertonic solution.

SUMMARY

An electron microscopic study of the smooth muscle of the guinea-pig taenia coli freshly dissected, exposed to Krebs solution (292 m-osmole) and a hypertonic Krebs solution (620 m-osmole) was performed.

No significant fine structural differences were noticed between fresh normal tissues and tissue incubated in Krebs solution.

Tissues incubated for 1 h in two times hypertonic solution showed an enlarged extracellular space, but the nexuses between smooth muscle cells were intact. This indicates that the nexus is a stable structure and, if it is the site of an electrical low-resistance pathway between cells, such a low-resistance pathway can persist in a hypertonic solution.

After incubation in a hypertonic solution the number of plasmalemmal vesicles was increased. They were enlarged and had a chracteristic narrow-necked, flask-like shape. The dense bodies were larger and the myofilaments were less clearly defined than in normal muscle cells, in which small and large size filaments could be distinguished.

There was no evidence that the muscle was in a contracted state in a hypertonic solution.

The author wishes to thank the Wellcome Trust for a Research Fellowship and Professor Edith Bülbring for her help and encouragement during the course of this study, which was carried out under a grant from the Medical Research Council. He also wishes to thank Dr S. Bradbury for his helpful criticism during the preparation of the manuscript.

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