EFFECTS OF CALCIUM DEPLETION ON THE JUNCTIONAL COMPLEX BETWEEN OXYNTIC CELLS OF GASTRIC GLANDS

ALBERT W. SEDAR, Ph.D., and JOHN G. FORTE, Ph.D.

From The Daniel Baugh Institute of Anatomy, Jefferson Medical College of Philadelphia, and the Department of Physiology, University of Pennsylvania Medical School, Philadelphia

ABSTRACT

In order to ascertain the role of calcium in maintaining the structure of the junctional complex between oxyntic cells, control gastric mucosae of the frog *Rana pipiens* were compared with those exposed to 1 to 4 mM ethylenediaminetetraacetic acid (EDTA). Changes in transmucosal potential difference and mucosal conductance were monitored. In one case a piece of EDTA-treated mucosa was washed and placed in a Ca^{++}-containing solution. Material from all three categories was prepared for electron microscopy (glutaraldehyde and OsO_4 fixation with Epon 812 embedding). Electron micrographs showed that after Ca^{++} depletion the intercellular electron-opaque material of the desmosome disappears and the walls of this component separate. Similar changes were observed in the *zonula adhaerens*. Consistent changes were difficult to detect in the *zonula occludens* although in some instances disorganization or separation within the tight junction was seen. These effects on the components of the junctional complex were reversible on readministration of Ca^{++}. The results indicate that Ca^{++} is important in maintaining the integrity of the junctional complex. A model correlating the fine structural changes with physiological data is presented.

INTRODUCTION

The importance of calcium on the permeability and secretory phenomena of cells has been well recognized (1–5). Recently, Forte and Nauss (6) have shown that the removal of calcium by a chelating agent caused large increases in the movement of substances across the mucosa which are normally restricted to the extracellular compartment (*e.g.*, sodium and sucrose). Concomitantly there was a fall in the transmucosal potential difference (PD) and a dramatic rise in the mucosal conductance, the latter approaching the same value as that of the bathing solutions. The same authors have proposed that the removal of calcium altered intercellular permeability and that the terminal bar and desmosome regions between acid-secreting cells would be likely places for a "calcium bridge."

Several authors have suggested a possible role in epithelial permeability for the components of the junctional complex between cells (7–13). This report is an attempt to establish a fine structural correlation with the observed physiological changes in permeability of the calcium-depleted gastric mucosa.

173

MATERIALS AND METHODS

The stomachs of two frogs (*Rana pipiens*) were removed and placed in physiological salt solution of the following composition: 89.3 mM NaCl; 3.4 mM KCl; 17.6 mM NaHCO$_3$; 0.2 per cent (w/v) glucose; and gassed with 5 per cent CO$_2$ and 95 per cent O$_2$. The concentration of CaCl$_2$ in one experiment (*A*) was 0.5 mM, while in the other experiment (*B*) 2.0 mM CaCl$_2$ was used. In both experiments, the gastric mucosa was removed from the underlying tunics and a portion of the tissue was immediately fixed and prepared for electron microscopy (see below). The remaining portion of the mucosa was mounted between two Plexiglass chambers and bathed with the respective physiological salt solution, and measurements of the PD and mucosal conductance were made (for details of apparatus and techniques, see Forte and Davies, 14).

These measurements were carried out for an initial control period and after the addition of ethylenediaminetetraacetic acid (EDTA; previously adjusted to pH 7.3 with NaOH) to both the nutrient (serosal) and the secretory (mucosal) bathing solutions. The initial control measurements were made until the PD and conductance had reached a fairly constant level (about 45 minutes). At this point, the procedure differed for the two experiments:

EXPERIMENT A: 1.0 mM EDTA was added to both bathing solutions and the measurements of PD and conductance were continued for 65 minutes when the mucosa was quickly removed from the chambers and fixed and prepared for electron microscopy.

EXPERIMENT B: 4.0 mM EDTA was added to both bathing solutions and the experiment was continued for 35 minutes, at which time a portion of the mucosa was fixed and prepared for electron microscopy (see Fig. 1). The remaining portion was washed once in physiological salt solution (2.0 mM CaCl$_2$) and allowed to incubate in the presence of
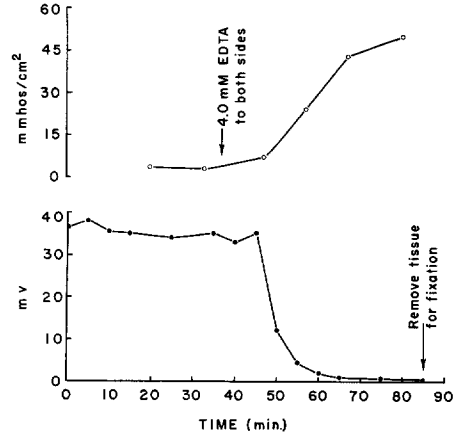

FIGURE 1 Experiment (*B*) showing effects of EDTA on the potential difference (*mv*) and mucosal conductance (*mmhos/cm^2*). The conductance is calculated as the current necessary to produce a 10-mv change across the mucosa $\left(\dfrac{\mu\text{amps/cm}^2}{\Delta \text{mv}} = \text{mmhos/cm}^2 \right)$.

calcium for 1.5 hour, after which it was also fixed and prepared for electron microscopy.

For electron microscopy, tissue specimens were placed for 1 hour, at 4°C, in 5 per cent glutaraldehyde buffered with 0.12 M Na$_2$HPO$_4$-NaH$_2$PO$_4$ at pH 7.4.[1] The tissue was then washed for 30 minutes at 4°C using three changes of the phosphate buffer. The washed specimens were then postfixed at 4°C in 1 per cent OsO$_4$ buffered at pH 7.4 with 0.12 M phosphate buffer. Rapid dehydration (10-minute intervals), starting with 50 per cent ethanol and con-

[1] Frog erythrocytes exposed to 5 per cent glutaraldehyde for as long as 1.5 hour showed no significant change in size (length and width) when compared to cells in the physiological saline (P $\gg$ 0.05).

FIGURE 2 Portions of three adjacent normal oxyntic cells facing the lumen (*L*) of a gastric gland are illustrated. The apical surface of the cells facing the lumen is differentiated into a number of microvilli or finger-like extensions of cytoplasm. The limits of the junctional complex between neighboring cells are indicated between pairs of *arrows*. An intercellular space (*is*) containing filamentous cytoplasmic projections of adjacent cells extends in a basal direction below the desmosome (*d*) of the junctional complex. Numerous mitochondria (*m*) are disposed randomly in the cytoplasm. A number of pale elliptical profiles found in the apical cytoplasm represent the zymogen granules (*z*) found in the amphibian oxyntic cell (20). The dense granules found freely scattered in the cytoplasm are identified as particulate glycogen (*G*). Granules of smaller diameter and exhibiting less electron-scattering represent the ribonucleoprotein component; characteristically it is not found in large amounts in this cell-type. The remaining portion of cytoplasm is occupied in large part by the tubular elements of the smooth surfaced endoplasmic reticulum; these elements are seen in longitudinal, oblique, and transverse planes of section. Part of a nucleus (*N*) is visible also. $\times$21,000.
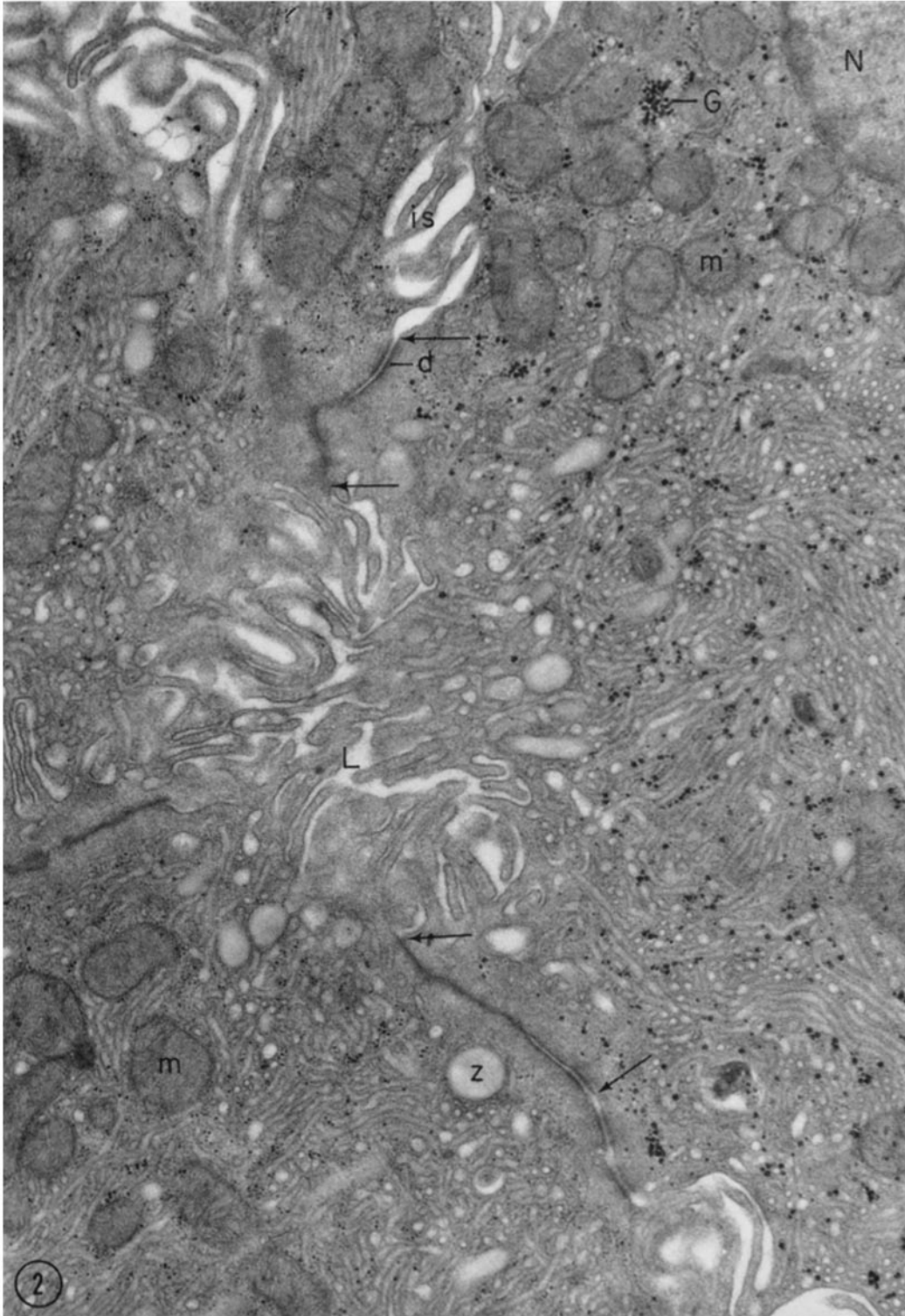
Figure 3   The junctional complex between normal oxyntic cells is shown. The desmosome (*macula adhaerens*) extends from arrow *1* to arrow *2*. The desmosome is composed of two parallel leaflets (unit membranes) separated by a distance of ~200 A; these leaflets exhibit an asymmetry, the outer membrane of the leaflet being less dense than the inner membrane. The intercellular space here contains material of a greater electron-scattering capacity than the larger intercellular space located on the basal side. A dense laminar plaque (*p*) is seen in the cytoplasm subadjacent and running parallel to each leaflet of the desmosome. The *zonula adhaerens* (intermediate junction) is located between arrow *2* and arrow *3*. The leaflets of this component, exhibiting an asymmetry similar to that of the desmosome, are arranged parallel to one another and separated by a distance of ~170 A. The intercellular material seen here is of lesser density than that contained in the desmosome of the junctional complex. The *zonula occludens* (tight junction) runs between arrows *3* and *4* adjacent to the lumen (*L*) of the gastric gland. It consists of five layers (three dark layers separated by two light layers) and contains a single central member which is less dense than but similar in diameter to either outer member. ×115,000.

tinuing through absolute ethanol (15-minute intervals) and propylene oxide (two changes in 1 hour), was performed at 4°C. Subsequent infiltration of the tissue with catalyst-containing resin was done at room temperature (~24°C), and the tissue embedded *via* the Epon embedding method of Luft using a 7:3 proportion of mixture A to mixture B with a "slow cure" (15). Sections 600 to 900 A thick (16) were cut from plastic blocks with the LKB Ultrotome and mounted on carbon-coated 150- to 200-mesh copper grids (17). These preparations were stained with lead for 10 minutes according to Millonig (18).

Electron microscopy was done with an RCA

EMU 3D containing a 1 mil platinum objective aperture and a 10 mil condenser aperture. The microscope was operated at ~100 kv and was equipped with an external bias control to regulate the amount of beam current.[2] Micrographs were obtained at original magnifications of 5,000 to 30,000 diameters and enlarged or reduced photographically as required.

---

[2] By reducing the beam current it was possible to control the amount of "beam damage" to the sections.

OBSERVATIONS

The methods employed for the preparation of the tissue for electron microscopy in these experiments provided a fine structural pattern for the oxyntic cells which was similar to that previously obtained with other methods (19–25). However, the fixation used in the present work was more favorable for the demonstration of an extensive, closely packed system of smooth surfaced tubules in the cytoplasm of the oxyntic cells (Figs. 2 and 5).
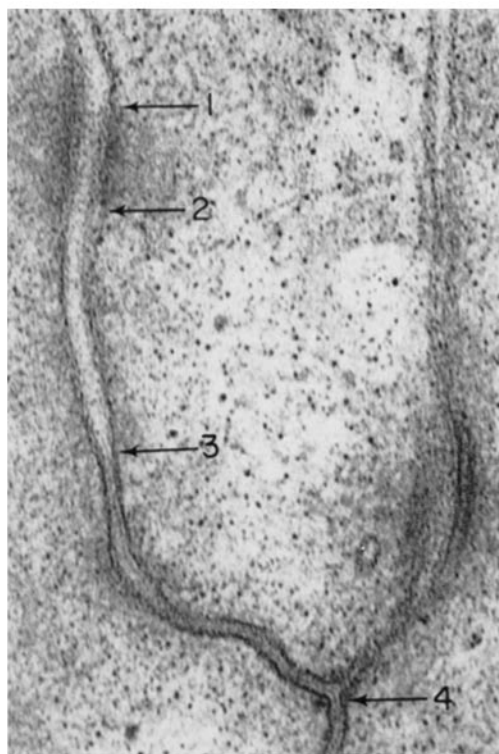

FIGURE 4 Portions of the junctional complex of three normal adjacent oxyntic cells are demonstrated. The desmosome stretches between arrows *1* and *2*, the *zonula adhaerens* between arrows *2* and *3*, and the *zonula occludens* between arrows *3* and *4*. Here the tight junction of this complex merges with the *zonula occludens* of the junctional complex of adjacent cells; the central member of this five-layered structure appears to be continuous through the union. Note in the region marked by the unnumbered arrow that the membranes of the *zonula occludens* have separated, providing an intercellular space bounded by two unit membranes. This type of separation was found infrequently in our preparations. ×115,000.

These elements are identified as the smooth surfaced endoplasmic reticulum of the cell.

JUNCTIONAL COMPLEX BETWEEN NORMAL OXYNTIC CELLS

Recently Farquhar and Palade (13) have published an extensive review of the structure of the junctional complex of various epithelia, including the gastric mucosa. These authors have proposed a terminology for the elements of the complex which will be used in this paper. Our results on oxyntic cells agree with their general findings. However, our dimensions for the intercellular spaces of the desmosome and *zonula adhaerens* are ~15 per cent lower than the corresponding values reported by these authors.

DESMOSOME (MACULA ADHAERENS): The desmosome is associated with the junctional complex in the apical zone between adjacent cells, but it is also found in intermediate and more basal zones between cells (Figs. 2 to 4). This element consists of two parallel leaflets (unit membranes as defined by Robertson, 26) separated by a distance of ~200 A and contains electron-scattering material in the intercellular space. These leaflets show an asymmetry, the inner membrane of the leaflet being more dense than the outer membrane. In the cytoplasm, subadjacent to each leaflet, a dense laminar plaque, running parallel to the membranes, is seen. Cytoplasmic fibrils are usually associated with these dense lamellae. The intercellular space is encountered below the level associated with the desmosome of the junctional complex.

ZONULA ADHAERENS (INTERMEDIATE JUNCTION): This intermediate element of the complex consists of two leaflets disposed in a parallel fashion and separated by a distance of ~ 170 A; the intercellular substance found here exhibits less electron-scattering than that found in the desmosome (Figs. 2 to 4). There is an asymmetry of the membranes of the *zonula adhaerens*, the inner membrane being more dense than outer membrane. Farquhar and Palade (13) have assumed that this element forms a continuous belt surrounding each cell.

ZONULA OCCLUDENS (TIGHT JUNCTION): This is the most apical component of the complex. It appears to be formed by the apposed membranes of adjacent cells. The two outer members of the unit membranes fuse into a single central membrane of approximately the same width as either

outer member (Fig. 3). The density of this central component is less than that of either inner member of the *zonula occludens.* These inner members are continuous with the corresponding inner component of the apical plasmalemma. (In the case of the apical cell membrane of oxyntic cells, the unit membrane consists of an inner membrane exhibiting greater density than the outer membrane.) Occasionally (Fig. 4), the membranes of the tight junction separate for a short distance, resulting in an opening bounded by two unit membranes (13).

PHYSIOLOGICAL EFFECTS OF EDTA

The changes in both the PD and conductance after the addition of EDTA were consistent with the findings previously obtained (6); the results of an experiment are graphically illustrated in Fig. 1. The chelating agent induced a marked increase in the mucosal conductance (the value of the conductance at the time the tissue was fixed for electron microscopy approached that of the Ringer's solution) and a decrease in the transmucosal PD.

JUNCTIONAL COMPLEX IN EDTA-TREATED CELLS

DESMOSOME (MACULA ADHAERENS): Seventy-seven per cent of the desmosomes measured after exposure to EDTA showed a marked separation of apposed leaflets (Figs. 5, 8, 10, and 12). In the remaining 23 per cent, a partial separation of the walls was observed in many of the desmosomes (Figs. 6 and 7). The unit membrane and associated cytoplasmic plaque of the desmosome wall are often obvious; however, in many instances the wall of the desmosome appears as a uniformly dense, amorphous structure lacking

differentiation of these elements (Fig. 7). Although it is possible that these results could be due to the plane of section, the frequency of the occurrence of the amorphous, dense appearance of the desmosome wall suggests a possible modification in structure. The intercellular dense material normally associated with the desmosome is no longer obvious in EDTA-treated preparations. It should be pointed out here that these alterations in the fine structure are seen in all desmosomes, in addition to the desmosome associated with the junctional complex (Fig. 5).

ZONULA ADHAERENS (INTERMEDIATE JUNCTION): Similar to the changes in the desmosome induced by EDTA, there is a widening of the intercellular space in the *zonula adhaerens* as a result of the separation of the apposed leaflets (Figs. 5, 6, and 9). Sacculations or invaginations of the wall into the adjacent cytoplasm(s) frequently accompany these changes (Figs. 7 and 8). When the leaflets are separated, the intercellular dense material is no longer evident in the intermediate junction. The asymmetry of the membranes does not appear to be altered by the chelating agent.

ZONULA OCCLUDENS (TIGHT JUNCTION): Structural changes in the *zonula occludens* were not so obvious and clearly seen as those observed in the other components of the complex (desmosome, *zonula adhaerens*). The dimensions and asymmetry of the EDTA-treated membranes of the tight junction are identical with those of the controls. The changes that are observed consist of local modifications within the component. For example, there appears to be an increased frequency of the openings or separations of the membranes (Figs. 10 to 12). In exceptional cases a disorganized fine

FIGURE 5   Portions of five adjacent oxyntic cells facing the lumen (*L*) of a gastric gland from an EDTA-treated mucosa are seen. The short and club-shaped apical microvilli (*mv*) observed here replace the corresponding filamentous and elongate microvilli of control preparations (see Fig. 2). At this relatively low magnification, separation of the desmosome walls (*d*) of both the desmosomes associated with the junctional complex and the more basally located desmosomes is obvious; the intercellular dense material found within the desmosome has disappeared. The walls of the desmosome at *x* are out of register. Arrows indicate places where the walls of the *zonula adhaerens* have separated; the intercellular dense material has disappeared here also. No changes in structure are noted in the *zonula occludens* (*zo*) at this magnification, but higher magnification micrographs (see Figs. 6, 11 to 13) sometimes exhibit separation of membranes in this zone. Obvious changes in structure were not observed in the smooth surfaced endoplasmic reticulum (*ER*), mitochondria (*m*), zymogen granules (*z*), and particulate glycogen (*G*). (Compare with Fig. 2). The intercellular canaliculi (*ic*) appear as typical as those in control preparations. ×21,000.
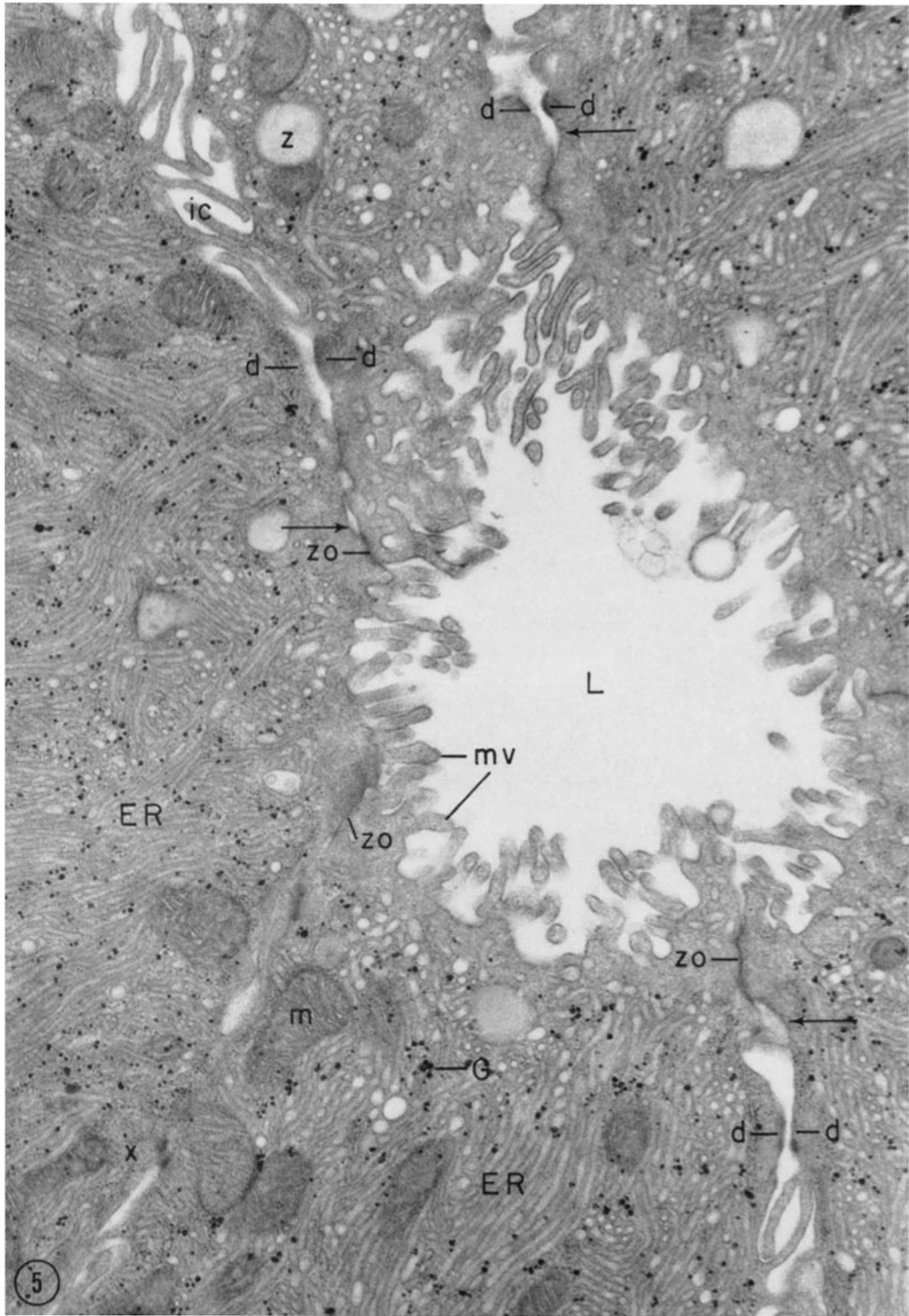
structure is seen within this zone, *e.g.* short lengths of unit membranes randomly oriented (Fig. 13). However, it should be pointed out that such images could result from tangential sectioning of membranes.

OTHER COMPONENTS OF THE CELL: No obvious changes were observed in the following components of oxyntic cells: mitochondria, smooth or rough surfaced endoplasmic reticulum, nuclei, freely scattered RNP particles, particulate glycogen, intramitochondrial granules, zymogen granules, or the asymmetry of cell membranes (including basal, lateral, and apical). However, the appearance of the microvilli on the apical surface of the cells bordering the lumen of the gastric gland were modified by EDTA. The elongate and filamentous microvilli seen characteristically in the control preparations (Fig. 2) are replaced by shortened, club-shaped extensions of cytoplasm (Fig. 5).

The Fine Structure of Oxyntic Cells Exposed to Calcium after Prior Treatment with EDTA

Electron micrographs of the junctional complex of oxyntic cells from experiment *B*, allowed to incubate in Ca^{++}-Ringer's solution after EDTA treatment, showed a fine structure for this area (Figs. 14 and 15) which was similar to that described for the normal cells. The recovery from the effect of the chelating agent was especially striking in the desmosome and the *zonula adhaerens*. The intercellular distance and the dense material contained therein were as characteristic as those found in untreated preparations.

It was noted that microvilli or apical projections of cytoplasm became more elongate and filamentous than in the normal cells. In addition, the apical cytoplasm exhibited numerous large vacuoles (as large as 3000 A) (Fig. 14).
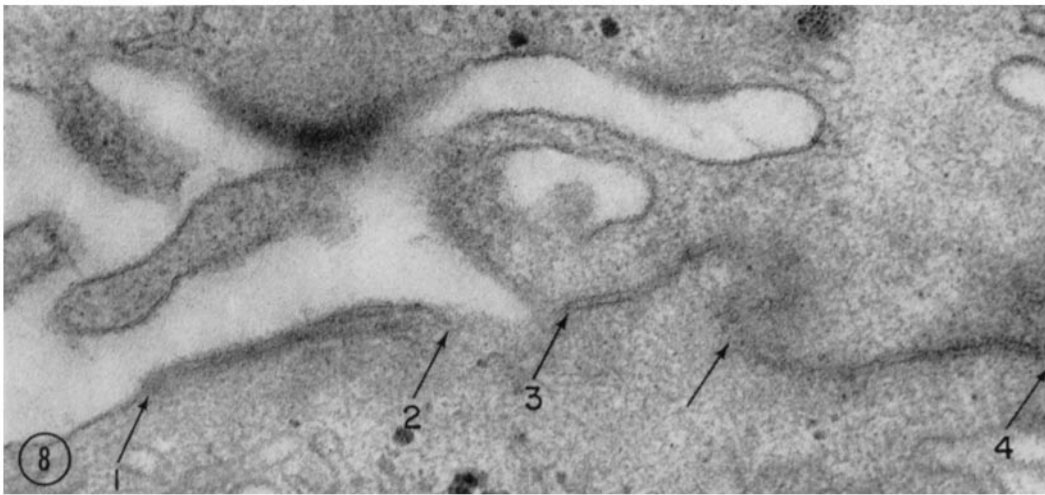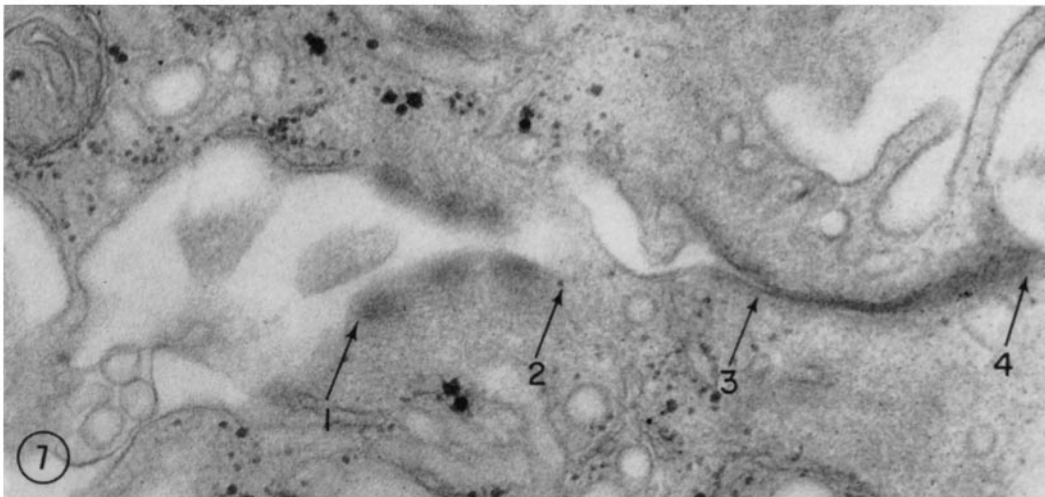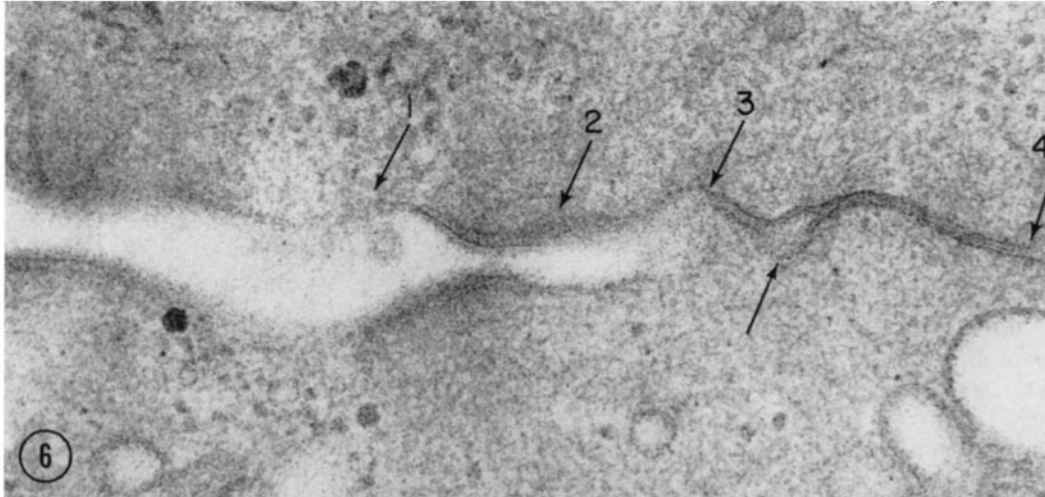
DISCUSSION

The results presented in this report concerning the fine structure of the junctional complex confirm, in general, the findings of other authors (27–31, 11, 32) who have described this structure in various epithelial cell-types. Specifically, these results, using oxyntic cells of the frog, extend the observations of Farquhar and Palade (13) to amphibian cells. However, the asymmetry noted by these authors for the plasmalemma of the oxyntic cells

---

FIGURE 6   The junctional complex between oxyntic cells from an EDTA-treated gastric mucosa is depicted. A separation of the membranes is observed, at the unnumbered arrow, within the *zonula occludens* which extends from arrow *3* to arrow *4*. Under these circumstances an intercellular opening is obvious, bounded on either side by a unit membrane. The *zonula adhaerens* is located between arrows *2* and *3*, and the desmosome of the complex between arrows *1* and *2*. The intermediate junction exhibits a separation of its walls and a relatively wide intercellular space. An alteration in structure is not conspicuous in the desmosome, although portions of the apposing walls of the structure have separated some distance. Apparently this particular desmosome was not completely affected by the chelating agent. ×99,500.

FIGURE 7   The junctional complex between neighboring oxyntic cells from an EDTA-treated gastric mucosa is represented. Although no obvious change in structure of the *zonula occludens* is seen (between arrows *3* and *4*), changes in structure of the desmosome (arrows *1* to *2*) and *zonula adhaerens* (arrows *2* to *3*) are apparent. It appears as though the leaflets and associated dense cytoplasmic plaques of the desmosome have lost their individuality. This could be due to the section plane, but the high frequency of occurrence seems to preclude the possibility (see also Fig. 5); the apposing walls of this desmosome are partially separated. The walls of the intermediate junction have separated and the intercellular space between them lacks appreciable density. ×58,500.

FIGURE 8   The junctional complex between adjacent oxyntic cells from an EDTA-treated gastric mucosa illustrates marked separation of the apposing walls of both the desmosome (arrows *1* to *2*) and the *zonula adhaerens* (arrows *2* to *3*). The tight junction extends from arrow *3* to arrow *4*. During a part of its course the section plane is parallel to the wall of this component. Here a number of tiny vesicles are seen (unnumbered arrow). This could represent a modification in membrane structure due to the chelating agent or perhaps a local manifestation of poor fixation of membrane. ×75,500.

of the rat was reversed in our results. As is well known, variations in symmetry of the plasmalemma of other cell types have been reported by a number of investigators (for further discussion and pertinent literature see reference 13).

The effects of EDTA on the structure of the desmosome and *zonula adhaerens* were more obvious than those noted on the *zonula occludens*. The marked separation of these former regions may be related to the disappearance of the dense intercellular material usually found here. It is possible that this intercellular material is still present although lacking electron-scattering properties. This could be (1) the result of a dilution of the electron-scattering substance in the enlarged intercellular space or (2) an indication that calcium itself is necessary for the electron-scattering properties of the substance.

The structure of the *zonula occludens* does not include an intercellular substance. In fact, the outer members of the apical plasmalemma appear to fuse here to form a single, central component. This arrangement presumably involves a stronger binding of adjacent cells. However, in EDTA-treated preparations there is an increased frequency of separations or openings of the membranes in this area. If these openings were continuous with the secretory surface, they could provide channels for the passage of materials between cells. Evidence for the existence of such channels

would be difficult to obtain because of the large number of serial sections required to demonstrate the continuity.

It would be useful for the cytophysiologist to know which of the components of the junctional complex is limiting for the passage of various substances between cells. Farquhar and Palade (13) have found that mass tracers (hemoglobin in kidney cells and zymogen in pancreatic cells) did not penetrate beyond the *zonula occludens*. With this observation they concluded that the *zonula occludens* was the barrier restricting flow. However, the mass tracers used in their experiments were present only on the apical side (closest to the occluding zone) and thus the evidence does not exclude other members of the complex as the major diffusion barrier. Both the *zonula adhaerens* and the *zonula occludens* are presumed to be continuous belt-like structures, encompassing cells (13). As such they would constitute a morphological barrier for diffusion. Although our results show that the changes induced by EDTA in the *zonula adhaerens* were more striking than those occurring in the *zonula occludens*, it is not possible to distinguish which of the two elements is most limiting to diffusion. On the other hand, the desmosome of the junctional complex, as well as other desmosomes found more basally, are not continuous structures surrounding the cells; they have been described as discontinuous button-like

FIGURE 9 The apical parts of three adjacent oxyntic cells from an EDTA-treated gastric mucosa are shown. The *zonula occludens* (*zo*) of the cells has fused into a wishbone-shaped figure, and otherwise appears normal in fine structure; the less dense central member of the junction appears continuous from one branch to another. The *zonula adhaerens* (*za*), on the other hand, shows a striking change in fine structure, in that its walls have separated some distance and it contains less dense intercellular material (compare with Fig. 3). The desmosome component of the junctional complex is not seen in this figure since the section plane is close to the luminal surface of the gastric gland. ×78,000.

FIGURE 10 A portion of the junctional complex between adjacent oxyntic cells from an EDTA-treated gastric mucosa is seen. The tight junction extends between arrows *3* and *4* and contains a prominent opening (unnumbered arrow). The walls of the *zonula adhaerens* and the desmosome (*d*) of the complex have separated some distance; both these elements have no visible intercellular dense material. ×100,000.

FIGURE 11 The *zonula occludens* of the junctional complex between two oxyntic cells is seen running between arrow *3* and arrow *4*. The mucosa had been exposed to EDTA and showed a marked increase in mucosal conductance prior to fixation of the tissue. Unnumbered arrows designate openings within the tight junction. The openings are bounded by unit membranes. If these openings were continuous with the secretory surface of the cell, they could provide a pathway for diffusion of substances. ×121,000.
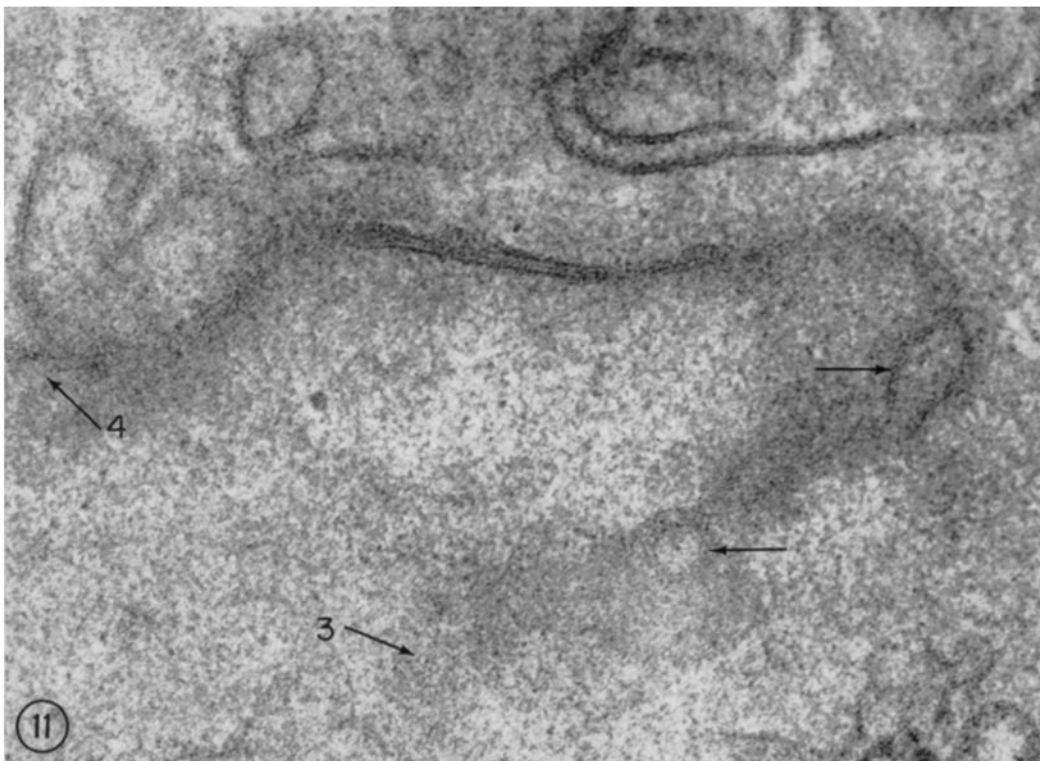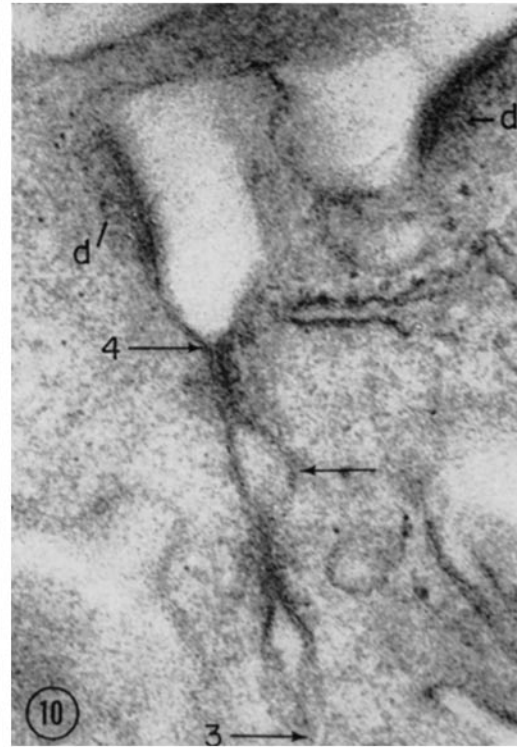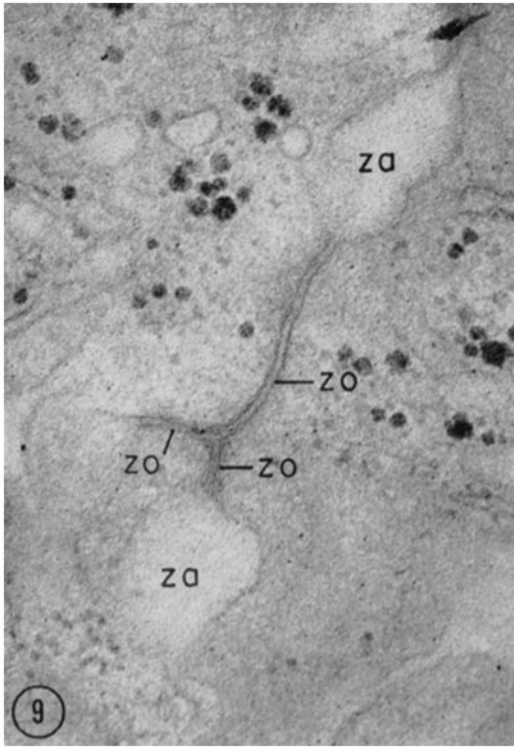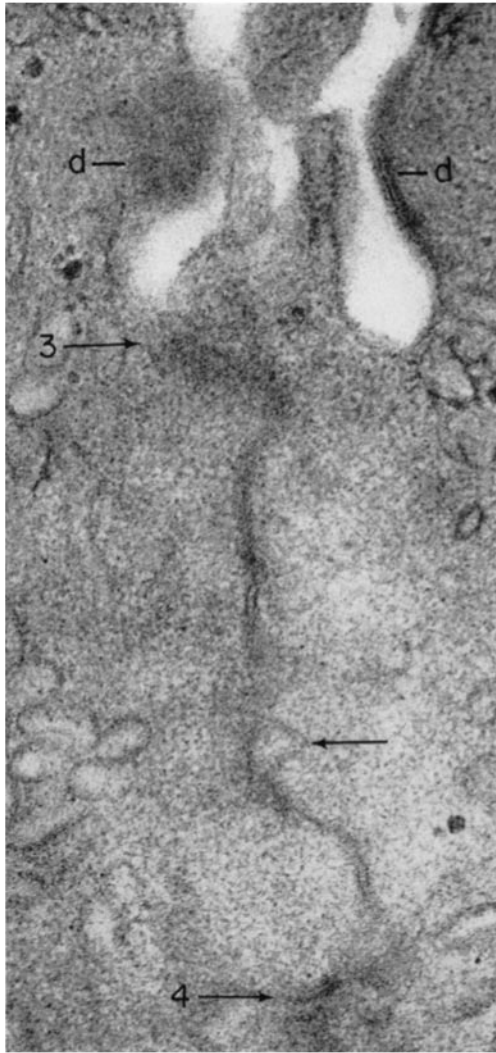
FIGURE 12 Another example of an opening (unnumbered arrow) within the tight junction of the junctional complex between oxyntic cells of the EDTA-treated gastric mucosa; the *zonula occludens* extends between arrows *3* and *4* although the exact position of arrow *3* is difficult to determine because of the section plane. The walls of the *macula adhaerens* (*d*) have separated some distance. The chelating agent appears to produce more obvious changes in fine structure of the desmosome of the junctional complex than of the *zonula occludens*. × 75,500.

structures (13, 32). Therefore, the desmosomes would not constitute an effective barrier to diffusion, in that materials could pass around them.

The desmosomes undoubtedly are points of attachment between the lateral apposed walls of cells. Without these zones of contact, the cells presumably become more plastic, as evidenced by both separated desmosomes and desmosomes out of register (Fig. 5). It is interesting to note that Dornfield and Owczarzak (33) have found evidence for the loss of cell membrane rigidity in EDTA-treated tissue culture cells.

Electron micrographs showed normal structure and dimensions for the components of the junctional complex in the experiments where calcium incubation followed EDTA treatment. This demonstrates that the effects of the chelating agent are reversible and that calcium ions are necessary for the preservation of the fine structures of the junctional complex.

The nature of the calcium complex which maintains structural and physiological integrity in the epithelium of the gastric glands is at present unknown. However, calcium specificity is indicated, in that magnesium, strontium, or barium cannot restore the calcium-depleted mucosa (6). Katchalsky (34) has pointed out that binding of divalent ions to macromolecules, such as proteins, may be either an electrostatic loose binding or a strong and specific binding. He suggested that the majority of anionic sites on a protein molecule would favor the former binding with calcium, but a few stereospecific regions (*e.g.* 2 carboxyls flanked by 2 hydroxyls) permit binding far stronger than the ordinary electrostatic type. It should be pointed out that the calcium "bridge" complex need not be *via* carboxyl groups alone, but other groups, such as phosphoryl, sulfhydryl, and sulfonate, may be involved.

Several authors have suggested that the region of the junctional complex in epithelial tissue serves as the intercellular barrier across which chemical and electrical gradients are maintained (8, 9, 11, 12). In the present experiments we noted dramatic changes in the resistance and PD induced by EDTA. These alterations were consistent with earlier observations (6) which also included measurements of the passage of sucrose and sodium across the mucosa. From all of these data, it is reasonable to conclude that EDTA produces a marked increase in the diffusion of materials between cells. The fine structural changes in the junctional complex are consistent with these physiological data.

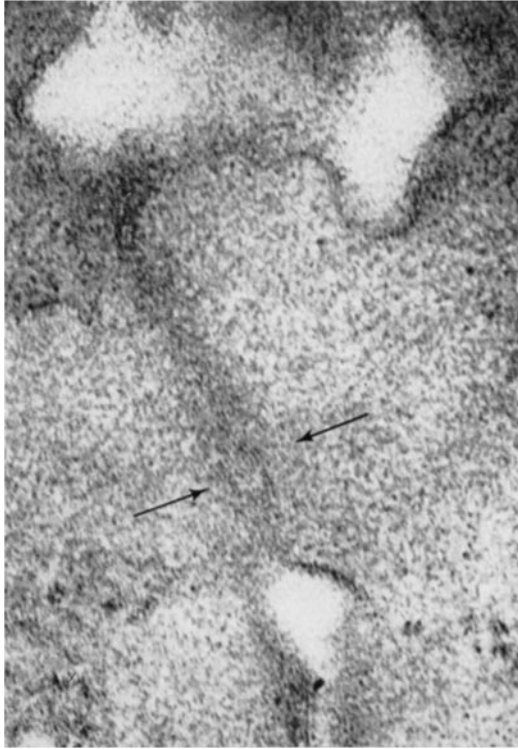A schematic model to correlate the physiological and morphological findings is presented in Fig. 16.

Figure 13 This micrograph demonstrates a disorganized fine structure within the tight junction between oxyntic cells of the EDTA-treated gastric mucosa. Note short lengths of unit membranes (arrows) which have a random orientation in this component of the junctional complex. × 121,000.

Both the normal junctional complex and one affected by EDTA are represented. Normally calcium maintains the integrity of the complex and allows minimal movement of charged substances between cells. Here the intercellular resistance (R_i) is greater than the resistance of the cell (R_c) and thus the PD between N and S across the mucosa (30 to 40 mv) would approach the value of the potential resulting from ion transport (e). The removal of calcium alters the fine structure of the junctional complex and results in the increased passage of charged and uncharged substances between cells. In this situation, R_i is much less than R_c, and the PD (between N' and S') approaches zero millivolts.

Our studies provide fine structural evidence for the necessary presence of calcium ions in maintaining both the junctional complex and lateral cell-to-cell attachments (desmosomes) of oxyntic cells. The importance of calcium in keeping cells together has been established for many cell-types (35–38, 12). For example, Heilbrunn (39) cites a reference of Ringer and Sainsbury (40) who commented on "the remarkable power which a minute dose of lime salt, $e.g.$ of lime phosphate, possesses in maintaining the integrity of the tissues." Since the junctional complex and desmosomes function as intercellular attachment devices in many cell types, it is reasonable to conclude the general importance of calcium ions in maintaining these structures.
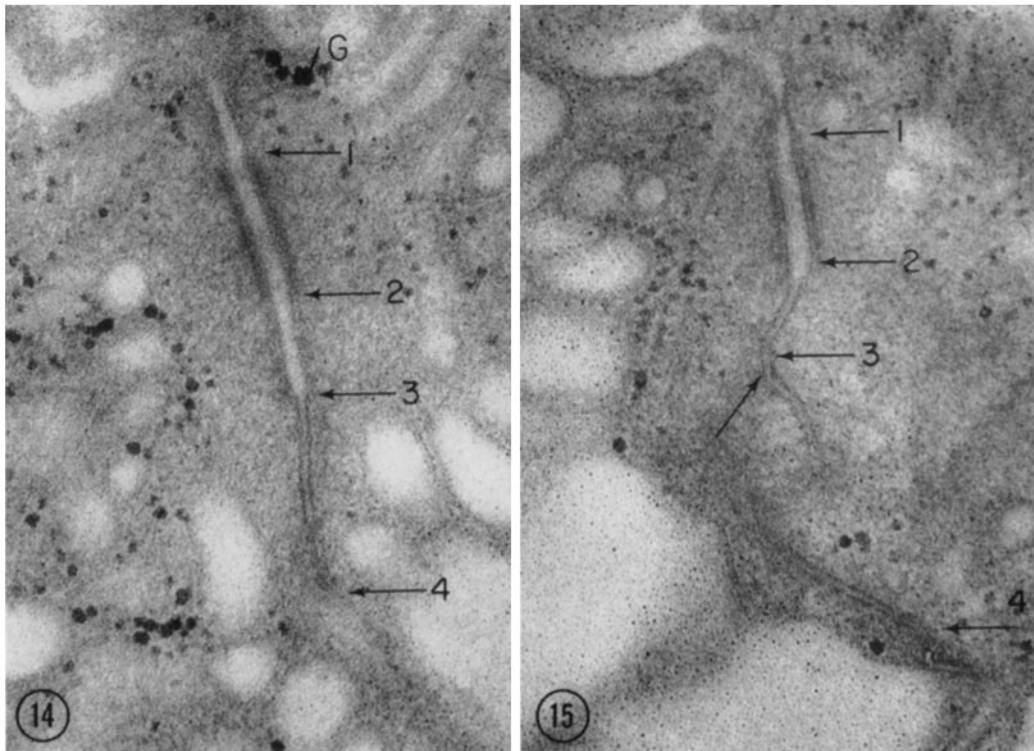
FIGURE 14   This micrograph shows the junctional complex between oxyntic cells of a gastric mucosa incubated in Ca-++-Ringer's solution after EDTA exposure. The components of the complex including the desmosome (arrows *1* to *2*), the *zonula adhaerens* (arrows *2* to *3*), and the *zonula occludens* (arrows *3* to *4*) exhibit a fine structure which is similar to that found between normal cells (compare with Fig. 3); the intercellular dense material within the desmosome is also similar to that within the intermediate junction. The cytoplasm of the cells contains a number of large vacuoles which were not observed in control or EDTA-treated preparations. Groups of large dense granules (*G*) are presumed to be particulate glycogen. ×78,000.

FIGURE 15   An example of a junctional complex between oxyntic cells of the gastric mucosa which was first incubated in an EDTA-containing Ringer's solution and then placed in a Ca++-Ringer's solution. The leaflets of the desmosome (arrows *1* to *2*) are shown to better advantage than in Fig. 14; the adjacent cytoplasmic dense plaques can be seen clearly on either side of these leaflets, separated by a less dense space. The length of the *zonula adhaerens* is short (arrows *2* to *3*). The tight junction runs a serpentine course between arrows *3* and *4*; the central member of this five-layered structure can be seen well in the region of the unnumbered arrow. The fine structure of the elements of the complex resembles that of normal cells (the fine dense particles seen in the micrograph are presumably due to "beam damage"). ×78,000.
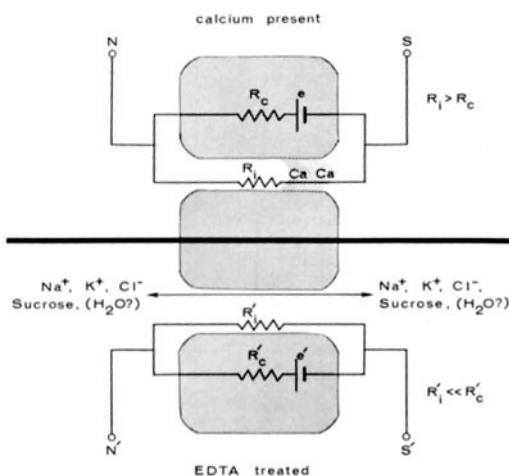
calcium present

EDTA treated

FIGURE 16 Schematic diagram showing the influence of calcium on physiological and cytological parameters of oxyntic cells. With calcium present, R_i (intercellular resistance) is greater than R_c (cellular resistance), and the PD across the mucosa (between N and S) approximates the value of e (potential resulting from cellular transport). EDTA removes calcium and alters the fine structure of the junctional complex between cells. Increased movement of materials between cells occurs and R_i' becomes much less than R_c', resulting in a decreased PD.

BIBLIOGRAPHY

1. Höber, R., Physical Chemistry of Cells and Tissues, Philadelphia, Blakiston, 1945, 243.
2. Heilbrunn, L. V., An Outline of General Physiology, Philadelphia, Saunders, 1952, 150.
3. Shanes, A. M., Electrochemical aspects of physiological and pharmacological action in excitable cells, Pharmacol. Revs., 1958, 10, parts 1 and 2, 59.
4. Whittembury, G., Sugino, N., and Solomon, A. K., Effect of anti-diuretic hormone and calcium on equivalent pore radius of kidney slice from Necturus, Nature (London), 1960, 187, 699.
5. Curran, P. F., and Gill, J. R., Jr., The effect of calcium on sodium transport by frog skin, J. Gen. Physiol., 1962, 45, 625.
6. Forte, J. G., and Nauss, A. H., Effects of calcium removal on bullfrog gastric mucosa, Am. J. Physiol., 1963, 205, 631.
7. Miller, F., Hemoglobin absorption by the cells of the proximal tubule in mouse kidney, J. Biophysic. and Biochem. Cytol., 1960, 8, 689.
8. Peachey, L. D., and Rasmussen, H., Structure of the toad's urinary bladder as related to its physiology, J. Biophysic. and Biochem. Cytol., 1961, 10, 529.
9. Gray, E. G., Ultra-structure of synapses of cerebral cortex and of certain specializations of neuroglial membranes, in Electron Microscopy in Anatomy, (J. D. Boyd, F. R. Johnson, and J. D. Lever, editors), London, Edward Arnold and Co., 1961, 54.
10. Kaye, G. I., Pappas, G. E., Donn, A., and Mallett, N., Studies on the cornea. II. The uptake and transport of colloidal particles by the living rabbit cornea in vitro, J. Cell Biol., 1962, 12, 481.
11. Muir, A. R., and Peters, A., Quintriple-layered membrane junctions at terminal bars between endothelial cells, J. Cell Biol., 1962, 12, 443.
12. Hays, R. M., and Singer, B., Oxygen consumption by toad bladder in the absence of calcium, Fed. Proc., 1963, 22, 623.
13. Farquhar, M. G., and Palade, G. E., Junctional complexes in various epithelia, J. Cell Biol., 1963, 17, 375.
14. Forte, J. G., and Davies, R. E., Oxygen consumption and active transport of ions by isolated frog gastric mucosa, Am. J. Physiol., 1963, 204, 812.
15. Luft, J. H., Improvements in epoxy embedding methods, J. Biophysic. and Biochem. Cytol., 1961, 9, 409.
16. Peachey, L. D., Thin sections. I. A study of section thickness and physical distortion produced during microtomy, J. Biophysic. and Biochem. Cytol., 1958, 4, 233.
17. Watson, M. L., The use of carbon films to support tissue sections for electron microscopy, J. Biophysic. and Biochem. Cytol., 1955, 1, 183.
18. Millonig, G., A modified procedure for lead staining of thin sections, J. Biophysic. and Biochem. Cytol., 1961, 11, 736.
19. Vial, J. D., and Orrego, H., Electron microscope observations on the fine structure of parietal cells, J. Biophysic. and Biochem. Cytol., 1960, 7, 367.
20. Sedar, A. W., Electron microscopy of the oxyntic cell in the gastric glands of the bullfrog (Rana catesbiana). I. The non-acid secreting gastric mucosa, J. Biophysic. and Biochem. Cytol., 1961, 9, 1.
21. Sedar, A. W., Electron microscopy of the oxyntic cell in the gastric glands of the bullfrog (Rana catesbiana). II. The acid-secreting gastric mucosa, J. Biophysic. and Biochem. Cytol., 1961, 10, 47.
22. Ito, S., The endoplasmic reticulum of gastric parietal cells, J. Biophysic. and Biochem. Cytol., 1961, 11, 333.
23. Sedar, A. W., The fine structure of the oxyntic

cell in relation to functional activity of the stomach, *Ann. New York Acad. Sc.*, 1962, **99**, 9.

24. SEDAR, A. W., Electron microscopy of the oxyntic cell in the gastric glands of the bullfrog, *Rana catesbiana*. III. Permanganate fixation of the endoplasmic reticulum, *J. Cell Biol.*, 1962, **14**, 152.

25. SEDAR, A. W., and FORTE, J. G., The fine structure of the histamine stimulated oxyntic cell of bullfrog *in vitro* gastric mucosa, *in* Fifth International Congress for Electron Microscopy (S. Breese, editor), New York, Academic Press, Inc., 1962, YY-4.

26. ROBERTSON, J. D., The unit membrane, *in* Electron Microscopy in Anatomy, (J. D. Boyd, F. R. Johnson, and J. D. Lever, editors), London, Edward Arnold and Co., 1961, 55.

27. FAWCETT, D. W., Structural specializations of the cell surface, *in* Frontiers in Cytology, (S. L. Palay, editor), New Haven, Yale University Press, 1958, 19.

28. ODLAND, G. F., The fine structure of the interrelationship of cells in human epidermis, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 529.

29. ZETTERQVIST, H., The ultrastructural organization of the columnar absorbing cells of the mouse intestine, Stockholm, Aktiebolaget Godvil, 1959.

30. KARRER, H. E., Cell interconnections in normal human cervical epithelium, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 181.

31. HAMA, K., The fine structure of the desmosomes in frog mesothelium, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 575.

32. TAMARIN, A., and SREEBNY, L. M., An analysis of desmosome shape, size, and orientation by the use of histometric and densitometric methods with electron microscopy, *J. Cell Biol.*, 1963, **18**, 125.

33. DORNFIELD, E. J., and OWCZARZAK, A., Surface responses in cultured fibroblasts elicited by ethylenediaminetetraacetic acid, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 243.

34. KATCHALSKY, A., *in* Membrane Transport and Metabolism, (A. Kleinzeller, and A. Kotyk, editors), London, Academic Press, Inc., 1961, 329.

35. HERBST, C., Über das Auseinandergehen von Furchungs und Gewebezellen in kalkfreiem Medium, *Arch. Entwcklungsmech.*, 1900, **9**, 424.

36. MEYER, H. H., Über die Wirkung des Kalkes, *Münch. med. Woch.*, 1910, **57**, 2277.

37. GRAY, J., The properties of an intercellular matrix and its relation to electrolytes, *Brit. J. Exp. Biol.*, 1926, **3**, 167.

38. ROBERTSON, J. D., The function and metabolism of calcium in the invertebrates, *Biol. Rev.*, 1941, **16**, 106.

39. HEILBRUNN, L. V., An Outline of General Physiology, Philadelphia, Saunders, 1952, 531.

40. RINGER, S., and SAINSBURY, H., The action of potassium, sodium and calcium salts on *Tubifex* rivulorum, *J. Physiol.*, 1894, **16**, 1.

41. SEDAR, A. W., and FORTE, J. G., The influence of calcium on the junctional complex between oxyntic cells in the gastric mucosa, Abstracts of papers presented at the Third Annual Meeting of the American Society for Cell Biology, New York City, November 6 to 8, 1963, *J. Cell Biol.*, 1963, **19**, 64A.