# Effects of Octylguanidine on Cell Permeability and Other Protoplasmic Properties of *Allium cepa* Epidermal Cells[1]

Beatriz E. Gómez-Lepe[2], OkYoung Lee-Stadelmann,
Jiwan P. Palta, and Eduard J. Stadelmann
*Laboratory of Protoplasmatology, Department of Horticultural Science and Landscape Architecture, University of Minnesota, Saint Paul, Minnesota 55108*

## ABSTRACT

Effects of octylguanidine (OG) were studied on the permeability of cells of the adaxial epidermis of *Allium cepa* bulb scales to water and methyl urea and on the protoplast surface. Interference of OG with the $Ca^{2+}$ and $Al^{3+}$ action on the cell surface was also investigated.

Permeability of the cell membrane for water and methyl urea increased nearly three times in presence of OG. The effect of OG on cell permeability depended on its direct contact with the protoplast surface.

The effect of OG on the interaction between the protoplast surface and the cell wall (wall attachment) was marked and rapid; OG (225 micromolar) decreased the time for protoplast detachment in hypertonic solutions from 420 to 120 seconds. The plasmolyzed protoplasts were immediately rounded off while the controls without OG remained heavily concave.

A considerable increase in protoplast detachment time and a decrease in rounding percentage were found when cells were plasmolyzed after pretreatment with $AlCl_3$ (0.05 molar for 2 minutes). This effect was partially reversed by KCl which was further enhanced by addition of OG.

Penetration of OG into the mesoplasm was manifested only after 10 to 15 minutes. Vacuolization and swelling of the protoplasm, fragmentation of the protoplast, and aggregation of the spherosomes, however, were observed only 30 minutes after transfer. No evidence for penetration of OG into the vacuole was found.

The results support earlier suggestions that OG acts primarily on the protoplast surface by interacting with membrane proteins as well as with phospholipids. In several aspects, OG acts on the cell surface similarly to a surfactant.

Octylguanidine has been shown to inhibit $K^+$ transport in excised roots of *Hordeum vulgare* (5) and *Avena sativa* seedlings (6) and it has been suggested that this inhibitor acts at the cell surface level by interfering with the plasma membrane ATPase complex. This conclusion was drawn from studies conducted on multicellular structures (excised roots; 5, 6) and on subcellular fractions (mitochondria; 11). Studies at the cellular level can provide information important for understanding the nature and mechanism of $OG^3$ inhibition. So far no such studies have been undertaken. In the present investigation, we attempted to observe and study the effect of OG at the cellular level by using various

plasmolytic techniques. We hoped that the results from direct microscopic evaluations of the living cell would allow us to determine a possible permeation (passage) of OG from the external solution to the cytoplasm, as well as effects of OG on the surface properties of the plant protoplasts. This type of research may also contribute to a better understanding of cell membrane structure and function.

Plasmolytic methods have long been used to study the permeability of plant cells for water and nonelectrolytes, osmotic properties of cells, and viscosity and cell wall attachment (for literature see ref. 15). These methods provide the opportunity to investigate the effect of external factors such as chemicals on the behavior of the living protoplasm.

During the early part of this investigation, OG was found to have a dramatic effect on the attachment between the protoplast surface and the cell wall. Cell wall attachment has been known to increase in presence of $AlCl_3$ (1). The strength of this attachment has been related to the electrical potentials between plasmalemma and cell wall (15). Also, OG is known to inhibit $K^+$ transport (6). Therefore, one of the objectives of this study was to investigate the effect of OG on the cell wall attachment in relation to various ions, e.g. $Al^{3+}$, $Ca^{2+}$, $K^+$.

## MATERIALS AND METHODS

Onions (*Allium cepa* L., cv. Downing Yellow Globe) were grown at the University of Minnesota Sand Plain Experimental Field without the use of herbicides or growth inhibitors. The bulbs were harvested in September of 1977 and stored at 5 C. Onions used for the work were brought to room temperature 1 day before the experiments.

### PREPARATION OF THE INNER (ADAXIAL) EPIDERMIS LAYER

The upper and lower quarters of the onions were discarded and the middle portion was used for the experiments. The third scale (counting inward from the outermost fleshy scale) was selected and several incisions of about 1 × 1 cm were made on the inner surface. The scale was then infiltrated for 2 min in 250 ml of spring water. Paired adjacent sections of the inner epidermis were peeled off and used as control and treated material. These epidermal strips were preconditioned prior to experiments for 30 min in Petri dishes containing spring water (approximately 80 $\mu g/g$ $Ca^{2+}$, 30 $\mu g/g$ $Mg^{2+}$, 14 $\mu g/g$ $Na^+$, and 6 $\mu g/g$ $K^+$ at about neutral pH).

### SOLUTIONS

For the permeability experiments, cytomorphological alteration and cell wall attachment studies, the solutions were prepared in spring water or otherwise in deionized $H_2O$. Osmolarities were determined by a freezing point depression osmometer (Advanced

[2] Present address: Instituto de Biología, Departmento de Biología Experimental, Universidad Nacional de México, México 20 D.F., México.
[3] Abbreviation: OG: octylguanidine.

Digimatic 3D). Chemicals were of reagent grade. OG was prepared using the procedure of Phillips and Clarke (12) by Dr. A. Peña, Instituto de Biología, Departmento de Biología Experimental, Universidad Nacional de México.

## PERMEABILITY MEASUREMENTS

A plasmometric method (15) was used. The epidermal sections were first plasmolyzed in 0.5 osmolar mannitol and were transferred after 30 min into 0.8 osmolar mannitol. The cells were deplasmolyzed in equiosmolar methyl urea solutions for methyl urea permeability studies and in slightly hypertonic mannitol solutions (0.5 osmolar) for water permeability measurements. A perfusion chamber (20) fixed on the stage of a Reichert Zetopan microscope was used during plasmolysis or deplasmolysis. Specific experimental conditions and the composition of the solutions are given under "Results" and in the table and figure legends. The change in length of the plasmolyzed protoplast during the time course of deplasmolysis was recorded using an eyepiece micrometer. In water permeability experiment, the protoplast expands in response to osmotic water flux and reaches a final length when the equilibrium between outside (0.5 osmolar) and inside concentration is reached. The time needed for reaching this equilibrium was designated as equilibrium time $T_{eq}$. Water and methyl urea permeability constants were calculated from the plot of protoplast length *versus* time (14, 15). Osmotic ground values were calculated as indicated (15). Two to four cells were evaluated for each treatment and the experiments were repeated at least three times.

## WALL ATTACHMENT AND PROTOPLASMIC VISCOSITY

Plant protoplasts separate from the cell walls when cells are exposed to hypertonic solutions (plasmolysis). Generally this separation occurs simultaneously at many parts of the inner cell wall surface, giving rise to a concave and angular shape of the protoplast (Fig. 2B). When left in the solution for a longer period of time, the protoplast generally assumes a convex (rounded) shape (Fig. 2A). The time required to come to a stable round shape is called rounding off time (7).

The strength of the wall attachment is determined primarily by the local structural peculiarities of the cell wall and the electrical potentials present between the cell wall and external protoplasmic surface (15). The protoplasmic viscosity on the other hand is dependent on its constituents. The rounding off time and the time for initiation of plasmolysis depend on both the wall attachment and the protoplasmic viscosity (15). By the method available to date, it is impossible to separate the contribution of the individual factors.

Effect of OG was studied on: (*a*) the time for initiation of detachment of the protoplast from the cell wall during plasmolysis (= time of incipient plasmolysis [19]) observed in almost all of the cells; (*b*) the rounding percentage, *i.e.* the percentage of cells that changed from heavily concave forms to convex rounded plasmolysis in a given time period after initiation of plasmolysis; and (*c*) the rounding off time when about 90% of the cells were rounded.

For cell wall detachment experiments, preconditioned onion epidermal strips were plasmolyzed in mannitol solutions in the presence or absence of OG using the perfusion chamber. For studies on protoplast shape, the epidermal strips were plasmolyzed in mannitol solutions in Petri dishes for 30 min, blotted between two layers of tissue paper, and transferred into a few drops of equiosmolar mannitol solution containing OG on a slide. The epidermal strips were then observed directly under the microscope. At various times after this transfer, photomicrographs were taken and evaluated for the percentage of cells with rounded protoplasts.

INFLUENCE OF $K^+$, $CA^{2+}$, AND $AL^{3+}$ ON CELL WALL ATTACHMENT AND PROTOPLASMIC VISCOSITY IN RELATION TO OG AND EDTA

**Effect of Ions.** Preconditioned epidermal strips were transferred

to each of the following equiosmolar solutions (0.8 osmolar): KCl, $CaCl_2$, mannitol + 0.05 M $AlCl_3$, KCl + $CaCl_2$ (9:1 mixture of 1 M solution of each, diluted to the desired concentration [17]) and mannitol + 225 $\mu$M OG. Times for initiation of detachment of protoplast from the cell wall and rounding percentage were recorded for the epidermal strips in each of these solutions individually.

**Pretreatment with $Al^{3+}$.** Preconditioned epidermal strips were transferred into 0.05 M $AlCl_3$ for 2 min. Several of these strips were then transferred to each of the following equiosmolar solutions: mannitol, KCl, $CaCl_2$, mannitol + OG, KCl + OG, and $CaCl_2$ + OG. Detachment time and protoplast shape were recorded for epidermal strips in each of these solutions individually.

**Interactions of $K^+$, OG, and EDTA with $Ca^{2+}$.** The epidermal strips were plasmolyzed for 10 min in 0.8 osmolar $CaCl_2$ solution and several of them were subsequently transferred into each of the following equiosmolar solutions: KCl, mannitol + 225 $\mu$M OG, mannitol + 10 mM EDTA, and mannitol + 225 $\mu$M OG + 10 mM EDTA. Rounding percentages at different times after transfer were recorded individually in each of these solutions.

Each series of these experiments were repeated at least three times to assure reproducibility.

## CYTOMORPHOLOGICAL ALTERATIONS AND CELL VIABILITY

The cytomorphological alterations occurring during plasmolysis in mannitol + 225 $\mu$M OG were closely observed and photographed. Cell viability was tested by the ability of the cells to plasmolyze and deplasmolyze, by protoplasmic streaming and vital staining with uranin (10).

# RESULTS

## METHYL UREA PERMEABILITY

When plasmolyzed cells were transferred to equiosmolar solutions of methyl urea, the protoplast started expanding because of the permeation of methyl urea from the outside solution into the vacuole (15). Examples of the time course of the protoplast expansion are shown in Figure 1. OG was added either to the plasmolyzing mannitol solution (Fig. 1A) or to the methyl urea solution, *i.e.* during deplasmolysis (Fig. 1C). The first part of the time course of deplasmolysis (approximately up to 9 min after transfer into the methyl urea + OG solution, and up to 25 to 30 min for control cells) shows a linear relationship between protoplast length and time, as may be expected from normal passive permeation processes (15). This linear part of the curve was used for calculation of the permeability constants (Table I). Data in Table I are from a single experiment. The experiment was repeated three times and similar results were obtained.

OG had no effect on methyl urea permeability ($K_s$) when it was present only during plasmolysis. This is clear from the initial slopes in Figure 1, A and B, as well as from the average $K_s$ values of $2.61 \times 10^{-7}$ and $2.87 \times 10^{-7}$ cm s$^{-1}$ for OG-treated and control cells, respectively. When OG was present during deplasmolysis, it increased the $K_s$ values for methyl urea to more than three times (from $2.92 \times 10^{-7}$ to $9.39 \times 10^{-7}$ cm s$^{-1}$), compared to the untreated cells. Also, there was a large variability in the $K_s$ values between different cells when OG was added during deplasmolysis (Table I).

For most of the OG-treated cells, the protoplast length *versus* time curves did not show a linear relationship after about 9 to 12 min in methyl urea (Fig. 1, A and C). This nonlinearity was observed occasionally in untreated control cells but only after about 25 min in methyl urea (Fig. 1B). These breaks in the curves are usually an indication of alteration in the membrane properties and a sign of damage to the cell protoplast, caused in these experiments by the combined action of OG and methyl urea.

## WATER PERMEABILITY

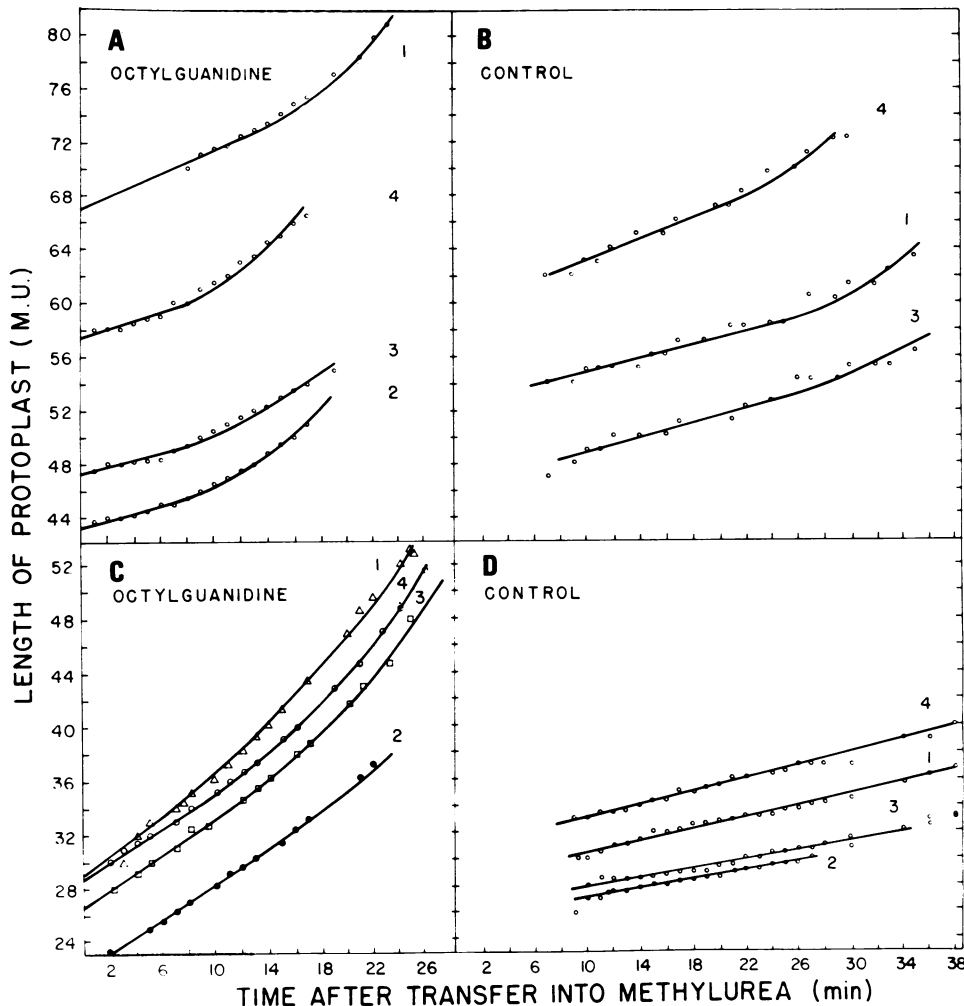A slightly higher permeability for water ($K_{wo}$) was observed

FIG. 1. Effect of OG (225 $\mu$M) on methyl urea permeability of onion epidermal cells. Cells were plasmolyzed for 1 h in mannitol solution and deplasmolyzed in equiosmolar methyl urea solution. A: OG applied during plasmolysis. C: OG applied during deplasmolysis. B and D: Controls. The length of the protoplast was measured in micrometer units. Number on the curve indicates the cell number.

Table I.  Effect of OG (225 $\mu$M) on methyl urea permeability of Allium cepa bulb scale adaxial epidermis cells.*

| | Methyl urea permeability constants $K_s$ $(10^{-7} cm\ s^{-1})$ | | | |
|---|---|---|---|---|
| Cell no. | Octylguanidine applied during plasmolysis | Control | Octylguanidine applied during plasmolysis | Control |
| 1 | 2.72 | 3.45 | 7.64 | 3.43 |
| 2 | 3.08 | -- | 11.09 | 2.45 |
| 3 | 2.52 | 2.52 | 8.77 | 2.99 |
| 4 | 2.12 | 2.57 | 10.08 | 2.82 |
| Mean | 2.6 ± 0.40 | 2.85 ± 0.52 | 9.39 ± 1.51 | 2.92 ± 0.41 |

*Experimental conditions as in Figure 1.  Data from one experiment.

almost consistently when OG was present during plasmolysis; the average $K_{wo}$ values were 8.03 × $10^{-4}$ and 6.83 × $10^{-4}$ cm s$^{-1}$ for treated and control cells, respectively (Table II). Data in Table II are from a single experiment. The experiment was repeated three times. Whereas absolute values for $K_{wo}$ varied from experiment to experiment, the difference between the treatments remained similar to that shown in Table II.

As in methyl urea permeability, when OG was present during deplasmolysis (i.e. the period when the water permeability was

measured), the increase in water permeability was much greater and $K_{wo}$ values increased to 1.6 times the average (8.57 × $10^{-4}$ to 14.37 × $10^{-4}$ cm s$^{-1}$, Table II). This increase was less striking than the one observed for methyl urea permeability under the same treatment. The equilibrium time ($T_{eq}$) was significantly shortened by both treatments and to a greater degree when OG was present during deplasmolysis. This shortening of $T_{eq}$ corresponds to the increase in water permeability observed in both treatments. The $K_{wo}$ values for treated cells are sometimes only approximate

because of the often more irregular time course for the deplasmolysis. Some protoplasts of the treated epidermis either burst or their protoplasm vacuolized, so that $K_{wo}$ and $T_{eq}$ could not be calculated (cell 2, Table II). The osmotic value of the cells (osmotic ground value) did not change significantly because of the OG treatment.

CELL WALL ATTACHMENT AND PROTOPLASMIC VISCOSITY

**Detachment Time.** The most striking effect of OG was on the detachment of the protoplast from the cell walls when the cells were plasmolyzed in the presence of OG. The time for the earliest observable detachment of the protoplast from the cell wall was

Table II. Effect of OG (225 µM) on water permeability constants ($K_{wo}$) and time for equilibrium ($T_{eq}$) for onion epidermis cells.*

| Cell no. | OG applied during plasmolysis | | Control | |
|---|---|---|---|---|
| | $K_{wo}$ $(10^{-4}$ cm s$^{-1})$ | $T_{eq}$ (min) | $K_{wo}$ $(10^{-4}$ cm s$^{-1})$ | $T_{eq}$ (min) |
| 1 | 8.69 | 12 | 7.79 | 16 |
| 2 | 8.63 | 13 | 8.08 | 16 |
| 3 | 7.39 | 12 | 6.33 | 16 |
| 4 | 7.41 | 14 | 5.14 | 16 |
| Mean | 8.03 ± 0.73 | 13 | 6.83 ± 1.37 | 16 |

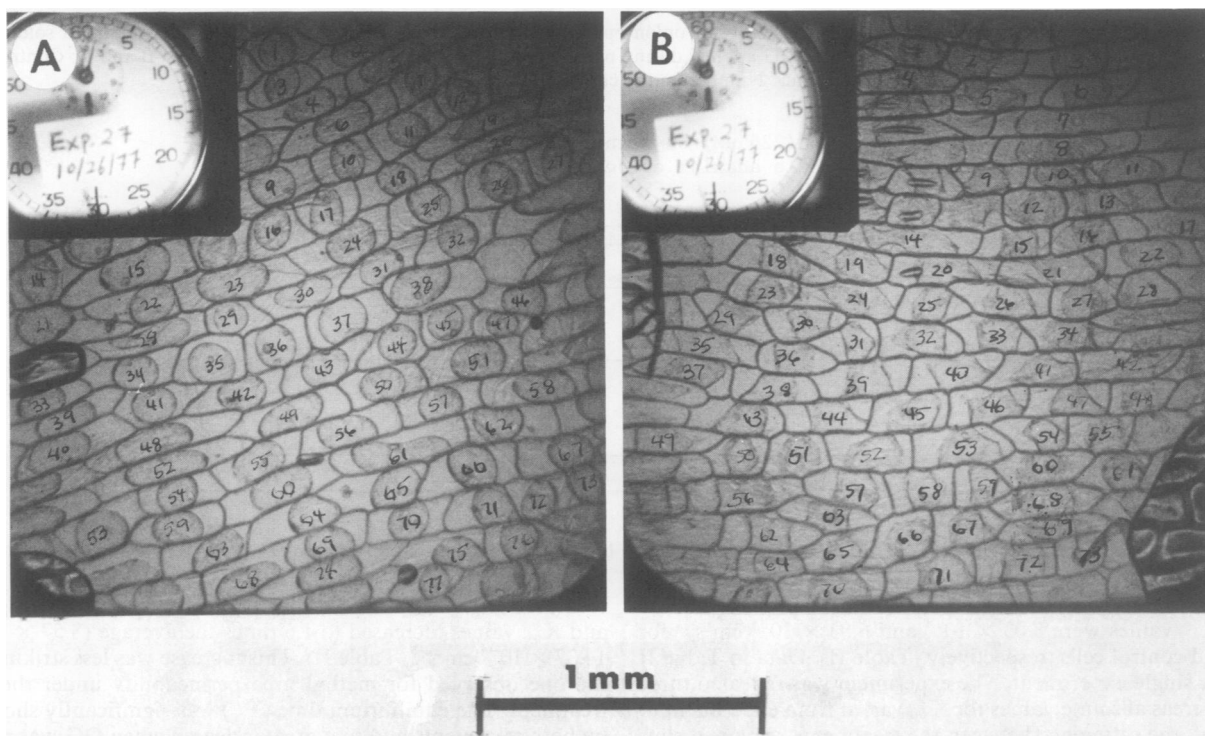| Cell no. | OG applied during deplasmolysis | | Control | |
|---|---|---|---|---|
| | $K_{wo}$ $(10^{-4}$ cm s$^{-1})$ | $T_{eq}$ (min) | $K_{wo}$ $(10^{-4}$ cm s$^{-1})$ | $T_{eq}$ (min) |
| 1 | 14.51 | 9 | 8.89 | 18 |
| 2 | damaged | -- | 9.07 | 13 |
| 3 | 13.76 | 11 | 7.75 | 15 |
| Mean | 14.37 ± 0.53 | 10 | 8.57 ± 0.72 | 15 |

*Data from one experiment.



FIG. 2. Effect of OG on the shape of plasmolyzed protoplasts. Cells were plasmolyzed for 30 min in 1.0 osmolar mannitol with (A) or without (B) 225 µM OG added.

reduced to half of the control value. Moreover, all of the proto-plasts were detached after 120 s by OG treatment compared to 420 s for controls.

**Protoplast Shape.** The plasmolyzed protoplasts of the untreated cells were generally heavily concave (Fig. 2B), but after transfer to OG containing mannitol solutions (180 $\mu$M and higher) the protoplasts were rounded off within 90 s (Fig. 2A). The percentage of rounded protoplasts at different OG concentrations is shown in Figure 3. At lower concentrations (0.45, 0.90 $\mu$M) OG had no effect on the protoplast shape. During the following 3.5 min the protoplast shape did not change significantly from the one found 90 s after transfer.

**Comparison of Ion Effects and OG.** Detachment time and rounding percentage (10, 120, and 300 s after the transfer of the cells from spring water to the plasmolyzing solutions) were tested and depended upon the kind of cation present. The following order was found for decreasing detachment time and increasing

rounding percentage:

$$\text{Mannitol} + 0.05 \text{ M AlCl}_3 \gg \text{mannitol} \geq \text{CaCl}_2$$

$$> \text{KCl} \gg \text{mannitol} + 225 \text{ }\mu\text{M OG}$$

The presence of AlCl$_3$ in the plasmolyzing mannitol solution increased the detachment time by a factor of 20 compared to OG (200 s compared to 10 s). Only 10% of the cells had rounded protoplasts 120 s after transfer when AlCl$_3$ was present compared to 100% for the cells in OG at the same time.

**Pretreatment with Al$^{3+}$.** A considerable increase in cell wall attachment was observed. Several plasmolyzing solutions were tested for their abilities to reverse the Al$^{3+}$ effect. The following order was found for the reduction of the Al$^{3+}$ effect on wall attachment:

$$\text{OG} + \text{KCl} > \text{KCl} \geq \text{mannitol} + \text{OG} > \text{CaCl}_2 \geq \text{mannitol}$$

Addition of OG to KCl enhanced the effect of KCl; CaCl$_2$ was least effective and none of the treatments reversed the AlCl$_3$ effect completely.

**Interaction of Ca$^{2+}$ with K$^+$, OG, and EDTA.** Samples plasmolyzed in CaCl$_2$ were transferred to various equiosmolar solutions. Rounding off times were in the following order:

$$\text{CaCl}_2 \geq \text{mannitol} \gg \text{mannitol} + \text{EDTA} \geq \text{mannitol} + \text{EDTA} + \text{OG}$$

$$\gg \text{mannitol} + \text{OG} \geq \text{KCl} + \text{OG}$$

Addition of OG, therefore, did not increase the effectiveness of EDTA in reducing the Ca$^{2+}$ effect on rounding percentage.

In epidermal strips pretreated for 10 min in 10 mM EDTA in spring water, the protoplasts of all cells rounded in subsequent plasmolysis with 0.8 osmolar CaCl$_2$ within 30 s compared to none in equiosmolar KCl + 225 $\mu$M OG.

### CYTOMORPHOLOGICAL ALTERATIONS

The most frequently observed cytoplasmic alterations in OG-treated (30 min) onion bulb scale epidermal cells were: aggregation of spherosomes (Fig. 4A), vacuolization (Fig. 4B), swelling of protoplasm, visibility of the ER, and formation of small partial protoplasts (Fig. 4, C and D). The latter may develop by fragmentation of the protoplast during plasmolysis or from breaking protoplasmic threads (Hecht strands). The first four alterations were generally reversible when OG was removed by washing out. In these altered cells, the ER which is usually not visible can be seen very clearly (cf. 18).

A typical plasmolysis experiment was conducted in a perfusion chamber in order to follow closely the time sequence of appear-
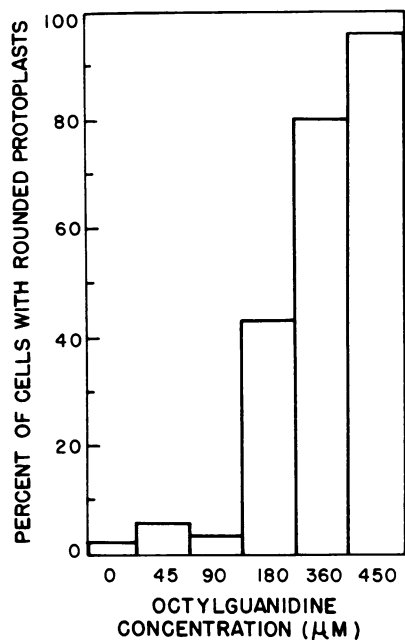


FIG. 3. Relationship between concentration of OG and the percentage of cells with rounded protoplasts. Cells were plasmolyzed for 30 min in 0.8 osmolar mannitol solutions and then transferred to an equiosmolar mannitol solution containing OG. Observations were made 90 s after this transfer.

Table III. Cytomorphological alterations produced by OG during plasmolysis of onion epidermal cells.
Cells were plasmolyzed in the perfusion chamber in 0.8M mannitol solution, in the presence of 225 µM OG. Photomicrographs were taken at different time intervals and evaluated.

|  | Time after initiation of plasmolysis (min) | Treatment | |
|---|---|---|---|
|  |  | OG | Control |
| Protoplast shape | 2 | convex | concave |
| Spherosome distribution | 34 | aggregation | homogenous |
| Small vacuoles in the protoplasm | 35 | present | absent |
| Other conditions of protoplasm | 35 | swollen, leaky | normal |
| Protoplasmic threads | 35 | formed protoplasmic droplets | present |
| Endoplasmic reticulum | 40 | visible | non-visible |
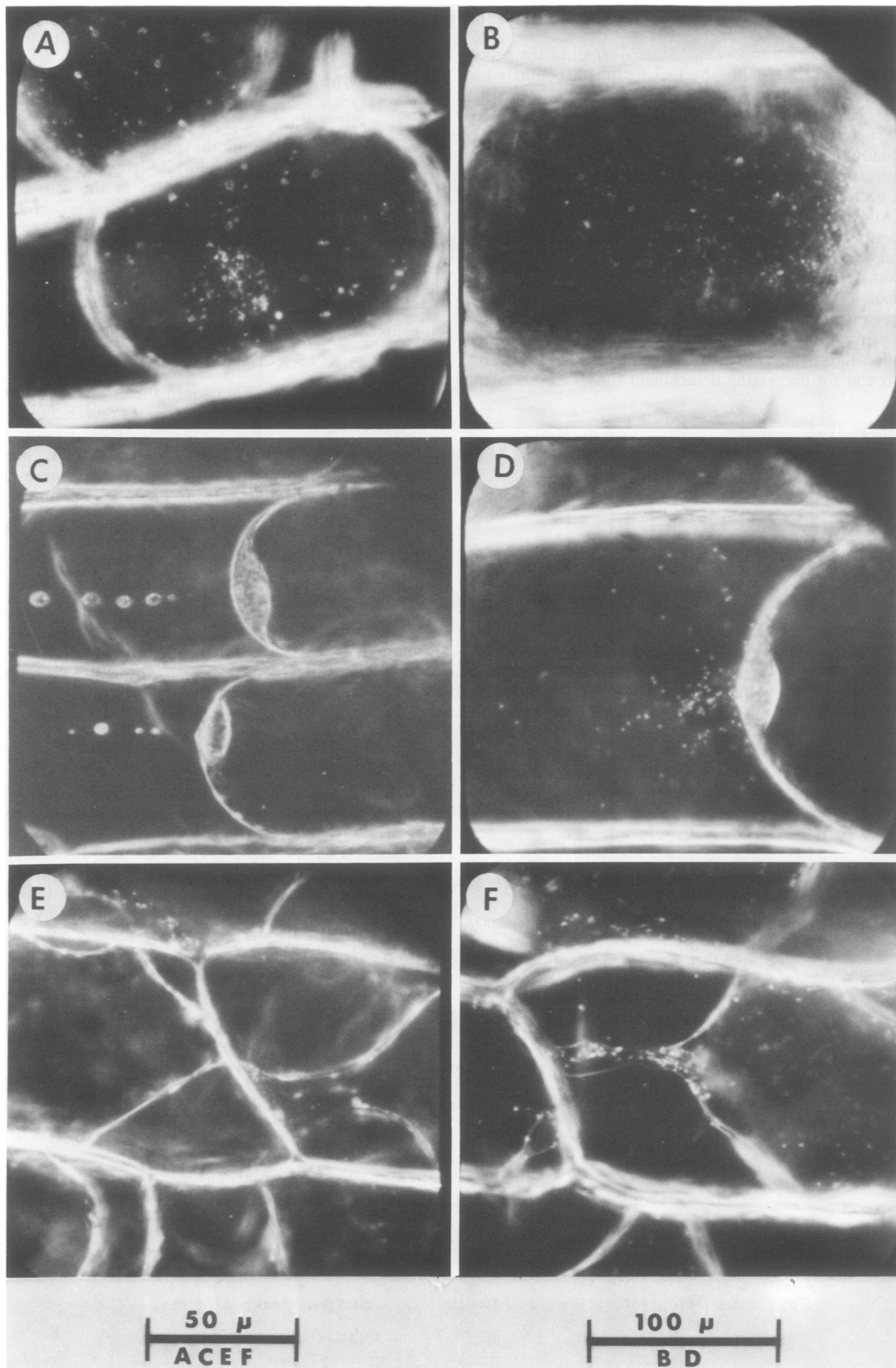
FIG. 4. Cytomorphological alterations by OG. Photomicrographs (dark field) were taken at various times after transfer to 0.8 osmolar mannitol solution containing 225 $\mu$M OG (A–D). A: Spherosome aggregation; B: vacuolization; C: protoplast swelling and formation of protoplasmic droplets; D: liberation of spherosomes; E–F: control cells in 0.8 osmolar mannitol solution.

136

ance of the cytomorphological perturbances. Results are presented in Table III. Even 24 min after transfer into the plasmolyzing solution, the control cells have a highly concave shape (Fig. 4, E and F).

**Protoplasmic Streaming.** When sections of the onion epidermis were transferred into mannitol solutions with 225 $\mu$M OG, the speed of protoplasmic streaming decreased within 15 min after transfer. After about 20 min, streaming stopped completely. The control tissue showed constant streaming during a period of at least 60 min.

**Vital Staining.** OG-treated cells initially showed normal fluorescence of the protoplasm by uranin staining. During an observation of 1 h, the fluorescence faded out gradually.

## DISCUSSION

**Effect of OG on Protoplast Surface.** A sharp reduction of the detachment time when OG is present in the plasmolyzing solution as well as the very short rounding off time (Fig. 2), indicate that the first action of OG is probably on the protoplast surface. Inasmuch as $AlCl_3$ is known to affect primarily cell wall attachment (1), the reduction of the $AlCl_3$ effect by OG is a further indication that OG influences the wall attachment. However, effect of OG on protoplasmic viscosity cannot be ruled out (see under "Materials and Methods").

How the protoplast is attached to the cell wall is still not clearly understood. It is probably the result of loose binding between cell wall $Ca^{2+}$ and membrane surface. It appears that OG in its protonized form is able to interact at the attachment sites and loosen the contact between the cell wall and protoplast surfaces by replacing $Ca^{2+}$ ions in the cell wall. This conclusion is based on the observation that a high concentration of $K^+$ is as effective as OG in reversing the $Ca^{2+}$ effect and that EDTA was most effective. The failure of OG to reverse the effect of $Ca^{2+}$ on the detachment time may be due to the small concentration of OG, compared to the high concentration of $Ca^{2+}$ applied in the experiment. Whether $Ca^{2+}$ binds with membrane proteins or phospholipids is not known, although there is some evidence that $Ca^{2+}$ binds to the polar heads of the phospholipid molecule (3, 13). Removal of the effect of $Al^{3+}$ by OG may suggest that $Al^{3+}$ and OG molecules are in competition at the protein moiety of the cell membrane. An action on cell membrane proteins has been suggested by Monné (8) as one of the mechanisms for surfactants; OG has a molecular structure similar to surfactants.

**Effect of OG on Membrane Permeability.** OG increases permeability considerably when it is present in the cell environment during the permeation process (deplasmolysis). The low effectiveness of OG on permeability when OG was available during the preceding plasmolysis may have been caused by washing out during the subsequent deplasmolysis or by a lack of opportunity for OG to interact with the membrane during the decrease of its surface area in plasmolysis.

It is now assumed that the rate of water and solute transport through the cell membrane and the tonoplast membrane is controlled by the lipid portion of the membrane (2, 16). The permeability measured is that of the whole protoplasm layer. The determining factor for this total permeability, however, is most likely the permeability of the cell membrane (cf. 17). The rapid effect of OG on the membrane permeability suggests an interaction of the hydrophobic moiety of OG with the phospholipid portion of the cell membrane. This interaction, however, is not damaging at first and does not seem to disrupt the intactness of the phospholipid layer of the membrane because the protoplast dilatation in methyl urea was proportional with time during the first 10 min. Time proportionality of protoplast dilatation during nonelectrolyte permeation is an indicator for membrane intactness (13). The enhanced exponential increase of methyl urea permea-

bility after about 10 min seems to result from secondary damages to the membrane by the OG molecules.

**Subsequent Action of OG.** Passive transport of OG, with its molecule containing a polar group can be expected to be relatively slow. OG does not inhibit respiration of excised roots in about 30 min (4), however, it does interfere with the energy metabolism of isolated mitochondria within 5 min (6). Protoplasmic streaming stopped after 20 min. Furthermore, visual cytoplasmic alterations (Fig. 4) were observed only after about 30 min (Table III). These findings suggest that the penetration of OG through the plasmalemma is slow. It can be visualized that the lipophilic moiety of the OG molecule facilitates its penetration through the plasmalemma and eventually OG reaches the different compartments of the cell.

**Mechanism of Action of OG.** The results of the present study (fast detachment of the protoplast from the cell wall, fast rounding of the protoplast and immediate change in passive permeability) complement and support the earlier findings (4–6) that OG acts on the cell surface.

Active transport of $K^+$ has been reported to be inhibited by OG (4–6). This inhibition is thought to be the result of an interaction between the charged portion of the OG molecule with the $K^+$ carrier proteins (ATPases) located at the plasmalemma (6). Furthermore, $K^+$ is also known to interact with surface proteins like ATPases (11). The observed additive effect of $K^+$ and OG on the partial reversal of the chemically induced increase in wall attachment provides additional support for the interaction of OG with surface proteins. The influence of OG on passive membrane permeability (Fig. 1, Table I, Table II) seems to indicate that OG interacts with the lipid portion of the membrane.

These findings can be explained by visualizing that the polar part of OG probably attaches itself to membrane proteins and the alkyl chain is then incorporated into the hydrophobic portion of the cell membrane. This may result in a disturbance of the spatial relationship of the protein-lipid complex which could be the cause for the effect of OG on the membrane protein for $K^+$ transport. The alkyl chain probably also greatly enhances the ability to OG to permeate eventually into the cytoplasm and act there on the proteins and lipids of the organelles as it does on the cell surface. The occurrence of pathological formations in the cytoplasm and the effect of OG on the ATPase complex of rat liver mitochondria (11) support this conclusion.

### LITERATURE CITED

1. BOEHM-TUECHY E 1960 Plasmalemma und Aluminiumsalz-Wirkung. Protoplasma 52: 108–142
2. COLLANDER R 1957 Permeability of plant cells. Annu Rev Plant Physiol 8: 335–348
3. DAWSON RMC, H HAUSER 1970 Binding of calcium to phospholipids. In AW Guthbert, ed, Calcium and Cellular Function, Academic Press, New York, pp 17–41
4. GÓMEZ-LEPE B 1976 Octylguanidine as inhibitor of barley seedling growth. Plant Physiol 57: S-430
5. GÓMEZ-LEPE B, TK HODGES 1978 Alkylguanidine inhibition of ion absorption in oat roots. Plant Physiol 61: 865–870
6. GÓMEZ-LEPE B, E JIMENEZ 1975 Alkylguanidines as inhibitors of $K^+$ transport in isolated barley roots. Plant Physiol 56: 460–463
7. HOEFLER K 1932 Plasmolyseformen bei Chaetomorpha und Cladophora. Protoplasma 16: 189–214
8. MONNÉ L 1947 The action of various detergents on the structure of the protoplasm. Ark Zool 38A(16): 1–12
9. OHKI S, D PAPAHADJOPOULOS 1970 Asymmetric phospholipid membranes: effect of pH and $Ca^{2+}$. In M Blank, ed, Surface Chemistry of Biological Systems. Plenum Publishing Corp, New York, 340 pp
10. PALTA JP, J LEVITT, EJ STADELMANN 1978 Plant viability assay. Cryobiology 15: 249–255
11. PAPA S, M TUENA DE GOMEZ-PUYOU, A GOMEZ-PYUOU 1975 On the mechanism of action of alkylguanidines on oxydative phosphorylation in mitochondria. Eur J Biochem 55: 1–8
12. PHILLIPS R, HT CLARKE 1923 The preparation of alkylguanidines. J Am Chem Soc 45: 1755–1757
13. STADELMANN EJ 1958 Zur Permeabilitaet der Epidermiszellen von Carduncellus eriocephalus. Protoplasma 50: 31–81
14. STADELMANN EJ 1963 Zu Vergleich und Umrechnung von Permeabilitaetskonstanten fuer Wasser. Protoplasma 57: 172–185
15. STADELMANN EJ 1966 Evaluation of turgidity, plasmolysis, and deplasmolysis of plant cells. In

DM Prescott, ed, Methods in Cell Physiology, 2. Academic Press, New York, pp 143-216

16. TRAEUBLE H 1971 The movement of molecules across lipid membranes. A molecular theory. J Membr Biol 4: 193-208

17. URL W 1971 The site of penetration resistance to water in plant protoplasts. Protoplasma 72: 427-447

18. URL W, F GABLER 1967 Beobachtungen an Pflanzenzellen im differentiellen Interferenzkontrast. Mikroskopie 22: 121-132

19. WEBER F 1929 Plasmolyse-Zeit-Methode. Protoplasma 5: 622-624

20. WERTH W 1961 Vergleichende Untersuchungen ueber die relative Permeabilitaet des Protoplasmas fuer Alkohol und Wasser. Protoplasma 53: 457-503