MATING IN CHLAMYDOMONAS: A SYSTEM FOR THE 
STUDY OF SPECIFIC CELL ADHESION

I. Ultrastructural and Electrophoretic Analyses of 
Flagellar Surface Components Involved in Adhesion

W. J. SNELL

From the Department of Biology, Yale University, New Haven, Connecticut 06510. Dr. Snell's present 
address is the Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218.

ABSTRACT

To determine the ultrastructural and biochemical bases for flagellar adhesiveness 
in the mating reaction in Chlamydomonas, gametic and vegetative flagella and 
flagellar membranes were studied by use of electron microscope and electrophoretic procedures. Negative staining with uranyl acetate revealed no differ-
ences in gametic and vegetative flagellar surfaces; both had flagellar membranes, 
flagellar sheaths, and similar numbers and distributions of mastigonemes. Freeze- 
cleave procedures suggested that there may be a greater density of intramem-
branous particles on the B faces of gametic flagellar membranes than on the B 
faces of vegetative flagellar membranes.

Gamone, the adhesive material that gametes release into their medium, was 
demonstrated, on the basis of ultrastructural and biochemical analyses, to be 
composed of flagellar surface components, i.e., membrane vesicles and masti-
gonemes. Comparison of vegetative (nonadhesive) and gametic (adhesive) 
“gamones” by use of SDS polyacrylamide gel electrophoresis showed both prep-
arations to be composed of membrane, mastigoneme, and some microtubule pro-
teins, as well as several unidentified protein and carbohydrate-staining compo-
nents. However, there was an additional protein of approximately 70,000 mol wt 
in gametic gamone which was not present in vegetative gamone. When gametic 
gamone was separated into a membrane and a mastigoneme fraction on CsCl 
gradients, only the membrane fraction had isoagglutinating activity; the masti-
goneme fraction was inactive, suggesting that mastigonemes are not involved in 
adhesion.

Specific cell adhesion in eucaryotes is a critically 
important cell surface process involved in gamete 
adhesion during fertilization (15, 24, 30) and 
possibly in morphogenesis (56). A variety of 
systems have been used to study specific cell 
adhesion: mating in Chlamydomonas (23, 59); 
conjugation in the ciliated protozoa Tetrahymena 
and Paramecium (29); yeast mating (7, 54, 62); 
slime mold aggregation (3, 37); sponge cell reg-
gregation (17, 33, 60) amphibian (55), chick, and 
mouse embryo cell reaggregation (32, 34, 38, 46, 
51, 52, 56); and sperm and egg interactions (15, 
24, 30). However, in only a few of these systems 
have the molecules responsible for the adhesion
The present study was initiated to further characterize the surface moieties responsible for flagellar adhesion. In this report, electrophoretic and electron microscope studies are presented which show that gamone, the adhesive material found in gamete medium, is composed of many different proteins and glycoproteins, several of which are identical to flagellar surface constituents. Moreover, although negative staining indicates that there are no differences in the surface ultrastructure of gametic (adhesive) and vegetative (non-adhesive) flagellar membranes, freeze-cleave techniques suggest that there may be differences in the density of intramembranous particles in these two types of flagella. A preliminary report of this research was presented at the thirteenth Annual Meeting of the American Society for Cell Biology (50); since then similar results have been presented by others (2, 28).

MATERIALS AND METHODS

Cultures

Stock cultures of strains 21gr (+ mating type) and 6145c (- mating type) of *Chlamydomonas reinhardtii* were maintained axenically on 1.5% agar slants supplemented with 2 g per liter of sodium acetate and 4 g per liter of yeast extract in medium 1 of Sager and Granick (41) at 15°C. Liquid cultures were inoculated from the agar stocks and were grown axenically in 250-ml flasks or 5-liter diphtheria toxin bottles in medium 1 of Sager and Granick supplemented with 3 g per liter of sodium acetate and five times the amount of phosphate buffer ("R" medium). Cultures were grown at 26°C with continuous aeration on a cycle of 13 h of light and 11 h of dark. Under these conditions the cells reproduced vegetatively (asexually) and divided synchronously during the dark part of the cycle to yield two-eight daughter cells from each parent cell (20, 61).

Harvesting of Cells

The cells were harvested from 100-ml cultures by centrifugation at 1,000 g (2,200 rpm). International Equipment Co., IEC, Needham Heights, Mass. centrifuge PR-6, rotor no. 253) for 4 min at 25°C in 50-ml conical polycarbonate centrifuge tubes. A DeLaval cream separator (model no. 104, Cow-to-Can, DeLaval Separator Co., Poughkeepsie, N. Y.) was used to harvest cells from large (4-16 liter) cultures grown in the diphtheria toxin bottles. The concentrated cells were washed out of the cream separator and concentrated by centrifugation at 1,000 g (2,200 rpm, IEC PR-6 centrifuge, rotor no. 254) for 6-8 min at 25°C in 250-ml round-bottomed polycarbonate bottles.

1 Originally obtained from Dr. Ruth Sager, Hunter College, New York.
Induction of Gametes

To form gametes, the cells were grown vegetatively in R medium to a density of 1-2 x 10^6 cells per ml and, after 6 h in the light part of their cycle, were placed in nitrogen-free medium (21). To do this, 100-ml cultures of cells were harvested in the IEC centrifuge as above, resuspended in 100 ml of Medium 1 of Sager and Granick at pH 7.6 with NH₄NO₃ omitted (M-N), and centrifuged again as above. These washed cells were then resuspended in 100 ml of fresh M-N and placed in continuous light for 15-18 h with aeration. A similar procedure was used for larger volumes of cells (4-16 liters) except that the cells were first collected in the cream separator. They were then washed with 1 liter of M-N Medium, resuspended in a volume of fresh M-N equivalent to the original culture volume, and placed in continuous light for 15-18 h with aeration.

Isolation of Flagella

The sucrose-pH shock method of Witman et al. (61) was used to isolate flagella from both gametes and vegetative cells. 4-16 liters of cells were harvested from their medium as described above, resuspended in 10 mM Tris buffer, pH 7.8 at 25°C (TB) and centrifuged at 1,100 g (2,500 rpm, IEC PR-6, rotor no. 253) for 11 min. This centrifugation sedimented the cell bodies to the bottom of the tubes and left the flagella in the 7% sucrose-TB layer. The 7% sucrose-TB layer was withdrawn by aspiration down to and including the interface, as it contained some intact flagella, diluted 1:1 with TB and centrifuged at 130,000 g (45,000 rpm, Spinco, L-2-65B centrifuge, rotor no. 50T) for 90 min to sediment the membranes. These were then resuspended in TB.

Light Microscopy

Mating cells were fixed with 2% glutaraldehyde in M-N and photographed with a Zeiss-Nomarski differential interference microscope on high contrast copy film (Eastman Kodak Co., Rochester, N. Y.) which was then developed in H & W developer.

Electron Microscopy

Negative Staining with Uranyl Acetate

Flagella and flagellar fractions: A drop of the suspension to be examined was placed on a carbon-over-Formvar-coated copper grid (Beiden Mfg. Co., S. Kilpatrick, Ill.). After 5-30 s the grid was held at a slight angle and washed with three drops of water, two drops of 0.02% cytochrome c in 1% n-amyl alcohol, and three drops of 1% uranyl acetate in H₂O. The uranyl acetate was quickly withdrawn with a piece of Whatman no. 1 filter paper, leaving a thin film of the stain on the grid, and the grid was air dried. The grids were examined in a Phillips 200 or 300 electron microscope.

Whole cells: The procedure used for negative staining of flagellar fractions did not work satisfactorily for whole cells, the cells being either swept off the grids or so heavily stained and clumped that all detail was lost. To stain whole cells a drop of a suspension of cells in their medium was placed on a carbon-over-Formvar-coated copper grid with a pasteur pipette; after 30-60 s, almost all of the drop was removed from above with a pasteur pipette and a drop of water was carefully added from above using a pipette, making sure not to let the drop fall from the grid; the water was immediately removed from the above with a pipette, leaving a film of water on the grid. A drop of 1% uranyl acetate was then placed on the grid and was very quickly and thoroughly removed from the side with a piece of Whatman no. 1 filter paper.

Collection of Flagellar Membrane Preparations with Isoagglutinating Activity

Flagella from 4-16 liters of gametes were isolated as described and resuspended in 20-30 ml of 7% sucrose-TB at 4°C; all subsequent steps were carried out at 4°C. The suspension of flagella was shaken overnight in a 50-ml round-bottomed polycarbonate centrifuge tube on a Super-mixer (Lab-line Instruments, Inc., Melrose Park, Ill.) at one-third speed to release membranes from the axonemes. 15-ml aliquots were layered over 15 ml of 40% sucrose-TB in 50-ml round-bottomed centrifuge tubes and centrifuged at 10,000 g (9,000 rpm, Sorvall RC-2 centrifuge, swinging bucket rotor no. HB-4) for 1 h to sediment axonemes and whole flagella. The supernate above the interface, containing membranes, was withdrawn (being careful to leave all of the material at the interface, as it contained some intact flagella), diluted 1:1 with TB and centrifuged at 130,000 g (45,000 rpm, Spinco, L-2-65B centrifuge, rotor no. 50T) for 90 min to sediment the membranes. These were then resuspended in TB.

Electron Microscopy

Negative Staining with Uranyl Acetate

Flagella and flagellar fractions: A drop of the suspension to be examined was placed on a carbon-over-Formvar-coated copper grid (Beiden Mfg. Co., S. Kilpatrick, Ill.). After 5-30 s the grid was held at a slight angle and washed with three drops of water, two drops of 0.02% cytochrome c in 1% n-amyl alcohol, and three drops of 1% uranyl acetate in H₂O. The uranyl acetate was quickly withdrawn with a piece of Whatman no. 1 filter paper, leaving a thin film of the stain on the grid, and the grid was air dried. The grids were examined in a Phillips 200 or 300 electron microscope.

Whole cells: The procedure used for negative staining of flagellar fractions did not work satisfactorily for whole cells, the cells being either swept off the grids or so heavily stained and clumped that all detail was lost. To stain whole cells a drop of a suspension of cells in their medium was placed on a carbon-over-Formvar-coated copper grid with a pasteur pipette; after 30-60 s, almost all of the drop was removed from above with a pasteur pipette and a drop of water was carefully added from above using a pipette, making sure not to let the drop fall from the grid; the water was immediately removed from the above with a pipette, leaving a film of water on the grid. A drop of 1% uranyl acetate was then placed on the grid and was very quickly and thoroughly removed from the side with a piece of Whatman no. 1 filter paper.
NEGATIVE STAINING WITH PHOSPHOTUNGSTIC ACID (PTA)

A drop of the material to be examined was placed on a carbon-over-Formvar-coated copper grid for 5-30 s and was withdrawn almost completely with a piece of filter paper. A drop of 0.02% cytochrome c in 1% n-amyl alcohol was added to the thin film remaining on the grid and quickly removed with a piece of filter paper, leaving a thin film of liquid. To this was added one drop of 4% PTA in 0.4% sucrose (pH 7.0 with KOH) which was immediately withdrawn to dryness.

FREEZE CLEAVING OF FLAGELLA

Flagella isolated according to the sucrose-pH shock method were fixed with 1.5% glutaraldehyde in 7% sucrose-TB for 15 min at 4°C, diluted 1:1 with TB and centrifuged for 15 min at 27,000 g (15,000 rpm, Sorvall RC-2, rotor no. SS 34) at 4°C for 20 min. The flagella were resuspended in 20% glycerol at 25°C for ½ - 1 h, placed on gold grids (Balzers High Vacuum Corp., Santa Anna, Calif.) and frozen in Liquid Freon 22 (E. I. duPont de Nemours & Co., Inc., Wilmington, Del.) cooled by liquid nitrogen. The specimens were freeze-cleaved at −115°C at 2 × 10⁻⁶ torr, and immediately after cleaving they were shadowed with platinum-carbon from a 45° angle and then with a carbon electrode from a 90° angle in a Balzers Freeze-etch apparatus (Balzers High Vacuum Corp.) according to the method of Moore and Mühlethaler (31). The specimens were digested away from the replicas by floating them onto 100% Chlorox and incubating them in the Chlorox at 25°C for 24-48 h. They were then moved into 50% Chlorox for 1-2 h, distilled water for 30 min, acetone for 30 min (the acetone step suggested by Dr. J. Hogan helps to remove any foreign material from the replicas), and finally water again for 5-15 min. The replicas were then pickled up with uncoated 200-mesh copper grids and examined in a Philips 300 electron microscope.

Gel Electrophoresis

The proteins of flagella or flagellar membrane fractions were analyzed on 3% gels with sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) according to the method of Fairbanks et al. (11). After dialysis against distilled water and lyophilization, the samples were taken up in 1% SDS, 1 mM EDTA, 40 mM dithiothreitol (DTT), 10 mM Tris, pH 8.0 and immediately heated in a boiling water bath for 2-5 min. The proteins were then separated by electrophoresis on 6 × 170-mm gels run at 6-8 mA per gel. The amount of protein layered on a gel varied from 40 to 150 μg. The gels were stained for proteins overnight in 50 ml per gel of 0.1% Coomassie brilliant blue in 25% isopropanol, 10% acetic acid. Destaining was carried out in 0.005% Coomassie brilliant blue in 10% isopropanol, 10% acetic acid for 6-8 h, followed by 10% acetic acid to complete the destaining.

To determine whether the proteins revealed by the Coomassie brilliant blue had any carbohydrate constituents, gels identical to those stained with Coomassie blue were stained using the periodic-acid-Schiff method described by Fairbanks et al. (11). The gels were photographed on a Polaroid MP-4 camera (Kodak) with 55 PN film using yellow [O(G)] and green [G(XI)] Hoya filters.

Isolation of Isoagglutinating Material (Gamone) from the Medium of Gametes

A modification of previously published methods was used to collect isoagglutinating material (gamone) from gametic cell medium (57). Gametes were induced as described above except that the cells were placed in a volume of M-N equal to one-half the volume of R medium in which they were grown. 18 h after the cells were placed in M-N they were harvested with the cream separator and the culture medium was centrifuged at 1,300 g (2,500 rpm, IEC PR-6 centrifuge, rotor no. 253) for 6 min at 25°C in 50-ml conical polycarbonate centrifuge tubes to sediment any remaining cells. The supernate was removed by aspiration and centrifuged at 18,000 g (13,000 rpm, Sorvall RC-2 centrifuge, rotor no. SS 34) at 4°C for 1 h in 50-ml round-bottomed polycarbonate centrifuge tubes. The upper 90% of this centrifugate was collected by aspiration and centrifuged at 75,200 g (31,000 rpm, Spinco L2-65B centrifuge, rotor no. 35) at 4°C for 90 min. The pellets from this centrifugation were resuspended in 10 ml of M-N, filtered through a 0.45 μm Millipore filter and centrifuged at 133,600 g (45,000 rpm, Spinco L2-65B, rotor no. 50 Ti) at 4°C for 1 h. The material sedimented by this centrifugation was resuspended in M-N and is referred to as gamone.

Collection of Vegetative "Gamone"

Cells were grown in R medium to a density of 1-2 × 10⁶ cells per ml, harvested, washed once with R medium and resuspended in R medium. 18 h later, vegetative "gamone" was collected from the culture medium exactly as described above for gametic gamone.

Determination of Protein Concentration

Proteins were determined by the Schacterle and Pollack (44) modification of Lowry et al. (25) using bovine serum albumin as a standard.

Determination of Cell Density

Chlamydomonas were fixed in Lugol’s iodine and were counted in a Levy hemocytometer (Clay Adams). At least 600 cells were counted for each density determination.

Visual Assay for Adhesion

To determine the mating ability of cells, 0.5 ml of (+) gametes were mixed with an equal volume of (−) gametes.
gametes at the same cell density and the amount of clumping (agglutination) was immediately assessed in a Zeiss phase-contrast microscope. To determine the ability of flagella or flagellar fractions to cause clumping (isoagglutination) of cells of the opposite mating type, the fraction to be tested was added to cells of the opposite mating type and the amount of clumping was assessed by phase-contrast microscopy. The flagella or flagellar fractions were also mixed with gametes of the same mating type as a control to detect any nonspecific clumping which may have occurred.

RESULTS

I. Description of the Mating Process

The cell body of *C. reinhardtii* (Fig. 1) is approximately 10 μm long and is covered with a thin glycoprotein cell wall (9). The flagella, the organelles responsible for motility and for the initial adhesion during mating, are approximately 12-14 μm long and are covered with thin, hairlike structures called mastigonemes (35) which are approximately 1 μm long and 16 nm wide. The flagella are enclosed by a plasma membrane and, just outside of the membrane, by a flagellar sheath (Fig. 2) (35, 61). The flagellar axoneme is composed of microtubules arranged in the 9 + 2 pattern typical of most eucaryotic cilia and flagella (Fig. 2).

After the cells undergo gametogenesis, which is brought about by resuspending vegetatively growing cells in N-free medium, the flagella of the resulting gametes are specifically adhesive (Fig. 3). If (+) gametes are mixed with (-) gametes the flagella of the two cell types stick together, forming large clumps of cells (Fig. 4 a). During this stage of the mating process, a cell wall lysin, liberated into the medium, brings about release of the cell walls (5, 49). This must occur before cell fusion takes place. Immediately after the release of the cell walls the flagellar collars (35, 36) slip off the flagella and are also released into the medium. Eventually, pairs of cells break away from the large clumps of cells (Fig. 4 b) and, within 2–3 min, the pairs fuse to form quadriflagellated zygotes (Fig. 4 c). The flagella, which seconds before had been highly adhesive, lose their adhesiveness, the flagellar beat becomes coordinated (13), and the zygote is motile. At some time during or after the cell wall is released the fertilization tubule is formed (Fig. 5) on (+) gametes, establishing cytoplasmic continuity between the two gametes (13). It is not clear whether the fertilization tubule is fully formed before the cell wall is lost or whether it attains its full length only after the cell wall is removed. Further study of the timing of fertilization tubule formation should be simplified by use of the whole cell negative-staining procedures reported here.

II. Comparison of Surface Ultrastructure of Vegetative and Gametic Flagella

To determine if there is an ultrastructural basis for the adhesiveness of the gametic flagella, the surface of nonadhesive flagella from (+) vegetative cells was compared to that of adhesive flagella from (+) gametic cells by use of negative-stain and freeze-cleave techniques.

NEGATIVE STAINING OF VEGETATIVE AND GAMETIC FLAGELLA: Attempts to use PTA as a negative stain for whole cells or isolated flagella led to immediate vesiculation of the flagellar membrane and apparent loss of the mastigonemes from the flagella. For these reasons uranyl acetate was used as a negative stain to visualize the flagella of whole cells. Examination of electron micrographs of flagella of (+) vegetative (nonadhesive) (Fig. 6) and (+) gametic (adhesive) (Fig. 7) cells showed no differences in the number, arrangement, or distribution of mastigonemes along the surface of the flagella. The flagellar membrane and sheath appeared to be the same in both (+) vegetative and (+) gametic cells and no structures were apparent on (+) gametic flagella that were not found on (+) vegetative flagella. Similar results were found for (-) gametic and (-) vegetative flagella. Therefore, when examined by use of uranyl acetate negative staining, all four types of flagella were ultrastructurally indistinguishable.

FREEZE CLEAVING OF VEGETATIVE AND GAMETIC FLAGELLA: Preliminary examination of freeze-cleaved flagellar membranes revealed many similarities between the vegetative and gametic membranes and possibly some differences. Both types of flagella had numerous intramembranous particles arranged at random on the A-face (outer aspect of the inner half) of the cleaved membranes (Fig. 8 and 9). There was no apparent particle alignment associated with the development of adhesiveness. On the other hand, the B faces (inner aspect of the outer half)
FIGURE 1 Electron micrograph of *Chlamydomonas* negatively stained with uranyl acetate. The mastigonemes extend laterally from the flagella. × 10,000.
**Figure 2** *Chlamydomonas* flagellar cross section showing the flagellar sheath (FS) and the flagellar membrane (MB) enclosing the 9 + 2 microtubular axoneme. × 400,000.

**Figure 3** Diagrammatic outline of *Chlamydomonas* gametogenesis, agglutination, and zygote formation.

(+) VEGETATIVE CELLS  (-) VEGETATIVE CELLS = NO AGGLUTINATION

NITROGEN-FREE MEDIUM

(+) GAMETES  (-) GAMETES = AGGLUTINATION

REMOVE GAMETES AND CENTRIFUGE MEDIUM TO OBTAIN "GAMONE"

**ZYGOTES**
FIGURE 4 Nomarski micrographs of three stages in the mating process in *Chlamydomonas.* (a) Immediately after being mixed, gametes of the two mating types adhere primarily at the tips of their flagella, forming large clumps of cells. (b) A pair of gametes in the initial stages of zygote formation. (c) Quadriflagellated zygote immediately after gametic fusion; the flagella are no longer adherent to each other. × 2,500.
of gametic membranes (Fig. 9) seemed to have many more particles than the corresponding B faces of the vegetative flagellar membranes (Fig. 8). At this time, this difference is based on qualitative observations, and a more detailed quantitative analysis of particle size and density on the A and B faces of both types of flagellar membranes is in progress.

Some exceptions to the random arrangement of particles were found in both vegetative and gametic flagellar membranes. A few of the flagella had particles aligned in rows on both the A and B faces of the membranes. In the flagellum shown in Fig. 10 there are three rows on the B face and one row on the A face, although the A face has numerous particles in a random arrangement. These rows of particles may represent attachment sites between the membrane and the underlying microtubules or they may be a reflection of attachment points for mastigonemes. Similar rows of particles have been observed on freeze-cleaved oral cilia membranes of Tetrahymena pyriformis (43).

Another feature that was apparent on the flagellar membranes of both gametic and vegetative Chlamydomonas were the rows of particles that surrounded the flagellum approximately 270 Å away from the outer surface of the flagellar membrane (Figs. 8 and 9). These particles might represent some aspect of the mastigonemes or they may be related to the extramembranous flagellar sheath. Sattler and Staehelin (43) have observed similar rows of particles associated with the surface of the oral cilia of Tetrahymena which, in thin section, have short bristles protruding from their surface.
FIGURE 6  Electron micrograph of flagellum of (+) vegetative cell negatively stained with uranyl acetate showing the mastigonemes and the flagellar sheath. × 41,000.

FIGURE 7  Electron micrograph of flagellum of (+) gametic cell negatively stained with uranyl acetate. In such negatively stained preparations (Figs. 6 and 7) (+) vegetative (nonadhesive) and (+) gametic (adhesive) flagella are indistinguishable. × 43,000.
III. Isolation of Flagellar Membrane Preparations with Isoagglutinating Activity

Various methods were used in attempt to isolate flagellar membranes with the adhesive properties of the intact, isolated flagella. Witman et al. (61) reported that *Chlamydomonas* flagellar membranes could be isolated by treating isolated flagella with the detergent Sarkosyl or by dialyzing the flagella against Tris-EDTA. However, membranes isolated from gametic flagella using these procedures had no isoagglutinating activity.

A method based on the application of shear forces to isolated flagella yielded preparations of flagellar membrane vesicles (Fig. 11 a) that had isoagglutinating activity. These membrane vesicles were not smooth but were covered with a material that appeared to be similar to the flagellar sheath.
material seen on negatively stained whole flagella (see Figs. 6 and 7). In addition to the vesicles, these membrane preparations also contained a substantial number of mastigonemes (Fig. 11 b).

IV. Electrophoretic Analysis of Flagellar Membrane Preparations

SDS PAGE of the adhesive flagellar membrane preparations revealed one major protein staining band which presumably represents the major membrane protein (Fig. 11 c and Witman et al. (61), and one or two minor bands. The most rapidly migrating minor protein was determined to be mastigoneme protein by comparison to gels of isolated, purified mastigonemes (Fig. 11 d).

V. Isolation of Isoagglutinating Material (gamone) from the Cell Culture Medium

It has been known for some time that gametes release a material into their medium, called gamone, that causes cells of the opposite mating type to isoagglutinate (12). A modification of previously published methods (57) was used to collect this isoagglutinating material from the medium of gametes. It was shown to be active in causing isoagglutination of gametes of the opposite mating type, but had no effect on gametes of the same mating type or on vegetative cells of either mating type. Examination of (+) gametic gamone, stained with uranyl acetate (Fig. 12 a) and PTA (Fig. 12...
FIGURE 12 Isolated (+) gametic gamone. (a) Negative staining with uranyl acetate (UA) shows gamone to be composed of membrane vesicles and some particulate material. The vesicles are similar to those seen in isolated flagellar membrane preparations (see Fig. 11a). × 30,000. (b) Negative staining of gamone with PTA reveals numerous mastigonemes in the gamone which are not revealed by uranyl acetate negative staining (see footnote 3). × 30,000.

b) revealed it to be composed of membrane vesicles, mastigonemes, and some particulate material. The membrane vesicles and mastigonemes of the gamone preparation were indistinguishable from those of the membrane preparations obtained from the isolated flagella described above and similarly stained (compare Figs. 11 and 12). This ultrastructural similarity, along with electrophoretic evidence presented below (Section VI), suggests that gamone is identical to flagellar surface constituents. Material isolated from vegetative cell medium by the same procedures (Fig. 13) appeared similar to gamone in that it was composed of membrane vesicles, mastigonemes and some particulate material; however, it was not active in causing isoagglutination. For convenience it is called vegetative "gamone", even though it was not adhesive.

VI. Electrophoretic Analysis of Vegetative and Gametic Gamone

(+) cell gamone: Gamone from (+) gametes was subjected to SDS PAGE and stained for protein with Coomassie brilliant blue. There was one major protein-staining band in the gamone (Fig. 14 GAM), and this band corresponded to the major membrane protein band seen on SDS gels of flagellar membranes (Fig. 14 MB). Bands corresponding to mastigoneme protein (Fig. 14 MS) and microtubule protein (Fig. 14 AX) were also seen in the gamone preparation; however, the unidentified protein

\[\text{\footnotesize W. J. SNELL} \ \text{Mating in Chlamydomonas, I} \]
Vegetative and gametic were electrophoretically identical. There was some variation from preparation to preparation in the relative amounts of several of the unidentified bands on the gels. The broad smear migrating ahead of tubulin (15+G, 70,000 mol wt) was especially prominent in this adhesive gamone preparation from (+) gametes. Plus vegetative gamone (Fig. 15+V) did not have this 70,000 mol wt band although it did have all of the other bands present in the (+) gametic gamone (Fig. 15+G).

(--) Cells: Gamone from (--)-gametic cells and gamone from (--)-vegetative cells were also analyzed electrophoretically. Negative staining revealed that (--)-gamone from both vegetative and gametic (--)-cells was composed of membrane vesicles, mastigonemes and some particulate material similar in appearance to the material isolated from (+)-vegetative and gametic cell media. Both the vegetative (Fig. 15-V) and gametic (Fig. 15-G) (--)-gamones lacked any significant amounts of band U which was present in (+)-gametic gamone (Fig. 15+G). Therefore, except for the prominence of band U in (+)-gametic gamone, all four types of gamone [(+)-vegetative and gametic and (--)-vegetative and gametic] were electrophoretically identical. There was some variation from preparation to preparation in the relative amounts of several of the unidentified bands on the gels. The broad smear migrating ahead of tubulin (15+G, 70,000 mol wt) was especially prominent in this adhesive gamone preparation from (+) gametes. Plus vegetative gamone (Fig. 15+V) did not have this 70,000 mol wt band although it did have all of the other bands present in the (+) gametic gamone (Fig. 15+G).

Figure 13 Electron micrograph of (+) vegetative gamone negatively stained with PTA. As with gametic gamone, membrane vesicles and mastigonemes are present in this material isolated from the medium of vegetative cells. x 36,000.

Figure 14 Electrophoretic analysis of (+) gametic gamone (GAM) on SDS gels shows it to be composed of several proteins. Gels of isolated (+) gametic flagellar membranes (MB), isolated mastigonemes (MS), and flagellar axonemes (AX) are included for identification of some of these proteins in the gamone (GAM) preparation. The membrane (mb), mastigoneme (ms), and tubulin (tb) bands are marked in both the gamone and the isolated preparations of these organelles. Band U, with a mol wt of approximately 70,000 daltons, is unique to (+) gametic gamone and cannot be identified in preparations of membranes (MB), mastigonemes (MS), or axonemes (AX).
FIGURE 15 Coomassie blue-stained SDS polyacrylamide gels of all four types of gamone: (+) gametic (+G); (+) vegetative (+V); (-) gametic (-G); and (-) vegetative (-V). Only (+) gametic gamone (+G) contains substantial amounts of band U; the other major bands on the gels correspond to membrane protein (mb), mastigoneme protein (ms), and tubulin (tb). The amount of tubulin in the gamone preparations varied, and, if only a small amount of tubulin was present, these long, 3% SDS gels separated the tubulin into bands representing tubulin 1 and tubulin 2 (61). The band between ms and mb* is unidentified, as are several other bands in these preparations of gamones.

FIGURE 15 Coomassie blue-stained SDS polyacrylamide gels of all four types of gamone: (+) gametic (+G); (+) vegetative (+V); (-) gametic (-G); and (-) vegetative (-V). Only (+) gametic gamone (+G) contains substantial amounts of band U; the other major bands on the gels correspond to membrane protein (mb), mastigoneme protein (ms), and tubulin (tb). The amount of tubulin in the gamone preparations varied, and, if only a small amount of tubulin was present, these long, 3% SDS gels separated the tubulin into bands representing tubulin 1 and tubulin 2 (61). The band between ms and mb* is unidentified, as are several other bands in these preparations of gamones.

VII. CsCl Density Gradient Fractionation of Gamone

To determine whether flagellar membranes or mastigonemes or both are responsible for the adhesiveness of (+) gametic gamone, the gamone was fractionated on CsCl density gradients. Gamone from (+) gametes was resuspended in 2.80 M CsCl in TB and centrifuged at 130,000 g (39,000 rpm, Spinco TL2-50 centrifuge, rotor no. SW50L) at 4°C for 24 h in 5-ml cellulose nitrate tubes. After centrifugation the bands of material visible in the gradient (Fig. 17 b inset) were withdrawn with a “J” needle and syringe and dialyzed overnight against M-N. Negative staining of the two fractions with PTA revealed that the top band was composed primarily of membrane vesicles (Fig. 17 a) with a few mastigonemes (see 17 a inset) and that the lower more dense band was composed of mastigonemes (Fig. 17 c). Uranyl acetate negative staining of the membrane fraction (Fig. 17 c) again confirmed the ultrastructural similarity of the membrane preparation obtained from isolated flagella with the membranes found in gamone. When samples from the membrane-enriched fraction of the gradient were tested for their isoagglutinating ability, they were shown to be very active. However, the mastigoneme fraction was not active in causing isoagglutination when comparable protein concentrations were tested for isoagglutinating ability.

VIII. Electrophoretic Analysis of Fractions from the CsCl Gradients

The ultrastructural analysis (above) of the two gamone fractions, indicating that they were com-
Figure 16 SDS polyacrylamide gel electrophoresis of (+) gametic (G+) and (+) vegetative (V+) gamones; the gels are stained for protein with Coomassie blue (cb) and for carbohydrate with PAS (ps). Membrane protein (mb) and mastigoneme protein (ms), as well as many of the other unidentified Coomassie blue-staining bands, also stain with PAS, indicating the presence of carbohydrate on these proteins. In addition, there are high molecular weight PAS-positive bands near the top of the gels which stain very little with Coomassie blue. They probably represent large molecular weight polysaccharides. Band U, present only on the (+) gametic gamone (G+), and tubulin (tb) do not stain with PAS.

DISCUSSION

In this report, methods have been described for the isolation and fractionation of gamone, the isoagglutinating material released from the flagella of Chlamydomonas gametes into the culture medium. Isolated flagellar membranes, gamone, and a gamone-like material from the medium of vegetative cells have been compared by use of SDS polyacrylamide gel electrophoresis, negative-stain procedures, and with a qualitative agglutination assay. Negative-stain and freeze-cleave procedures were used to examine the flagellar surfaces of vegetative and gametic cells.

Similarity of Flagellar Membranes and Gamone

Ultrastructural and electrophoretic comparison of isolated flagellar membranes with gamone obtained from the culture medium of gametes suggested that the major constituents of gamone were identical to flagellar surface components. When the gamone was observed by negative-stain procedures with the electron microscope, it was found to be similar to the isolated flagellar membrane preparation: both contained primarily membranes and mastigonemes. Perhaps of more importance, gamone and isolated flagellar membranes had

The relative amount of this unidentified protein appearing between bands ms and mb varies from preparation to preparation. Since it appears with mastigonemes on the CsCl gradients, one possible function may be to attach mastigonemes to the membrane.

posed of either membranes or mastigonemes, was corroborated by SDS PAGE. The membrane fraction (Fig. 18 MB) of (+) gametic gamone contained primarily band mb, the major membrane protein, while the mastigoneme fraction (Fig. 18 MS) contained band ms, the mastigoneme protein. In addition the band which was observed to migrate between ms and mb in preparations of membranes or gamones appeared with the mastigonemes. Both fractions of (+) gametic gamone contained a significant amount of band U, the protein prominent only in unfracticated (+) gametic gamone. Moreover, neither the membrane fraction nor the mastigoneme fraction of (+) vegetative gamone (Fig. 18, VEG) contained band U.
FIGURE 17 Fractionation of (+) gametic gamone into membrane and mastigoneme fractions by CsCl gradient centrifugation (61). (a) PTA negative staining of the membrane fraction shows it to contain primarily membrane vesicles with a few mastigonemes (see footnote 3). × 26,000. Inset: some of the membrane vesicles have attached mastigonemes. × 41,000. (b) Uranyl acetate negative staining of the membrane fraction of the gamone shows that these membrane vesicles are indistinguishable from those of the isolated flagellar membrane preparation shown in Fig. 11 a. × 28,000. (c) Electron micrograph of mastigoneme fraction from CsCl gradient negatively stained with PTA. Inset: CsCl gradient of (+) gametic gamone shows the membrane band (mb) and the mastigoneme band (ms). × 27,000.
similar patterns on SDS polyacrylamide gels: the gamone preparation was composed of those proteins known to be the major components of purified membranes and mastigonemes. The electrophoretic results are the first direct evidence that gamone contains proteins and glycoproteins identical to flagellar surface constituents. The similarity to flagellar membranes was further reinforced by the experiments which showed that (+) gametic flagellar membranes had isoagglutinating activity similar to that of (+) gametic gamone.

Although it is possible that gamone is also similar to cell body membranes as well as to flagellar membranes, two observations suggest that this is not true: (a) gamone contains mastigonemes attached to membrane vesicles, and mastigonemes have not been observed on cell body membranes, (b) the flagellum is the only part of the cell involved in isoagglutination; the cell body membrane is never observed to be involved in this process. Since gamone is specifically adhesive, it is not likely that it originates from a nonadhesive part of the cell.

A Protein Unique to (+) Gametic Gamone

No ultrastructural differences between gamones from vegetative and gametic (+) cells (Figs. 12 and 13) could be detected with uranyl acetate negative-staining procedures. However, electrophoretic analysis revealed that gametic gamone from (+) cells contained a protein (band U, Fig. 15) which was not present in the vegetative material. Moreover, neither gametic nor vegetative gamone from (−) cells contained significant amounts of this protein. This suggested that band U protein may be involved, at least in part, in the adhesive properties of (+) gametic gamone.

Because of the similarity between flagellar membranes and gamone, it was expected that the membranes purified from isolated (+) gametic flagella (section IV) would also contain band U protein. However, electrophoretic analysis of the (+) gametic flagellar membranes did not confirm this prediction, suggesting that band U is not involved in adhesion. In fact, rather than being a protein related to adhesiveness, band U may be related to some other aspect of gametogenesis. Alternatively, band U may be an artifact of the gamone isolation procedure, e.g., a proteolytic fragment of a larger protein. A more complete understanding of the origin of band U protein and of its possible role in adhesion will require further study.
Ultrastructural Comparison of Adhesive and Nonadhesive Flagella

Freeze-cleave procedures offered a means of looking for ultrastructural differences in the internal portions of (+) vegetative and (+) gametic flagellar membranes. The results showing that there may be a greater particle density on the B faces of the (+) gametic flagellar membranes than on the corresponding faces of the (+) vegetative flagellar membranes are consistent with the adhesiveness of the gametic flagella. Freeze-cleave studies by Gregg and Nesom (14) on the slime mold Dictyostelium discoideum have shown that adenosine 3':5' cyclic monophosphate, which induces aggregation in these cells, also induces an increase in intramembranous particle size. While the findings presented here relate to particle density, both results suggest a relationship between intramembranous particles and adhesiveness. Additional studies will be required to determine whether there is a functional relationship between intramembranous particles and adhesiveness in this system.

Comparison of the flagella of vegetative and gametic cells with uranyl acetate negative staining showed both to have similar flagellar membranes, flagellar sheaths, and a similar number and distribution of mastigonemes. Evidently, the changes in the flagellar surface are too subtle to be revealed by such procedures. This similarity between vegetative and gametic flagella when negatively stained with uranyl acetate conflicts with earlier reports by McLean (27) that negative staining with PTA revealed particles near the distal ends of gametic but not vegetative flagella. On occasion it was observed in the studies reported here that PTA treatment of longer than 5-10 sec caused vesiculation of the membranes of both vegetative and gametic flagella, giving the impression that these flagella had particles aligned on their surfaces. Cunningham et al. (8) have reported similar PTA-induced artifacts in studies with isolated golgi membranes. However, McLean et al. (28) have since confirmed the initial report (50) of the studies presented here that no surface differences between vegetative and gametic flagella are revealed by negative staining with either PTA or uranyl acetate under conditions that maintain the integrity of the flagellar membrane.

Fractionation of Gamone

Electrophoretic analysis of gamone fractions from CsCl gradients revealed that both the membrane and the mastigoneme fractions of (+) gametic gamone contained band U protein (Fig. 18 GAM). If band U is related to adhesiveness, this would suggest that both fractions should have isoagglutinating ability. Yet, only the membrane fraction caused isoagglutination; the mastigonemes were inactive. An agglutination assay, however, cannot rule out the possible role of mastigonemes in adhesion if mastigonemes are univalent and bind to flagella without causing isoagglutination. Structures with only one available binding site might be detected through their ability to inhibit agglutination, but since an agglutination assay only measures the number of cells interacting and not the number of binding sites interacting, such an assay might not be sensitive enough to detect univalent structures. In the following report (48) a radioactive assay is described for measuring the binding ability of gametic flagella to flagellated gametes of the opposite mating type. Eventually, by use of such a binding assay, it should be possible to directly determine which of the flagellar surface structures are capable of specifically binding to gametes of the opposite mating type.

The author would like to express his gratitude to Dr. Joel L. Rosenbaum for his guidance, advice, encouragement, and constructive criticism during the course of these investigations and preparation of the manuscript. The author would also like to acknowledge the expert guidance of Dr. James Hogan in the use of the freeze-cleave apparatus generously provided by Dr. V. T. Marchesi.

These investigations were supported by National Institutes of Health training grant HD 00032-11 to Yale University and NIH grant GM 14642 and National Science Foundation grant GB 36758 to Joel L. Rosenbaum in whose laboratory this research was carried out. The author was a recipient of an NSF predoctoral fellowship and a Connecticut State Graduate Award. These investigations were performed in partial fulfillment of the requirements for the Ph.D. degree, Yale University.

Received for publication 23 April 1975, and in revised form 22 August 1975.

REFERENCES

1. BALSAMO, J., and J. LILJEN. 1974. Embryonic cell aggregation: kinetics and specificity of binding of enhancing factors. Proc. Natl. Acad. Sci. U.S.A. 3:727–731.

2. BERGMAN, K., and U. GOODENOUGH, 1974. A
membrane-bound agglutinating substance from the flagella of Chlamydomonas reinhardii gametes. J. Cell Biol. 63:23a (Abstr.).

3. BEUG, H., F. E. KATZ, A. STEIN, and G. GERISCH. 1973. Quantitation of membrane sites in aggregating Dicyostelium cells by use of tritiated univalent antibody. Proc. Natl. Acad. Sci. U.S.A. 70:3150–3154.

4. CHANG, K. S., J. R. KATES, R. F. JONES, and N. SUEOKA. 1970. On the formation of a homogenous zygotic population in Chlamydomonas reinhardii. Dev. Biol. 22:655–669.

5. CLAES, H. 1971. Autolysis der Zellwand bei den Gameten von Chlamydomonas reinhardii. Arch. Mikrobiol. 78:180–188.

6. COLEMAN, A. W. 1962. Sexuality. In Physiology and Biochemistry of Algae. R. A. LEWIN, editor. Academic Press, Inc., New York. 711–729.

7. CRANDALL, M. A., and T. D. BROCK. 1968. Molecular aspects of specific cell contact. Science (Wash. D. C.). 161:473–475.

8. CUNNINGHAM, W. P., L. A. STAHELIN, R. W. RUBIN, R. WILKINS, and M. BONNEVILLE. 1962. Linkage maps in Chlamydomonas reinhardi. Genetics. 47:531.

9. DAVIES, D. R. 1972. Electrophoretic analyses of wall glycoproteins in normal and mutant cells. Exp. Cell Res. 73:512–516.

10. EBERSOLD, W. T., R. P. LEVINE, and M. A. OLMSTED. 1962. Linkage maps in Chlamydomonas reinhardi. Genetics. 47:531.

11. FAIRBANKS, G., T. L. STECK, and D. F. H. WALLACH. 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry. 10:2606–2616.

12. FORSTER, H., L. WIESE, and G. BRAUNITZER. 1956. Über das agglutinierend wirkende Gynogamyon von Chlamydomonas eugametos. Z. Naturforsch. Teil. B. Anorg. Chem. Biochem. Biophys. Biol. 11:315–317.

13. FRIEDMAN, I., A. L. COLWIN, and L. H. COLWIN. 1968. Fine-structural aspects of fertilization in Chlamydomonas reinhardi. J. Cell Sci. 3:115–128.

14. GREGG, J. H., and M. G. NESOM. 1973. Response of Dicyostelium plasma membranes to adenosine 3′:5′ cyclic monophosphate. Proc. Natl. Acad. Sci. U.S.A. 70:1630–1633.

15. HARTMAN, J. F., R. B. L. GWATKIN, and C. F. HUTCHINSON. 1973. Early contact interactions between mammalian gametes in vitro: evidence that the vitellus influences adherence between sperm and zona pellucida. Proc. Natl. Acad. Sci. U.S.A. 69:2767–2769.

16. HENKART, P., S. HUMPREYS, and T. HUMPREYS. 1973. Characterization of sponge aggregation factor. A unique proteoglycan complex. Biochemistry. 12:3045–3055.

17. HUMPREYS, T. 1963. Chemical dissolution and in vitro reconstruction of sponge cell adhesions. I. Isolation and functional demonstration of the components involved. Dev. Biol. 8:27–47.

18. JOHNSON, U., and K. PORTER. 1968. Fine structure of cell division in Chlamydomonas reinhardi. Basal bodies and microtubules. J. Cell Biol. 38:403–425.

19. JONES, R. F. 1970. Physiological and biochemical aspects of growth and gametogenesis in Chlamydomonas reinhardi. Ann. N.Y. Acad. Sci. 175:648–659.

20. KATES, J. R. 1966. Biochemical aspects of synchronized growth and differentiation in Chlamydomonas reinhardi. Ph.D. Thesis. Princeton University, Princeton, N. J.

21. KATES, J. R., and R. F. JONES. 1964. The control of gametic differentiation in liquid cultures of Chlamydomonas. J. Cell. Comp. Physiol. 63:157–164.

22. LEWIN, R. P., and W. T. EBERSOLD. 1960. The genetics and cytology of Chlamydomonas. Ann. Rev. Microbiol. 14:97–216.

23. LEMIN, R. A. 1954. Sex in unicellular algae. In Sex in Microorganisms. I. F. Lewis and J. R. Raper, editors. Amer. Assoc. for the Advancement of Science, Washington, D. C. 10–133.

24. LILLIE, F. R. 1913. The mechanism of fertilization. Science (Wash. D. C.). 38:524–528.

25. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265–275.

26. McCLAY, D. R., and A. A. MOSCONA. 1974. Purification of the specific cell-aggregating factor from embryonic neural retina cells. Exp. Cell Res. 87:438–443.

27. McLEAN, R. J. 1972. Studies of the gamete contact mechanism in Chlamydomonas. J. Cell Biol. 55:172a (Abstr.)

28. McLEAN, R. J., C. J. LAUGENDI, and R. M. BOWW, Jr. 1974. The relationship of gamone to the mating reaction in Chlamydomonas moewusii. Proc. Natl. Acad. Sci. U.S.A. 71:2610–2613.

29. METZ, C. B. 1954. Matin substance and the physiology of fertilization in ciliates. In Sex in Microorganisms. C. B. Metz and A. Monroy, editors. American Assoc. Adv. Sci., Washington, D. C. 84–334.

30. METZ, C. B. 1967. Gamete surface components and their role in fertilization. In Fertilization: comparative Morphology, Biochemistry and Immunology. C. B. Metz and A. Monroy, editors. Academic Press, Inc., New York. 163–224.

31. MOORE, H., and K. MÜHLETHALER. 1963. Fine structure in frozen-etched yeast cells. J. Cell Biol. 17:609–628.

32. MOSCONA, A. A. 1957. Development in vitro of chimaeric aggregates of dissociated embryonic chick and mouse cells. Proc. Natl. Acad. Sci. U.S.A. 43:184–194.
33. Moscona, A. A. 1963. Studies of cell aggregation: demonstration of materials with selective cell binding activity. Proc. Natl. Acad. Sci. U.S.A. 49:742-747.
34. Orr, C. W., and S. Roseman. 1969. Intracellular adhesion. I. A quantitative assay for measuring the rate of adhesion. J. Membrane Biol. 1:109-124.
35. Ringo, D. L. 1967. Flagellar motion and fine structure of the flagellar apparatus in Chlamydomonas. J. Cell Biol. 33:543-571.
36. Roberts, K., M. Gurney-Smith, and G. J. Hills. 1974. Ciliary membrane differentiation in Tetrahymena pyriformis. Tetrahymena has four types of cilia. J. Cell Biol. 62:473-490.
37. Schacterle, G. R., and R. L. Pollack. 1973. A simplified method for the quantitative assay of small amounts of protein in biologic material. Anal. Biochem. 51:654-655.
38. Schiemer, E. T., D. M. Baumgartel, and S. H. Howell. 1973. Gametic differentiation in Chlamydomonas reinhardtii. Cell cycle dependency and rates in attainment of mating competency. Dev. Biol. 31: 31-37.
39. Shimada, Y., A. A. Moscona, and D. A. Fischman. 1974. Scanning electron microscopy cell aggregation; cardiac and mixed retina-cardiac cell suspensions. Dev. Biol. 36:428-446.
40. Smith, G. M., and D. C. Regeney. 1950. Inheritance of sexuality in Chlamydomonas reinhardtii. Proc. Natl. Acad. Sci. U.S.A. 71:246-248.
41. Snell, W. J. 1976. Mating in Chlamydomonas. II. A radioactive flagella-binding assay for quantitation of adhesion. J. Cell Biol. 68:70-79.
42. Snell, W. J., W. L. Dentler, L. T. Haimo, L. I. Binder, and J. L. Rosenbaum. 1974. Assembly of chick brain tubulin onto isolated basal bodies of Chlamydomonas reinhardtii. Science (Wash. D.C.). 185:357-360.
43. Snell, W. J., S. A. Kroop, and J. L. Rosenbaum. 1973. Characterization of adhesive substances on the surface of Chlamydomonas gamete flagella. J. Cell Biol. 59:327a (Abstr.).
44. Steinberg, M. S. 1963. Reconstitution of tissues by dissociated cells. Science (Wash. D.C.). 141:401-408.
45. Steinberg, M. S. 1970. Does differential adhesion govern self-assemble processes in histogenesis? Equilibrium configurations and emergence of a hierarchy among populations of embryonic cells. J. Exp. Zool. 173:395-434.
46. Suez, N., K. S. Chiang, and J. R. Kates. 1967. Deoxyribo nucleic acid replication in meiosis of Chlamydomonas reinhardtii. J. Mol. Biol. 25:47-66.
47. Taylor, N. W. 1964. Specific, soluble factor involved in sexual agglutination of the yeast Hansenula wingei. J. Bacteriol. 87:863-866.
48. Townes, P. L., and J. Holtfreter. 1955. Directed movements and selective adhesion of embryonic amphibian cells. J. Exp. Zool. 128:53-118.
49. Trinkaus, J. P. 1969. Cells into organs: the forces that shape the embryo. Prentice-Hall, Inc., Englewood Cliffs, N. J.
50. Wiese, L. 1965. On sexual agglutination and mating-type substances (gamones) in isogamous heterothallic Chlamydomonas. I. Evidence of the identity of the gamones with the surface components responsible for sexual flagellar contact. J. Phycol. 1:46-54.
51. Wiese, L. 1969. Algae. In Fertilization. C. B. Metz and A. Monroy, editors. Academic Press, Inc., New York, 2:135-188.
52. Wiese, L. 1974. Nature of sex specific glycoprotein agglutinins in Chlamydomonas. Ann. N.Y. Acad. Sci. 234:383-394.
53. Wilson, H. V. 1907. On some phenomena of coalescence and regeneration in sponge. J. Exp. Zool. 5:245-258.
54. Witman, G. B., K. Carlson, J. Berliner, and J. L. Rosenbaum. 1972. Chlamydomonas flagella. I. Isolation and electrophoretic analysis of microtubules, matrix, membranes, and mastigonemes. J. Cell Biol. 54:507-539.
55. Yen, P. H., and C. E. Ballou. 1973. Composition of a specific intercellular agglutination factor. J. Biol. Chem. 248:8316-8318.