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Electron Microscope Observations of Intracellular Responses to Immobilization Antibody in Tetrahymena pyriformis

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Electron Microscope Observations of Intracellular Responses to Immobilization Antibody in *Tetrahymena pyriformis*[1]

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Abstract. Through the coupling of high resolution electron microscopy with the sensitive and flexible techniques of agar diffusion it is possible to study in great detail the nature, site, and degree of intracellular damage caused by exposure to antibody. *Tetrahymena* is well suited for this type of study since pure cultures are readily grown axenically in the laboratory. Control cells exposed to saline solutions, neutral serum, or heterologous antiserum showed few differences from normal cells. In cells treated with homologous antibody a greatly increased number of cavities appear in the cytoplasm along Pitelka's primary meridian. It is suggested that these cavities are the results of osmotic stress produced by pellicle modification by the antibody, and are therefore not entirely homologous to protrichocysts.

Literature has accumulated describing antibody responses in a variety of cells using the light microscope (Robertson, 1939; Sonneborn, 1950; Beale and Kacser, 1957; Loefer et al., 1958; Elliott and Byrd, 1959; Margolin, et al., 1959). Several studies have been made of ciliate antigen systems using agar diffusion techniques (Preer and Preer, 1959; Finger, 1960). Latta (1958, 1959), using the electron microscope, has recorded antibody reactivity throughout the embryonic chick heart fibroblast. To my knowledge no studies of antibody responses in unicellular organisms have been attempted. This paper presents evidence supporting the coupling of the extremely sensitive and flexible technique of agar diffusion with high resolution electron microscopy to study completely the nature of intra-cellular responses to antibody.

MATERALS AND METHODS

Antigen

Cultures of *Tetrahymena pyriformis* were isolated from pure clones maintained as "testors" in the laboratory of Dr. A. M. Elliott. Mating types one and two of variety one [N(1)1/I, N(1)1/II] and mating type one of variety two (H2/I) were selected for use since they had been observed in a previous study (Gurion, for Elliott, unpublished) to have intravariety cross reactivity at all dilutions of antisera based on the criterion of agglutination observable under the light microscope. Once ini-

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tial stocks had been isolated they were grown axenically in 200-ml to 1000-ml Erlenmeyer flasks for 24 to 48 hours, and all results presented here pertain to these specific cultures. Twelve hours prior to exposure to antisera, the ciliates were washed with glass-distilled water and transferred to flasks of sterile glass-distilled water. Suspensions of cells for treatment with antisera were prepared by twice washing in glass-distilled water to remove soluble contaminants and dead organisms, and finally were resuspended in glass-distilled water in a concentration of 9,000 animals/ml.

Antiserum

The initial batch of antiserum was prepared by Gurion by repeated injections, into the peritoneal cavity of rabbits, of ruptured ciliates in amounts up to 3-4 ml of packed cells.

Subsequent antisera were prepared, after final growth of the ciliates in glass-distilled water for 12 hours, by twice washing and then resuspending in 1 ml of glass-distilled water at a concentration of approximately 0.95% protein. The cells were lysed by repeated freezing and thawing. This suspension was then emulsified with Freund's Complete Adjuvant[3] (Freund, 1951), and 1-ml quantities of the emulsion containing 0.25 ml of antigenic mixture (approximately 50,000 ruptured cells) were injected under the right scapula of previously unused rabbits; this was followed by an identical booster injection after one week.

Blood was first collected after one month and at irregular intervals thereafter through the ear vein; the serum was passed through a Seitz Filter and stored with, and without, 1:1000 merthiolate in a freezer. All observations were made of these unabsorbed antisera.

Agar Diffusion

Antisera were screened using the Ouchterlony agar diffusion technique (Wilson and Pringle, 1956), as modified by Nace (personal communication) for use on 1" x 3" glass microscope slides. Protein concentration of the antigen was $0.93 \pm 0.1\%$.

Macroscopic Observations

Titrations of antisera were made in 3 ml test tubes, leaving 1 ml of antiserum in each tube, adding 1 ml of the suspension of living animals, and noting the nature of the precipitation, if any, with the naked eye.

Light Microscope Observations

Antisera were titrated into ten-depression glass slides, and observations were made with low power and high-dry objec-

[3] Difco Laboratories, Detroit, Michigan.

tives of any whole-animal responses to antiserum. Cell dimensions were measured with an ocular micrometer.

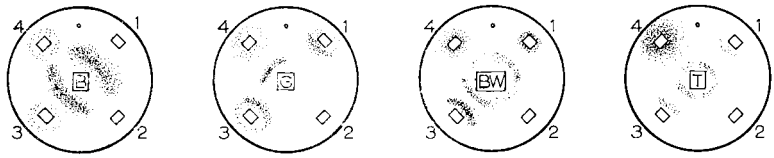
Electron Microscope Observations

Forty combinations of animals of 3 mating types with 2 antisera, neutral serum, or 0.9% NaCl in 3 dilutions (1:1, 1:10, or 1:20) were prepared. All controls were handled simultaneously and exactly as the experimental animals to assure validity in comparisons. After varying periods of exposure to test solutions (1 minute, 15 minutes, 1 hour, 2 hours), the cells were fixed according to the standard technique of Palade (1952) utilizing 1% OsO_4, buffered with acetate-veronal and brought to the general range of the tonicity of the cells with 0.34% sucrose. They were then dehydrated and infiltrated at 5°C for 24 hours or more. The blocks were sectioned with a Porter-Blum Ultramicrotome and studied with RCA EMU 3D and EMU 2 electron microscopes.

Results

Agar Diffusion

Screening of the serum from the four rabbits yielded the results depicted in Figure 1.



Depression	Contents	Rabbit	Inoculation	Interpretation
1	- Antigen 1/I	B	1/I	Anti- 1/I
2	- 2/I	G	1/II	?
3	- 1/I	BW	1/I	?
4	- 1/II	T	1/II	Anti- 1/II
Center	- Antisera			

Figure 1. Line drawing of the results of agar diffusion screening of antisera produced in rabbits injected with cells of a single mating type. The number, location, and density of precipitation lines is indicative of antibodies present. Interpretations of specificity of antiserum were made on the basis of lack of any degree of cross-reactivity.

Macroscopic Observations

With concentrated antiserum (1:1) the ciliates agglutinated within 2 to 10 seconds, the cells in homologous antiserum with complement fixed forming an immediate dense white column of agglutinated cells in the center of the test tube which slowly collapsed to the bottom after several minutes. The reaction in heterologous antiserum at 1:1, with complement fixed, produced fine flocculent clumps of cells that appeared throughout

the tube and slowly settled. At lower concentrations of homologous antisera, agglutination was proportionally slower if it occurred at all; times were not recorded. The tubes adjudged to contain "no reaction" were held before a light source and viewed without supplementary lenses. These observations are summarized in Table 1.

Table 1. Macroscopic Observations of Agglutination of Cells in Homologous and Heterologous Antisera

Serum	Dilutions (antibody diluted with distilled water; added in equal volume to suspension of cells (4500 individuals/ml)			
	1:1	1:10	1:20	1:40
Homologous Antibody	+++	+	++ (animals found dead under light microscope)	0
Heterologous Antibody	++	+	0	0

++ = degree of density of agglutination.
0 = no agglutination.

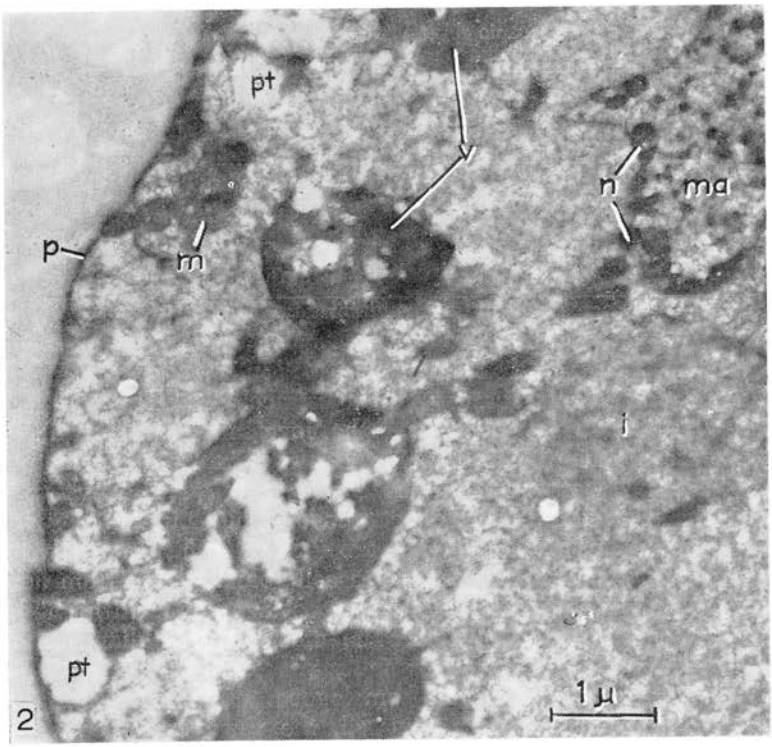
Light Microscope Observations

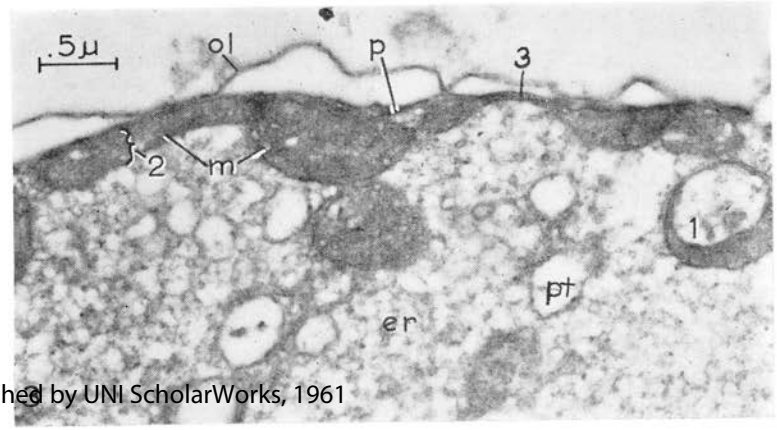
Table 2 presents observations of extracellular effects of antibody on the living ciliates and the concomitant whole animal responses. It may be seen that a pattern of intensity of reaction exists, homologous antiserum 1:1, with complement active, giving the strongest response, heterologous antiserum 1:80, with complement inactivated, giving the weakest. This is exactly as one would expect. The decrease in cell volume, aside from dorso-ventral flattening, was slight, and occurred only in the higher concentrations of antisera. The cross-sectional diameter at the widest point, taken as an index of cell volume, decreased from $\bar{x}=45\mu$ to $\bar{x}=35\mu$. In homologous antiserum at 1:1 and 1:10, with complement inactivated, the vacuoles indicated in Table 2 formed slowly and reached terminal diameters of 20μ to 60μ, often grossly distorting the shape of the cell. Cytolysis, a common effect of unfixed complement, occurred frequently even with complement inactivated. Total loss of mobility was the most common terminal point.

Electron Microscope Observations

Controls. Control cells treated with 0.9% NaCl and neutral serum at 1:1 and 1:20 for 1 minute and for 2 hours showed no deviations from normal untreated cells (Figure 2). All structures compared favorably with published observations of *Tetrahymena pyriformis*, e.g., Elliott and Tremor (1958) and Pitelka (1961). Cilia remained complete and attached to the cells, the pellicle showed little or no peeling or rupturing, and the cytoplasm appeared evenly dispersed and homogeneous in nature. Mitochondria showed a possible slight reduction in diameter.

Heterologous antibody. Exposure of cells to predetermined non-cross reactive heterologous antiserum produced few major changes in cell structure (Figure 3). Cilia tended to be more "brittle", many breaking off during fixation procedures. The pellicle showed the usual blisters but little or no rupturing. In-





ternally, the cytoplasm remained dispersed and homogeneous in texture, whereas the mitochondria appeared to flatten against the pellicle. Interspersed among the mitochondria were an increased number of spaces or cavities over the number observed in controls. Generally, however, it may be stated that the cells appeared normal.

Homologous antibody. Antiserum reactive with a given mating type produced profound modifications in cell structure (Figures 4-10).

Cilia. An extreme brittleness of the cilia was evidenced by the total lack of any cilia on most sections viewed. Both dilutions of antibody yielded this result when the cells were fixed after one minute. However, at both dilutions after 2 hours cilia seemed to have lost most of this fragility, since sections showed a nearly normal number of cilia. It is interesting to note that cilia associated with the oral membranelles appeared not to be affected by any of the various concentrations of serum used.

Pellicle. Both dilute and concentrated homologous antibody caused gross changes in the pellicular structure and, likely, in its function. Figures 6, 7, 8, and 9 show that the outermost layer became more osmophilic and apparently quite brittle, tending to buckle, peel and rupture over the entire surface of the animal. Normal blisters were difficult to distinguish. The appearance suggests that the cells tended to shrink away from this osmophilic layer, allowing it to rupture. The underlying layers did not take up extra osmic acid and tended to remain flexible, following the cell materials in shrinkage or distortions. The greatest amount of rupturing of the outer layer of the pellicle

Figure 2. Segment of an oblique cross section through a control cell treated with neutral serum at a concentration of 1.1 for two hours, showing the pellicle as united layers closely affixed to the underlying cytoplasm. Although no cilia appear in this figure, most control cells retained their cilia throughout fixation and sectioning. The cytoplasm is homogeneous in appearance and evenly dispersed throughout the cell. A few protrichocysts always occur at the periphrey. The membrane of the macronucleus appears as a double contoured membrane, and the nuclear contents are evenly dispersed with the presumptive RNA nucleoli (n) always contiguous with the nuclear membrane. A number of inclusions are present: vacuoles (v) of densely staining material, possibly fat or food, and an unidentified but regularly seen fibrous structure (i). Although present here, fat vacuoles were rarely seen in any cells. The general appearance of this cell typifies control cells exposed simultaneously with the experimental animals to neutral serum or to 0.9% NaCl solutions in the same concentrations as antisera, ABBREVIATIONS: *c,* cilia; *dm,* double membrane; *er,* endoplasmic reticulum; *i,* inclusion; *ls,* lamellar structure; *m,* mitochondria; *ma,* macronucleus; *mi,* micronucleus; *n,* presumptive nucleolus; *ol,* osmophilic layer; *p,* pellicle; *pt,* protrichocyst; *s,* space or cavity; *sh,* sheath; *v,* vacuole.

Figure 3. Portion of cross section through cell treated with heterologous antiserum, 1:1, for 10 minutes, demonstrating that the general appearance is little changed from that of the control cells. The outer layer (ol) of the pellicle (p) raises up somewhat more than in control cells, through it rarely ruptures and shows no greater osmophilia than do the underlying layers. The double set of double-contoured membranes appears as the underlying layers of the pellicle near (p). The unidentified structure indicated at 1 may be a vesiculate mitochondrion. The extreme flattening of the mitochondrion at 2 and 3 suggests that it may be in replication by a process of "fission." Normal numbers of protrichocysts are present (pt).

Table 2. Light Microscopic Observations of Whole-Animal Responses to Dilutions of Homologous and Heterlogous Antibody.

Serum	Dilutions (antibody diluted with distilled water; added in equal volume to suspension of cells (4500 animals/ml) 1:1	1:10	1:20	1:40	1:80
Homologous Antibody (Complement active)	Time* Observations **0 Dorso-ventral flattening Slight loss of volume Motion slowed Spiral rotation about the anterior-posterior axis 8 Volume regained 10 Immobilization 15 Lysis (few cells)	Time Observations 0 Slight D-V flattening Spinning about "cemented" posterior end 4 Motion slowed 6 Further flattening with loss of volume 10 4 cells agglutinate 15 Most normal; volume slightly reduced	Time Observations 0 No reaction 8 Some loose agglutination 10 Partial immobilization Cilia active	Time Observations 1 Immobilization of most cells Some dorso-ventral flattening 2 Spinning about "cemented" posterior end	Time Observations 3 Motion slightly slowed
(Complement inactivated)	0 Dorso-ventral flattening Immobilization 6 Agglutination; cilia active 10 Large vacuoles form Cilia cease activity 15 (Dead)	1 Dorso-ventral flattening Motion slowed Large vacuoles form slowly 7 Some loose agglutination 10 Twitching; much agglutination	1 No reaction 2-8 Motion slowed 9 Slight wrinkling 10 Few cells agglutinated	2-5 Motion slowed Some spinning about "cemented" posterior end 20 Immobilization of nearly all cells	0 No reaction 3 Motion slightly slowed 20 Immobilization of most cells
Heterologous Antibody (Complement active)	0 Dorso-ventral flattening Slight loss of volume Spiral rotation about the Anterior-posterior axis 1 Volume regained 5 Normal	0 Spiral rotation about the anterior-posterior axis 1-15 Normal	0 No reaction 2-12 Motion slightly slowed	0-15 No reaction	
(Complement inactivated)	0 No reaction 1 Motion slightly slowed 2 Immobilization (few cells)	0 No reaction 3 Immobilization 10 Nearly normal activity	0 Momentary immobilization 1-15 Normal	0 Momentary immobilization 1-15 Normal	0-15 No reaction

* expressed in minutes
** occurring within seconds; nearly immediate

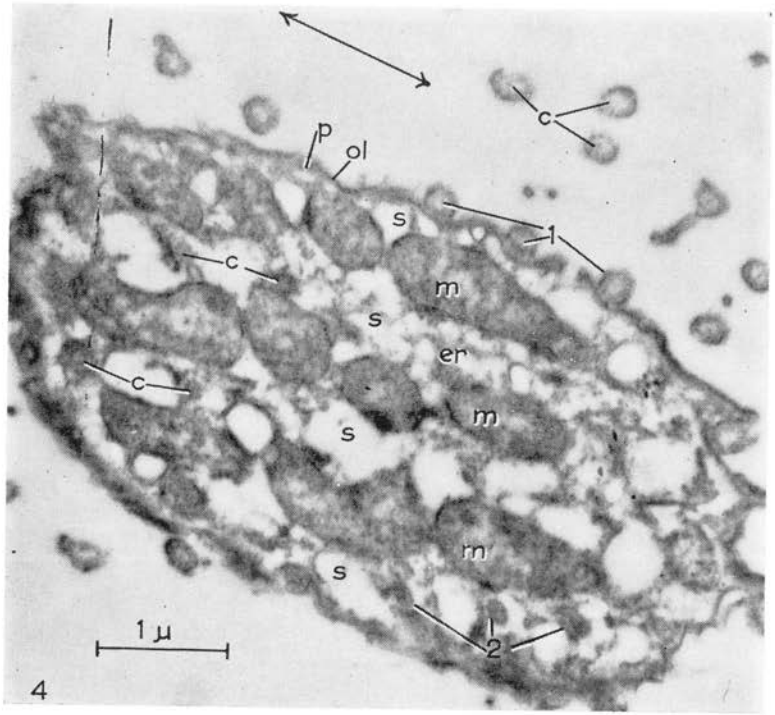


Figure 4. Tangential section through the pellicle and the underlying mitochondrial-alveolar layer in mid-body of a cell treated with homologous antibody, 1:1, for about 15 minutes. Although mitochondria (m) do migrate centrally, it may be seen here that when peripheral they take up positions in rows alternating with rows of kinetosomes (c). The subpellicular spaces (s) apparently intersperse among the basal bodies in great profusion. The longitudinal axis of the animal is indicated (arrow).

occurred peripheral to, or in the vicinity of, the greatest profusion of intracellular spaces.

No accumulations of material were observed on the cilia or the pellicle in cells treated with concentrated antibody. However, several cells subjected to dilute antibody for 2 hours showed a thick hyaline sheath which in some cases appeared to lie inside of the "osmophilic layer" of the pellicle (Figures 6*a* and *b*).

Intracellular cavities. Probably the most striking effect of homologous antibody on cells was the production of tremendously increased numbers of "spaces" immediately inside of the pellicle and among the mitochondria (Figures 4, 6, 7, 8, and 9). These remained discrete and were lost only gradually over a period of time. Although these are probably the protrichocysts described by Pitelka, their behavior suggests they are of another origin. Therefore, the designation "spaces" is substituted for protrichocysts. Surrounding membranes lining these

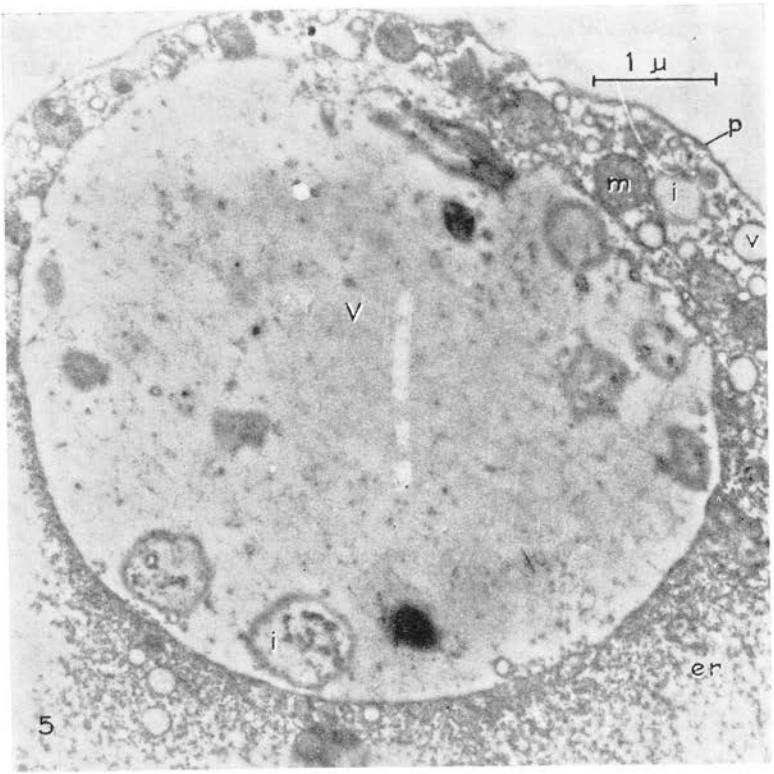


Figure 5. Segment of cross section through cell exposed to homologous antibody, 1:10, for about 15 minutes showing a large vacuole (v) about 6 u in diameter, presumably filled with water. Vacuoles with diameters up to 20-60 u were observed but not photographed.

spaces is presented in Figures 9 and 10. Since many spaces had no membranes, especially those fixed immediately after antibody treatment, there is the possibility that these spaces were forming after contact of the cell with antibody and that stages of membrane formation are represented in the figures. Another phenomenon was the formation of the giant vacuoles in cells exposed to high concentrations of homologous antibody (Figure 5). Often these were observed 4 or 5 per cell with diameters up to about 40μ, producing pronounced distortion of the cell shape.

Endoplasmic reticulum. From the normal condition of general homogeneity of cytoplasm shown in Figure 2, the endoplasmic reticulum often took on a punctate granular appearance in homologous antiserum. This was not entirely lost in 2 hours. It is possible that this condition was preliminary to cell lysis, and since it was most frequently seen in cells treated

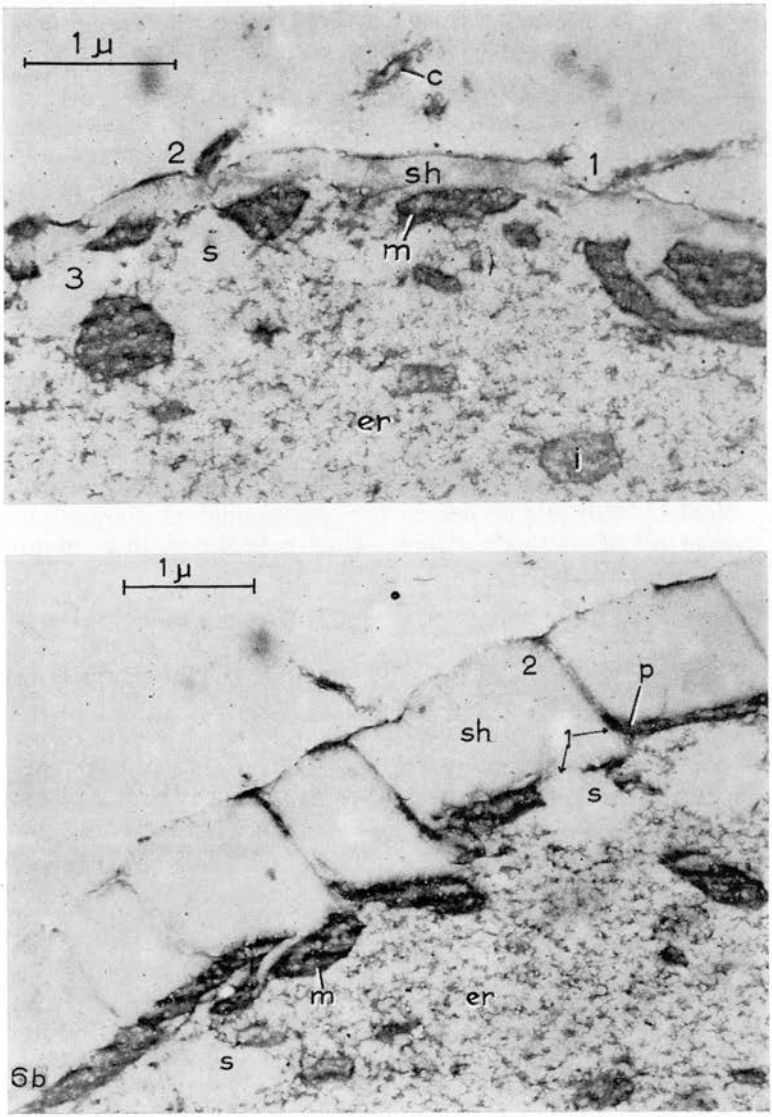


Figure 6. Segments of cross sections of cells treated with homologous antiserum, 1:20, for 2 hours. A thick hyaline sheath (sh) covering the outside of the cells appears external to the pellicle, although the osmophilic layer, and possibly the entire pellicle at points, may be raised up by the sheath (6*b*, at 1 and 2). As shown at 1 and 2 in 6*a*, the cilia apparently penetrate the sheath and may have accumulations of material on them distal to the sheath. The spaces (s) beneath the pellicles and at 3 in 6*a* indicates that the sheath has a greater density (or osmophilia) than do the spaces. Note the stellate granular appearance of the cytoplasm.

with homologous antibody, it may be that homologous antibody enhanced autolysis, even with the complement inactivated.

What are considered bizarre configurations of the endoplasmic reticulum are shown in figures 10*a*, *b* and *c*. These formations occurred only infrequently, taking on patterns resembling the layers of an onion, or a profusion of coiled tubules around mitochondria and unidentified inclusions.

Mitochondria. There seems to have been little effect on the mitochondria by any of the procedures used. Very slight reductions in mitochondrial diameter may have occurred, suggesting possible osmotic effects. However, recent observations suggest that rather than being reduced in volume the mitochondria tended to flatten somewhat. A very few cells showed vacuolation of mitchondria, but this is felt not to represent more than terminal effects of antibody.

Nuclei. Neither the micro- nor macro-nucleus showed any sign at all of antibody effects. They maintained their normal appearance throughout all of the experiments.

Discussion

Robertson (1939) laid the ground work for much of the future work dealing with antibody effects and specificities. Table 2 is presented in some detail to further supplement existing reports of antibody effect in ciliates as seen with the light microscope. Electron microscope observations tend to confirm predictions made by other investigators working with both ciliates and metazoan cells. For example Robertson (1939) and Beale and Kacser (1957) described the collection of a "sticky" exudate on the cilia and pellicle, and Robertson attempted to link this exudate to the "alveolar layer" beneath the pellicle. In a very recent paper, Pitelka (1961) presents electron-micrographic evidence to substantiate her view that the cavities of the alveolar layer are "protrichocysts". In the present study, the observation that homologous antibody greatly increases the number of such cavities would suggest that antibody is active in this layer, or that these cavities are the indirect result of the antibody treatment.

If the antigen is constantly being produced, as is suggested by Beale and Kacser, a certain number of cavities would be expected to appear under the pellicle at sites where the antigenic material is synthesized or stored. These may be the protrichocysts described by Pitelka. The antigen, when extruded without force, would likely cover the pellicle and cilia and be constantly dissolved away; but when extruded forcibly it may

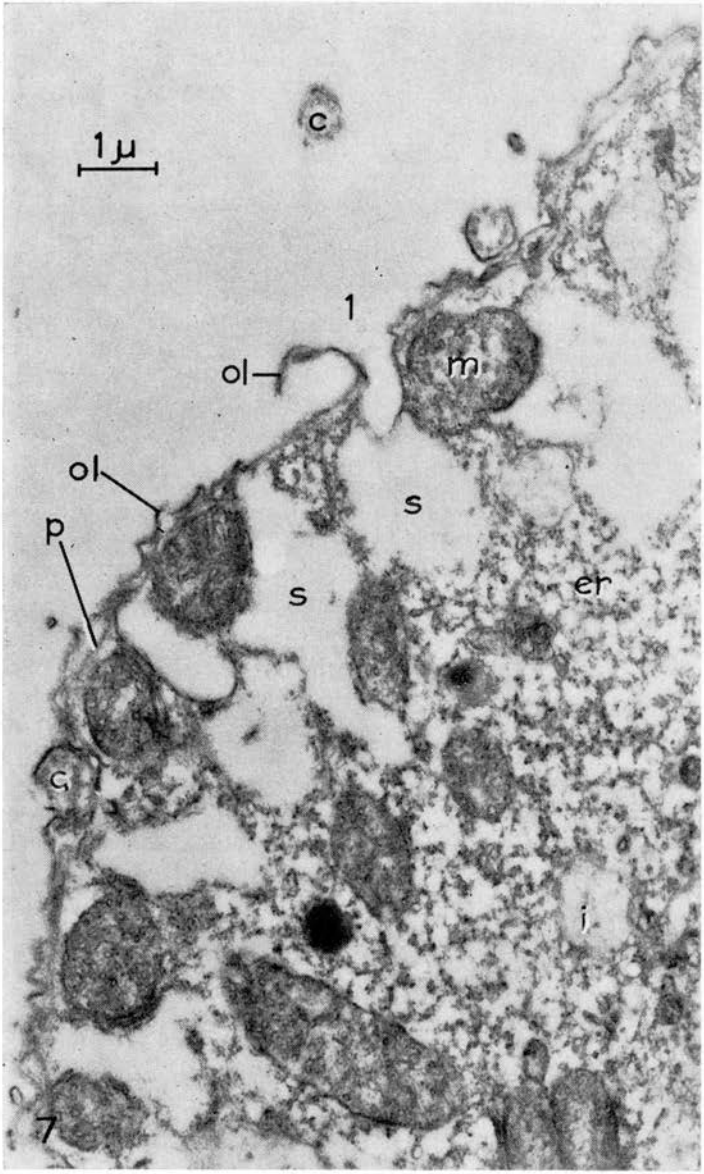


Figure 7. Segment of oblique section through a cell exposed to homologous antiserum, 1:1, for about 15 minutes. Note the profusion of spaces (s) immediately inside of the pellicle. At 1, the osmophilic layer has ruptured while the inner layers remain flexible and are forced inward. The mitochondria remain normal, but the endoplasmic reticulum has a distinctly granular nature.

produce the familiar trichocysts. If antibody specific to this material were introduced, then the complex would congeal and collect on the cilia and pellicle to form accumulations visible with the light microscope. Such an accumulation on the pellicle may be shown in Figure 6, *a* and *b*. However, since these sheaths occurred only infrequently, no easy explanation is possible.

Latta (1952) and Rebuck (1953) indicated that interaction of the antigen and antibody at the surface of red blood cells caused increased brittleness and fragility. If a similar reaction occurs in ciliates on the outer layer of the pellicle, which also encloses the cilia (Pitelka, 1961), then fragility and consequent loss of cilia and the outer layer of the pellicle during fixation confirm the presence of antigen-antibody interaction at these sites. Loss of the outer layer of the pellicle would remove whatever protective properties it affords.

It is well known that *Tetrahymena* can maintain itself over a surprisingly wide range of salt concentrations without showing any significant signs of osmotic stress. Routinely these animals are starved down in glass-distilled water for 12 hours in the laboratory before various procedures (Elliott, personal communication), yet some workers (Preer and Preer, 1959, and Finger, 1960) wash cells or cell fractions in 0.9% NaCl. This suggests that the animal must possess a rather remarkable osmoregulatory apparatus or that it is euryhaline, adjusting readily to varying salinities within physiological limits, or that it is little affected by changes of salt concentration in these ranges because of some specific structure. This latter possibility seems rather likely in light of the results obtained here. If the pellicle of the animal were to serve as a passive resistance to water diffusion, then the external medium could be varied within the range in which the pellicle offers protection, and the internal salt concentrations would remain relatively unchanged. Further, if some agent served to render the pellicle permeable to water, then immediate internal osmotic problems would be expected to arise. It may be possible that the pellicle coated with the antigenic substance offers a rather high degree of impermeability to water and that rupture produces increased water permeability. Indeed, as noted above, the greatest profusion of spaces in the cytoplasm seemed to occur in the vicinity of actual ruptures and buckling of the osmophilic layer. It may be that these spaces are in fact produced by inrushing of water and are therefore not all exactly homologous to protrichocysts, whether or not they function in the end in a fashion similar to protrichocysts. That these cavities are not due solely to di-

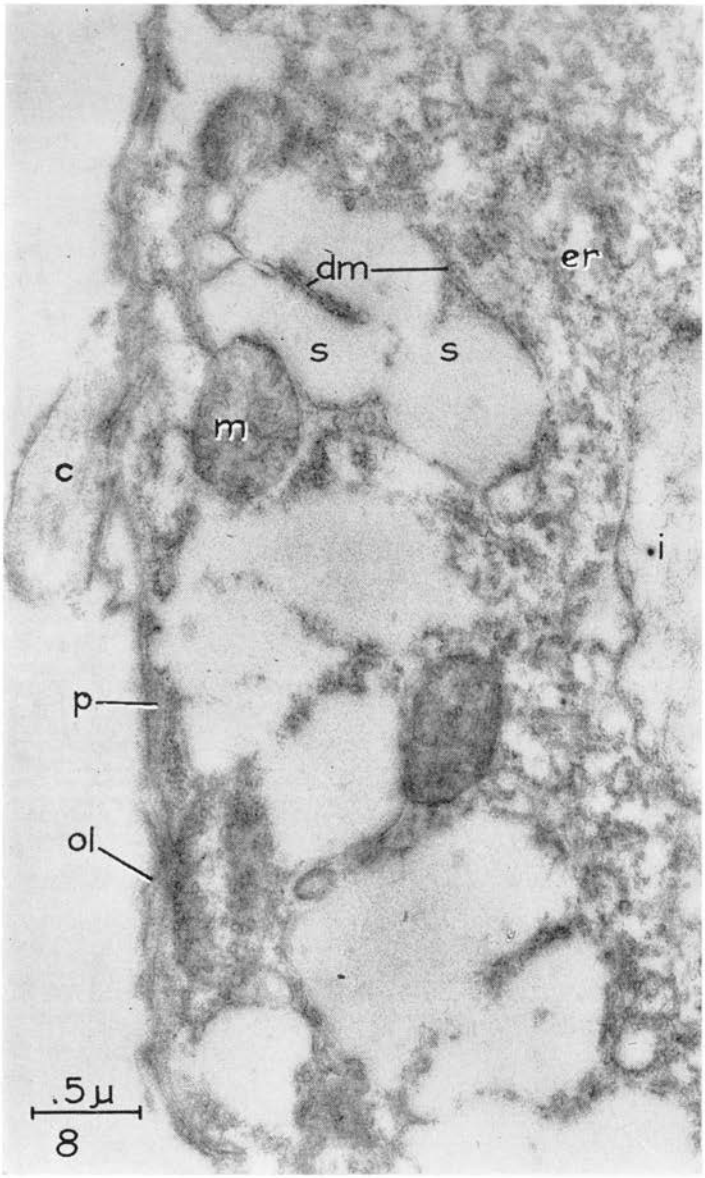


Figure 8. Segment of cross section of cell treated with homologous antibody, 1:1. for about 15 minutes, showing the profusion of subpellicular spaces (s) which do not tend to unite as long as strands of cytoplasm remain between. It may be seen that some cavities have double membranes beginning to form at the interface between cytoplasm and the fluid filling the space, whereas other cavities are not yet bounded by an observable double membrane.

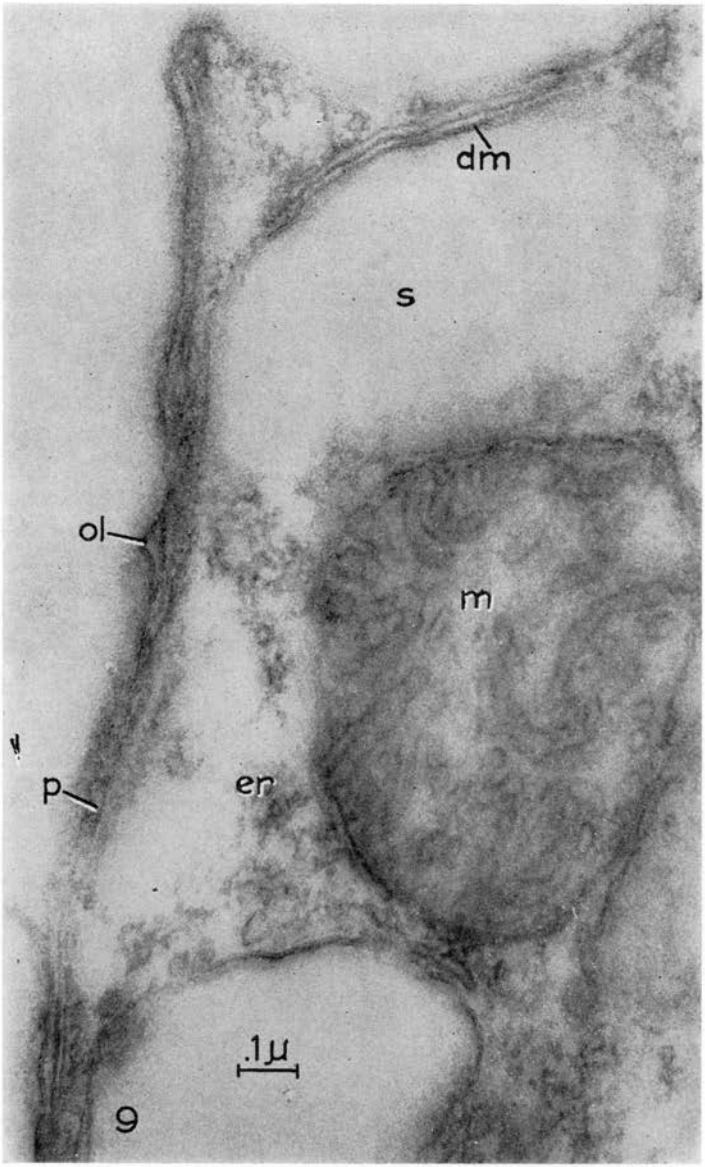


Figure 9. High resolution electronmicrograph of a small portion of the pellicle and underlying structures of a cell treated with homologous antibody, 1:1, for about 15 minutes. Ultrastructure of the pellicle appears to be made up of three sets of double-contoured membranes. Each double membrane consists of two dense layers, each approximately 50 A thick, separated by a less densely stained area approximately 40 A wide. The entire double structure is separated from the next by a lightly staining gap approximately 80 A wide. At (ol) the outer double-contoured layer may be seen buckled up from the inner layers. The dimensions of the double-contoured membranes of the pellicle are approximately the same as those of the double-membranes (dm) surrounding the sub-pellicular spaces.

rect osmotic stress is apparent in that saline and neutral serum controls showed distinctly fewer cavities. The fact that the cavities show varying degrees of formation of membranes lining them suggests that these cavities are new and that these double membranes are forming *in situ*. It is possible that protrichocysts are constantly forming in normal cells and dispelling their contents slowly as an excretory or cuticle-producing mechanism.

A number of techniques immediately suggest themselves for further testing of this hypothesis. Responses to antisera specific to various fractions such as prepared by Preer and Preer, and by Finger, should be studied with the electron microscope to ascertain exact specificity and site and mode of action of antibody. Cryofixation and low temperature preparation techniques perfected by Fernandez-Moran (1959*a*, 1959*b*) could presumably stop antibody response at any point and the formation of the cavities might be accurately described. Finally the localizations of antibody reactivity might be demonstrated by labeling the antibody with a radioactive substance such as I^{131} and applying techniques of high-resolution autoradiography, using stripping film laid over thin sections of fixed cells.

Latta (1959) used the electron microscope to study effects of antibody on various types of vertebrate cells. Many of his results compare favorably with those obtained here. He found buckling and disruption of the plasma membrane, loss of granularity of cytoplasm, destruction of mitochondria, and vesiculation of the nuclear membrane. Only one indication that parts other than the cilia and pellicle were directly affected by antibody was found in the present study, although it is presumed that other sites of activity would be observed if antisera to specific fractions were applied to whole cells. As found by Latta, bizarre irregular configurations of the endoplasmic reticulum were presumably a result of antibody effect. It is felt that these configurations, although similar to lamellar structures observed by others (Rudzinska and Trager, 1959; Hamilton and Gettner, 1958; Dipple, 1958), have no functional significance and possibly represent only the effect of breaking attachments of the endoplasmic reticulum normally maintained in a "stretched" and evenly dispersed state.

The question of criterion of antibody reaction and specificity is pertinent here. On the basis of fluorescent light microscopy, Beale and Kacser suggested that the name "ciliary antigen" be changed to "immobilization antigen" since they observed the presence of antigen-antibody complex on both cilia and pellicle. They suggested further that the antibodies that produce

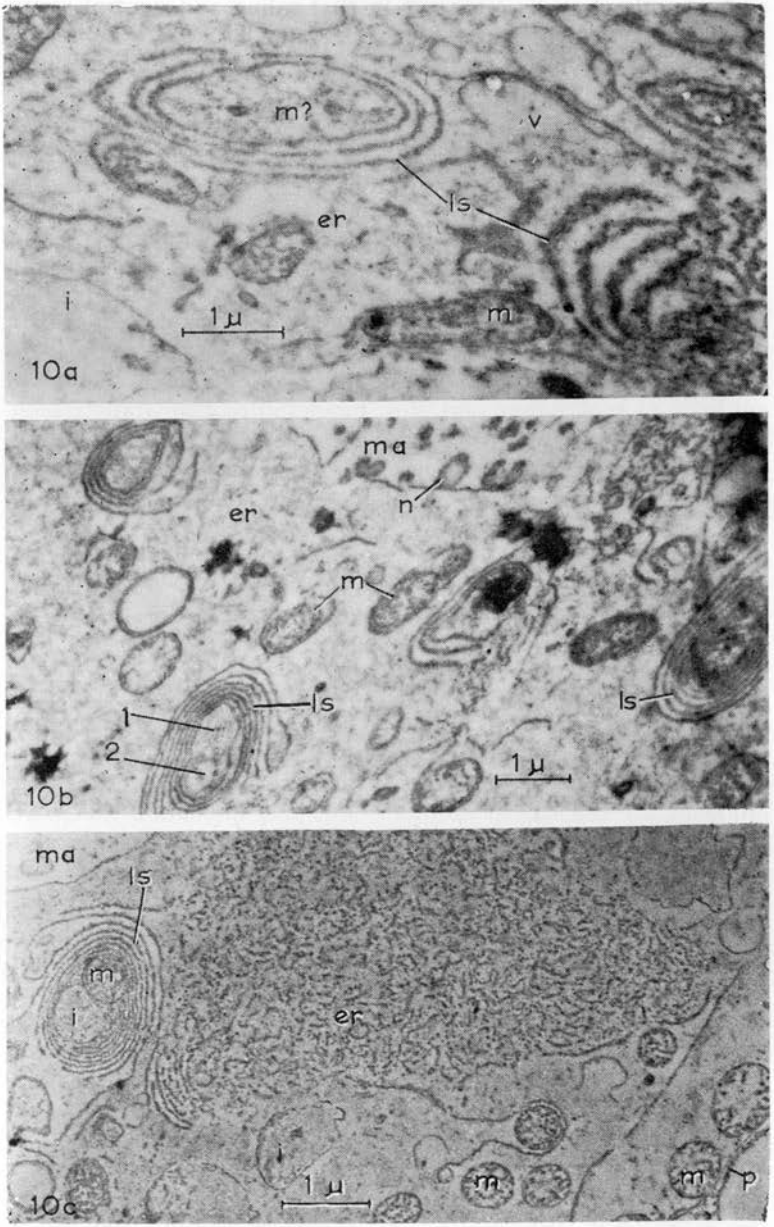


Figure 10. Ultrathin sections of cells exposed to homologous antibody, 1:1, for about 15 minutes, showing bizarre configurations of the endoplasmic reticulum (er). Although much of the endoplasmic reticulum retains its normal homogeneous appearance, some appears to "draw up" into long filamentous (10 *c*) or lamellar structures (10 *a*, *b*, *c*). Generally, lamellar structures form around some cytoplasmic incusion or an organelle, e.g., mitochondria and other unidentified structures.

agglutination may be separate from those that produce immobilization. Latta (1958) recorded similar conclusions working with chick cells in tissue culture. The results obtained by Finger (1960) and Preer and Preer (1959) certainly indicate the presence of antigenic materials in all constituents of cells. Results obtained in this study, and especially in Latta's (1959), lend strong support as presumptive evidence that antibody was active at diverse sites in the cells. Of the several antisera tested for agglutination, immobilization, and intracellular response, not all antisera that produced responses seen with the electron microscope produced immobilization, and relatively few produced agglutination consistently in several trials. It is therefore felt that "immobilization antigen" is a better title for the most common antigenic substance associated with the celia and pellicle, formerly called "ciliary antigen." However, discussion of the immobilization antigen of ciliates must not preclude the persence of a large number of other antigenic substances in the cells.

There is no reason *a priori* that every host must produce an identical antiserum to cells of a given species, variety, or mating type. Indeed, although the number of cells injected may not be too critical (Beale and Kacser, 1959), the preparation of the cells may be all important (Cushing and Campbell, 1957) since highest titers may develop to one component of whole living cells and to an entirely different component in ruptured cells. Gel diffusion techniques offer an excellent and sensitive method of testing cross-reactivity.

Preer and Preer (1959) found four distinct antigens among their fractions with cross-reactivity, indicating common antigens to the various fractions. Finger (1960) confirmed these findings and indicated that it appeared that mitochondria and "small granules" altered their specificity spontaneously and reversibly during cell production, ". . . reminiscent of the immobilization serotype which can transform one to another during clonal growth." Still further lability of the antigens of ciliates is demonstrated by Margolin et al. (1959), who found three different antigens elaborated in cells grown at three specific temperatures and that transformations from production of one antigen to another took place. Therefore, because of the lability of antigenic nature, the multiplicity of antigens, and the specificity of antibody to these antigens, it is strongly advocated that the electron microscope be considered an essential tool in studying antibody response, and that agglutination or immobilization are neither valid nor adequate criteria for determining true specificity of antisera, even when they are absorbed with presumptive cross-reactive cells or cell fractions.

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