Membrane proteins within cell fractions of Tetrahymena have been separated by polyacrylamide gel electrophoresis and the turnover of these proteins has been studied in nongrowing cells using double labeling procedures. Lactoperoxidase iodination and two-dimensional gel separations have revealed over 20 surface-labeled proteins, two of which are responsible for the single peak found in one-dimensional gels. The same proteins were found in ciliary and nonciliary surface membranes. Turnover measurements show that there is heterogeneity in the rates at which membrane proteins turn over. The data suggest that larger proteins may turn over at higher rates in the microsomal fraction, as previously shown in mammalian cells. This was not found in the surface membrane fractions of Tetrahymena. However, membrane-associated cytoskeletal proteins had lower turnover rates than integral membrane proteins, and significant heterogeneity was found among the integral proteins measured. The implications of these findings for possible modes of membrane biogenesis in Tetrahymena are discussed.

The primary concern in our studies of Tetrahymena membranes has been to understand more about the dynamic nature of eukaryotic cell membranes. In particular, more information is needed about the intracellular mechanisms involved in the biogenesis and turnover of the various membrane systems in these cells. The usefulness of the ciliated protozoan Tetrahymena in studies of this kind has been pointed out in a recent review, and the current information available from studies of this cell type has been summarized (1). Although a considerable amount of work has been done on Tetrahymena cell membranes, relatively little information is presently available on the protein constituents of these membrane systems. The present study is, therefore, concerned with the isolation and identification of membrane proteins in Tetrahymena, together with an attempt to obtain basic information concerning the metabolic properties of these important cell constituents.

The primary result is the identification of over 20 surface membrane proteins in Tetrahymena and the indication that these proteins may be regulated independently of each other. In the past, both homogeneous (2-5) and heterogeneous (6-8) turnover rates have been noted among the proteins within a single membrane system in eukaryotic cells. The most recent studies report homogeneous turnover rates for the proteins within the surface membrane of hepatoma tissue culture cells (5, 9, 10). Doyle and co-workers (9) have suggested the surface membrane proteins may, therefore, be added to the surface in aggregate by the fusion of internal membrane vesicles with the cell surface membrane. Although the present results with Tetrahymena do not rule out the involvement of vesicle fusion in surface membrane growth and turnover, they provide evidence which at least suggests that the postulated precursor vesicles do not represent fully differentiated surface membrane in this cell system. Additional mechanisms must, therefore, be involved in the formation and turnover of surface membrane in Tetrahymena.

**MATERIALS AND METHODS**

Tetrahymena thermophila (formerly Tetrahymena pyriformis, syngen 1), strain WH-14, was grown at 25°C in an enriched proteose-peptone medium on a shaker as described previously (11). Iodination and turnover experiments with radioactive leucine were carried out in the inorganic medium of Hamburger and Zeuthen (12). Cells in growth medium were centrifuged 365 × g and resuspended in an equal volume of inorganic medium at 25°C. They were then centrifuged again and suspended in the desired final volume of inorganic medium. Cultures were incubated on a rotary shaker.

**Fractionation Procedures**—Cell fractionation was performed as described previously (13), except that pellicles and mitochondria were also prepared in the present study. These were obtained by the following modification of an earlier method (14).

Deciliated cells from two cultures were homogenized in 10 ml of cold 0.2 M phosphate buffer, pH 7.2, containing 0.1 M NaCl and 3 mM diaminod EDTA. After diluting to 20 ml and centrifuging the homogenate at 19,000 × g for 10 min, the supernatant was used to prepare microsomes and postmicrosomal supernatant. The sediment was briefly homogenized in 0.25 M sucrose and loaded on a discontinuous sucrose gradient (15 ml of 1.46 M sucrose, 15 ml of 1.0 M sucrose, and 10 ml of 0.34 M sucrose). Following centrifugation at 4,920 × g for 10 min, the pellet band at the interface between 1.46 M sucrose and 1.0 M sucrose was removed with a syringe, diluted 1:5 times with the 0.2 M phosphate buffer, and centrifuged at 4,000 × g for 5 min. The pellet consisted mostly of microsomes with a few mitochondria. It was resuspended in 10 ml of the phosphate buffer and centrifuged at 164,000 × g for 1 hr to sediment pure microsomes. Mitochondria were isolated from the bottom half of the 0.34 M sucrose layer of the discontinuous gradient described above. That layer was collected and centrifuged at 14,000 × g for 5 min. The sediment consisted of mitochondria with a few cilia and smaller particles, thought to be lysosomes and peroxisomes. It was not purified further.

The isolated cell fractions were analyzed for purity by phase microscopy and electron microscopy employing techniques similar to those already described (14). There were no significant differences...
between the contents of the starved cells and fractions and of the  
prepared from growing cells. There was no measurable change in the 
proportions of total protein in the various cell fractions during the  
starvation period studied.

Cilia were subfractionated into axonemes, membrane vesicles, and  
soluble matrix by the Triton X-100 procedure described earlier (13).  
An alternative procedure not involving detergents was also developed.  
A pellet of detached cilia from four 200-ml cultures was resuspended in  
30 ml of the deciliation buffer (14) and centrifuged at 14,600 \( \times g \) for  
5 min. The cilia were resuspended and washed again in the same way.  
The length of the cilia was then reduced to 1 ml of 25 mM Tris-HCl, pH 8.0,  
0.1 ml of 1 M KCl, and 0.15 M KCl, 4 mM MgSO\(_4\), 0.5 mM disodium EDTA,  
0.5 mM mercaptoethanol, and 2 mM Tris-HCl buffer, pH 8. In this, the  
membranes near the basal end of the cilia swelled, giving the cilia a  
shape resembling minuscule tadpoles. The preparation was quickly  
refrozen in a Dry Ice-acetone bath and then slowly thawed over a 5- to  
10-min period. The freeze-thaw step was repeated. Finally, the  
preparation was centrifuged at 43,500 \( \times g \) for 10 min to sediment insoluble proteins.

**Iodination of Membrane Proteins**—The iodination of surfacemembrane proteins was carried out by modifying the procedure of Phillips and Morrison (15). A 37-h old culture of Tetrahymena was  
harvested, the cells were washed once with 20 ml of inorganic medium at room temperature, and then they were resuspened in 50 ml of fresh inorganic medium (about 5 \( \times 10^5 \) cells/ml). To this suspension were added 1.0 mg of lactoperoxidase (Calbiochem) freshly dissolved in 0.5 ml of 2.5 mM Tris-HCl, pH 8.0, 0.1 ml of 1 M KCl, and 0.5 mCi of Na\(^{125}\)I (carrier free, New England Nuclear Corp.) dissolved in 0.5 ml of 1 M Na\(_2\)SO\(_4\). The reaction was initiated by the addition of 50 \( \mu \)l of freshly prepared 20 mM H\(_2\)O\(_2\). The addition of H\(_2\)O\(_2\) was repeated four more times at 1-min intervals. The iodination was allowed to continue for 3 min after the last addition of H\(_2\)O\(_2\) and was terminated by the addition of 100 ml of growth medium containing 50 ml KCl and 1.2 mM disodium EDTA. The cells were then centrifuged at 365 \( \times g \) for 5 min and fractionated immediately or were washed and resuspended in inorganic medium.

It was necessary to add the unlabeled iodide to get a high degree of iodination (16). It was also found that some batches of the enzyme preparation caused deciliation of the cells immediately after the  
reaction if EDTA was not added. The cells iodinated by the above procedure behaved normally during starvation in inorganic medium and during cell fractionation.

In one experiment isolated cilia and pellicles were iodinated after  
sonication. Pellets of these fractions containing 1 mg of protein were  
suspended in 5 ml of inorganic medium, sonicated for 2 min, diluted  
to 10 ml, and then iodinated in the usual way.

**Electrophoresis**—Samples of cell fractions to be analyzed by one- 
dimensional polyacrylamide gel electrophoresis were solubilized in  
hot sodium dodecyl sulfate and otherwise treated according to the  
procedure of Laemmli (19). The proteins were separated in 8% tube  
gels and otherwise treated according to the procedure of Scherbaum (17) after spotting 50- to 100-\( \mu \)l aliquots of a suspension of  
the deciliation buffer (14) and centrifuged at 14,600 \( \times g \) for  
5 min. The freeze-thaw step was repeated. Finally, the preparation  
causwas centrifuged at 500 \( \times g \) for 5 min, and the pellet was  
resuspended in 70 ml of inorganic medium. After a 5-min recovery  
period, 500 \( \mu \)l of DL-[\(^{14}\)C]leucine (67.4 mCi/mmol, ICN) was added,  
and the culture was placed back in the 25°C shaker for 60 min. The  
culture was then chased by adding 1 ml of 50 mM L-leucine, and the  
cells were again centrifuged at 500 \( \times g \) for 5 min. The pellet was  
resuspended in 200 ml of fresh growth medium 1 mm in unlabeled L-  
leucine. After 2 h (to allow intracellular distribution of the labeled  
proteins), the cells were centrifuged again and resuspended in 200 ml  
of inorganic medium which was 1 mm in L-leucine.

Following a further 9-h incubation, the cells were centrifuged,  
resuspended in 200 ml of inorganic medium (to wash out the remaining  
cold leucine), centrifuged again, and resuspended in 70 ml of inorganic medium. Two millilitres of 1-[\(^{3}\)H]leucine (59 Ci/mmol,  
Schwarz/Maxx) were added and incubation was resumed for 60 min.  
Then, 1 ml of 50 mM L-leucine was added, and the cells were centri-  
fuged and resuspended in 200 ml of inorganic medium 1 mm in L-  
leucine. Incubation was continued for 3 h before chilling and fractionating the cells. The samples were subjected to SDS-polyacrylamide gel electrophoresis and the radioactive profiles of these gels were  
run at constant power, 4 watts/slab, for approximately 2.5 h. The gels  
were cut into the agarose adjacent to the isoelectric focusing gel and  
the location of \(^{25}\)I-labeled proteins in one-dimensional gels  
were identified in a Packard Tri-Carb scintillation spectrometer. Quench was determined using an external standard.

The relative rates of turnover for surface membrane proteins were  
also determined in two-dimensional polyacrylamide gels using double  
labeling. In these experiments, the cells were cultured with  
7 \( \mu \)Ci of DL-[\(^{14}\)C]leucine, washed, and chased for 9 h in 1 mm L-leucine  
and then pulsed another 4 h using 20 \( \mu \)Ci/ml of \(^{3}\)H]leucine. Cilia and  
pellicles were isolated and the proteins separated in two dimensions  
using the method of O'Farrell (22). The gels were then stained and  
Coomassie blue spots corresponding to iodinated surface membrane  
proteins were cut out and counted for tritium and \(^{14}\)C as described  
above.

**Electron Microscopy**—The cells were fixed in 1.3% glutaraldehyde  
in 0.07 M cacodylate buffer, pH 7.15, containing ruthenium red at 500  
ppm according to the method of Luft (23). They were postfixed in  
2.5% osmium tetroxide in 0.1 M cacodylate buffer containing ruthenium  
red, dehydrated, and embedded in Epon. Sections were examined  
without staining using a Philips EM300 electron microscope.

**RESULTS**

**Identification of Membrane Proteins**—In order to obtain information about membrane protein metabolism, it was first
necessary to identify as many membrane proteins as possible. The first step was to isolate membrane-rich fractions from *Tetrahymena* and examine the constituent proteins using one-dimensional SDS-polyacrylamide gel electrophoresis. *Tetrahymena* microsomes, pellicles (cell "ghosts"), and cilia were treated in this way, and the proteins of these fractions are shown as they appear in one-dimensional gels in Fig. 1.

Tubulin is found in abundance in both cilia and pellicles, and ciliary dynein is the major protein seen in the upper region of the cilia gel in Fig. 1. The pellicle proteins labeled A, B, and C are protein components of the membrane-associated cytoskeleton previously identified in *Tetrahymena* (24, 25). Many of the remaining bands in the gels of cilia and pellicles probably represent membrane proteins, but the structural complexity of these fractions means that some may be from other structures as well. In an attempt to identify proteins in ciliary and pellicle fractions which are integral membrane proteins, living cells were subjected to the $^{125}$I-lactoperoxidase procedure of Phillips and Morrison (15). This method is widely believed to iodinate only externally exposed polypeptides, therefore, radioactive profiles of polyacrylamide gels prepared from the cilia or pellicles of labeled *Tetrahymena* can be expected to reveal the presence and identity of exposed surface membrane proteins in these cells. The assumptions were checked initially by determining the distribution of labeled proteins among the various cell fractions immediately following the in vivo reaction with $^{125}$I. It was found, as expected, that only the cilia and pellicle fractions were heavily labeled (Table I).

The identity of individual surface-labeled proteins was next sought by comparing radioactive profiles of one-dimensional gels prepared from $^{125}$I-labeled cilia and pellicles with their optical density scans (Fig. 2). The examples presented in Fig. 2 show that this procedure reveals a single major peak of radioactivity in both pellets (top) and cilia (bottom) and further suggest that the labeled protein(s) may be the same in the two fractions. Gels prepared with molecular weight markers included indicate an apparent molecular weight for the iodinated protein(s) of about 48,000. Control experiments were carried out which show that the unlabeled proteins of cilia and pellicles are potentially reactive, but do not become labeled in vivo only because they are not exposed. Both cilia and pellicles were sonicated prior to lactoperoxidase iodination and then the proteins were separated on one-dimensional gels. The radioactivity profiles and density scans of these gels show that all major density peaks become heavily labeled under these conditions.

The suggestion that both cilia and pellicles contain a single

### Table I

<table>
<thead>
<tr>
<th>Cell fraction</th>
<th>% of whole cell specific radioactivity</th>
<th>% of whole cell protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole cells</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cilia</td>
<td>1046</td>
<td>0.6</td>
</tr>
<tr>
<td>Ciliary supernatant</td>
<td>110</td>
<td>7.1</td>
</tr>
<tr>
<td>Microsomes</td>
<td>126</td>
<td>7.1</td>
</tr>
<tr>
<td>Postmicrosomal supernatant</td>
<td>9</td>
<td>41.4</td>
</tr>
<tr>
<td>Pellicles</td>
<td>535</td>
<td>~20</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>73</td>
<td>~23</td>
</tr>
</tbody>
</table>

Data of Subbaiah and Thompson (13).

Estimated.

![Fig. 1. Proteins of *Tetrahymena* cell fractions separated by SDS-polyacrylamide gel electrophoresis.](http://www.jbc.org/)

![Fig. 2. Identification of surface membrane proteins after iodination by the lactoperoxidase method of Phillips and Morrison (15).](http://www.jbc.org/)
Turnover of Membrane Proteins in *Tetrahymena*

I externally exposed membrane protein of molecular weight 48,000 was then tested using the two-dimensional gel system of O'Farrell (22). The results, presented in Fig. 3, show that there are over 20 iodinated proteins in each preparation, and that two major proteins differing considerably in isoelectric point are probably responsible for the 48,000 molecular weight peaks in one-dimensional gels. Although the pellicle gels (top) typically show more background and fainter spots in some areas than the cilia gels (bottom), careful examination of the original autoradiograms leads to the conclusion that all spots in the one preparation can be found in the other. The present results, therefore, suggest that the two fractions contain the same surface-exposed membrane proteins.

Isolated cilia and pellicles from *125*I-labeled cells were subfractionated into membrane vesicles, residual insoluble materials, and soluble supernatant fractions by dilute detergent and freeze-thaw procedures in order to determine whether vesicle preparations offered any advantage for the metabolic studies described in the next section. Significant quantities of *125*I were found in all three fractions, which suggests that some membrane proteins may be lost from the vesicles during subfractionation. We, therefore, decided to determine turnover rates of membrane proteins using SDS-polyacrylamide gel electrophoresis of intact cilia and pellicles; these fractions are more likely to retain their normal protein constituents in their original proportions.

**Fig. 4.** Surface membrane fractions from *Tetrahymena* of the type presented in the previous figure, this time stained with Coomassie blue. Taking the cilia fraction (A) and the pellicle fraction (B) together, a total of eight Coomassie blue spots correspond to iodinated proteins found in the autoradiograms. Only one of the iodinated surface proteins (S1) was visible in both the cilia (S1a) and pellicles (S1b) when the gels were stained with Coomassie blue. The turnover measurements presented in Table II were obtained using this group of membrane proteins. The slight differences between Figs. 3 and 4 are due to dehydration of the gels in Fig. 3.

The double labeling procedures adopted for the turnover studies require that at least some membrane proteins in the two-dimensional separations be identifiable without the use of labeled iodine. We, therefore, stained gels of this type with Coomassie blue (Fig. 4) and looked at the correspondence in pattern between stained gels and gels visualized by autoradiography. The reliability of the lactoperoxidase iodination method for marking only surface membrane proteins is reflected in the presence of proteins in the stained gel which are not present in the autoradiograms. The major result was that seven of the proteins could be found repeatedly in both stained gels and autoradiograms. These are indicated by the arrows in Fig. 4 and are numbered so they can be dealt with individually in the turnover studies described below. One of the surface proteins (S1) was visible in both the cilia (S1a) and pellicle (S1b) fractions when the two-dimensional gels were stained with Coomassie blue; the remaining six were found either in pellicles or cilia, but not both. The proteins visible in autoradiograms which are not seen in the stained gels must be highly radioactive membrane proteins present in quantities too small to stain with Coomassie blue under the conditions employed. The seven membrane proteins identified in Coomassie blue-stained gels are listed in Table II, along with their isoelectric points and apparent molecular weights in SDS-polyacrylamide gels.

**Turnover of Membrane Proteins**—The first experiments were concerned with comparing the species of protein synthesized by normal and starved cells. To maintain consistency...
Turnover of Membrane Proteins in Tetrahymena

Relative rates of turnover for surface membrane proteins determined in two-dimensional polyacrylamide gels using double labeling

Coomassie blue spots corresponding to iodinatable surface membrane proteins were cut out of two-dimensional polyacrylamide gels and used to determine the ratio of tritium to 14C after double labeling in non-nutrient medium. The cells were pulsed for 4 h with 7 μCi/ml of [14C]leucine, washed and chased for 9 h in 1 mM DL-leucine, and then pulsed another 4 h using 20 μCi/ml of [3H]leucine. The cilia and pellicles were then isolated and the proteins separated in two dimensions using the O'Farrell procedure (22). Both tritium and 14C counts were between 10 and 100 times background in all protein samples isolated. H and 14C ratios are presented from three separate experiments.

Table II

<table>
<thead>
<tr>
<th>Protein</th>
<th>M.</th>
<th>pH</th>
<th>3H/14C Ratios</th>
<th>X ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1a</td>
<td>48,000</td>
<td>5.9</td>
<td>1.69 ± 0.89</td>
<td>2.09</td>
</tr>
<tr>
<td>S1b</td>
<td>48,000</td>
<td>5.9</td>
<td>1.45 ± 1.90</td>
<td>1.70</td>
</tr>
<tr>
<td>S2</td>
<td>41,000</td>
<td>5.5</td>
<td>1.45 ± 1.99</td>
<td>1.13</td>
</tr>
<tr>
<td>S3</td>
<td>36,000</td>
<td>5.2</td>
<td>1.40 ± 1.62</td>
<td>1.51 ± 0.11</td>
</tr>
<tr>
<td>S4</td>
<td>35,000</td>
<td>4.9</td>
<td>1.22 ± 1.50</td>
<td>1.36 ± 0.14</td>
</tr>
<tr>
<td>S5</td>
<td>58,000</td>
<td>4.6</td>
<td>2.25 ± 2.95</td>
<td>3.11</td>
</tr>
<tr>
<td>S6</td>
<td>48,000</td>
<td>4.5</td>
<td>1.98 ± 2.88</td>
<td>2.93</td>
</tr>
<tr>
<td>S7</td>
<td>43,000</td>
<td>4.5</td>
<td>2.16 ± 2.80</td>
<td>3.11</td>
</tr>
</tbody>
</table>

with earlier work with lipids (26) and proteins (13), 5-min pulses of radioactive leucine were administered to growing cells and fastig cells, and the electrophoretic gel patterns in one-dimensional separations were compared using both denaturing tracings and radioactivity profiles. Unfortunately, the relatively slow uptake of [3H]leucine by the cells and the even slower intracellular distribution of newly synthesized proteins to certain fractions of interest, such as cilia and pellicles, resulted in low yields of radioactivity in the proteins even when cells were incubated with as much as 2 mCi of [3H]leucine/experiment. Our conclusion from these experiments must be considered tentative, but indications were that all parts of the cell are provided with the same variety of proteins in approximately the same proportions whether the cells are growing or merely replacing the protein constituents without any net synthesis.

The turnover of individual proteins in nongrowing cells was next studied in one-dimensional gels using double labeling experiments. Logarithmically growing cells were pulsed for 1 h with [14C]leucine, chased with unlabeled leucine for 2 h in growth medium, and a further 9 h in inorganic medium. The cells were then labeled for 1 h with [3H]leucine. After an additional 3 h to permit intracellular distribution of the 3H-proteins, the cells were fractionated, the proteins were separated by SDS-polyacrylamide gel electrophoresis, and radioactivity was determined along the length of the gels in 1-mm slices. Proteins within a gel having the highest 3H/14C ratios will be those undergoing turnover at the greatest rate.

The complete data for one pellicle preparation are presented in Fig. 5. The radioactive profiles of 14C and 3H across the gel in the photograph are presented near the top, just above the densitometric scan. A plot of the 3H/14C ratio throughout the gel is presented at the top. It can be seen that there are significant differences in the rate of turnover among the various pellicular proteins. The cytospetkeletal proteins (slices 5 through 20) show relatively low turnover rates, whereas the presence of pronounced peaks on either side of tubulin suggests that integral membrane proteins which run in this region of one-dimensional gels may have relatively high turnover rates, and that there may be heterogeneity within this group. This was tested using two-dimensional gels (described below).

A second pellicle preparation is presented in Fig. 6B, along with a control pellicle preparation in which [14C]leucine and [3H]leucine were administered simultaneously (Fig. 6A). In this control, both labels were added during the late labeling period in the protocol; another control in which both labels were added during the initial labeling period gave a similar result (not presented). Heterogeneous turnover rates among the proteins of the other cell fractions included in Fig. 6 are less apparent than for pellicles. There appears to be a tendency for larger proteins to turn over at higher rates in the microsomal fraction, but whether this is in fact true for this or other fractions awaits further analysis.

The question of heterogeneity in turnover rates among surface membrane proteins was rigorously tested in double labeling experiments using two-dimensional gel separations. Cells in inorganic medium were pulsed for 4 h with 7 μCi/ml of [14C]leucine, washed, and chased for 9 h in 1 mM DL-leucine and then washed and pulsed again for 4 h using 20 μCi/ml of [3H]leucine. The cilia and pellicles were then isolated and the proteins were separated in the two-dimensional system of O'Farrell (22). Following this, the gels were stained with Coomassie blue and the identifiable membrane proteins (S1

![Fig. 5. Relative rates of turnover for pellicle proteins determined in one-dimensional polyacrylamide gels using double labeling. A densitometric scan and a photograph of a gel are presented in the lower part of the figure. The scan above this presents the radioactivity profiles of 3H and 14C throughout the gel. A plot of the 3H/14C ratios is presented at the top. In these experiments, cells were pulsed with [14C]leucine at 7.14 μCi/ml for 1 h, chased for 9 h in inorganic medium containing 1 mM unlabeled leucine, and then pulsed again for 1 h using [3H]leucine at 28.5 μCi/ml. Proteins with relatively high turnover rates are those showing elevated 3H/14C ratios. The position of tubulin is indicated by the arrow.](http://www.jbc.org/)
Fractions of the type illustrated in Fig. 5 were run on the gel. A pellicle preparation of leucine and ["C"]leucine were given together and isolated pellicles were run in the gel. A pellicle preparation (B) which is different from the one presented in Fig. 5 illustrates the high degree of reproducibility found in these measurements. The greatest heterogeneity in 3H/14C ratios is seen in the pellicle (B) and ciliary (C) fractions. Less heterogeneity is apparent in the microsomal proteins (D), ciliary supernatant (E), and postmicrosomal proteins (F) using this method. Although these plots utilized absolute 3H/14C ratios, nearly identical patterns resulted when the ratios were normalized after setting the ratio for total proteins in each cell fraction equal to 1.

Through S7, Fig. 4) were removed with a cork borer. The levels of tritium and 14C were determined by scintillation counting. The data from three separate experiments, presented in Table II, indicate that there is heterogeneity within this group of known surface membrane proteins. The ratios should be very accurate because the level of radioactivity within the protein samples was in every case between 10 and 100 times background levels (regions of the gel without protein). The most clear example of this heterogeneity is the significant difference in turnover rate between the acid proteins (S5, S6, and S7) and the others which was observed in all three experiments. The data show that these acidic proteins turn over very rapidly, and the similarity in their turnover rates suggests that these three proteins may be coordinately regulated. Unfortunately, it is not clear whether the one protein found in both cilia and pellicle gels stained with Coomassie blue turns over at the same or different rates in the two fractions. The lowest turnover rates were found in the proteins S2, S3, and S4. Measurements were also made by cutting out several spots not seen in the autoradiograms. The 3H/14C ratio found for these proteins was in every case significantly less than the ratio seen in the most stable membrane protein, S4. This confirms the indication that the surface membrane proteins in general show higher turnover rates than nonmembrane proteins in these fractions.

Ultrastructural Observations—Tetrahymena cells were fixed and stained with ruthenium red and then sectioned and observed with the electron microscope in order to determine which regions of the cell might be accessible to externally applied lactoperoxidase iodination. Ruthenium red is an intensely colored, low molecular weight compound frequently used to reveal the presence of surface coat material on cell membranes. This result also shows that the alveolar membrane system may be exposed to the environment, at least under some conditions, and the alveolar membrane proteins may be subject to labeling when whole cells are treated with the lactoperoxidase iodination procedure. A, subsurface alveoli adjacent to a nonciliated basal body: × 70,000. B, distended subsurface alveoli: × 37,000.

Identification of Membrane Proteins—Three relatively high molecular weight proteins which are readily seen in one-dimensional SDS gels have been identified previously as elements of the surface membrane-associated cytoskeleton (24, 25).
Turnover of Membrane Proteins in Tetrahymena

Following the convention of Vaudaux (24), these are labeled A, B, and C in Fig. 1. Proteins B and C appear to be the major component of the fibrogranular layer of material which underlies the surface membranes in *Tetrahymena*. This layer, called "epiplasm" in ciliates, structurally resembles the jor proteins seen in membrane fractions prepared in this way which underlies the surface membranes in fractions shows that all proteins in these fractions are potentially mobile and many of the bands and spots seen in one- and two-dimensional gels have been able to identify and partially characterize (molecular weights and isoelectric points) a relatively large number of externally exposed surface membrane proteins from *Tetrahymena*.

Lactoperoxidase iodination is generally accepted as iodinating only those proteins exposed on the surface of the cell (3, 5, 15). Several lines of evidence in the present study confirm this in *Tetrahymena*. First of all, only cilia and pellicle fractions were heavily labeled after iodination (Table I). Similarly, many of the bands and spots seen in one- and two-dimensional Coomassie blue-stained gels were not radioactive after iodination of whole cells, although the experiment with sonicated fractions shows that all proteins in these fractions are potentially iodinatable. Electron micrographs of cell sections following staining with ruthenium red, however, suggest that iodinated proteins might possibly be recovered from the surface-associated alveolar membrane system, as well as from the plasmalemma. The results with ruthenium serve to raise the question of a possible intimate relationship between the surface membrane and the subsurface alveolar membrane system in vivo at this type.

Turnover of Membrane Proteins—Appreciation of the dynamic nature of rongrowing membranes followed the important investigations of Omura et al. (6), Arias et al. (29), and Warren and Glick (30). In these studies, and in later publications by these and other authors, it was demonstrated that the membranes of nonproliferating mammalian cells renew their proteins and lipids at significant rates. From the beginning, it was noted that individual membrane proteins may show turnover rates which are independent of one another. Omura et al. (6) found significant differences in the half-lives of several different microsomal enzymes from rat liver. Subsequently, Dehlinger and Schimke (7) observed that different proteins within rat liver cell membranes, microsomes, and mitochondrial membranes showed markedly different rates of turnover. They found that there was a definite tendency for larger proteins to show higher turnover rates, a situation similar to that observed for soluble proteins. A similar result has been reported by Mendolosi (8), who estimated that the half-lives of rough microsomal membrane proteins in guinea pig pancreatic acinar cells vary from approximately 4.5 days for high molecular weight components to 28 days for small proteins.

In the present study, we obtained preliminary evidence for a direct relationship between protein size and turnover rate in the microsomal fraction and possibly also in the supernatant fractions. Careful studies using two-dimensional gels will be required to confirm this. Such a pattern was not apparent, however, in similar studies of the surface membrane fractions (cilia and pellicles). Nevertheless, the data obtained do not support the notion that protein turnover rates are homogeneous within these fractions. The cytoskeletal proteins of the pellicular fraction appeared to turn over with rates significantly slower than the integral membrane proteins, but, more importantly, heterogeneity in turnover rates was found within the group of known integral proteins. Although the number of integral proteins which could be measured using double labeling with [3H]leucine and [14C]leucine in combination with two-dimensional gel electrophoresis was relatively small (Table II), the separation and identification methods were rigorous and the measurements are likely to be highly accurate. The number of proteins measured is adequate to establish that all membrane proteins of the cell surface of *Tetrahymena* do not turn over at the same rate.

Homogeneous turnover in membrane components suggests the co-polymerization of membrane elements, perhaps by vesicle fusion, and this has been reported in a number of systems. For example, extensive work by Doyle and co-workers (5, 9, 10) using hepatoma tissue culture cells has led to the suggestion that these cells interiorize and degrade surface membrane as a unit; the interiorized membrane is then replaced with presassembled units of like composition. According to this model, secretory proteins and membrane proteins arrive at the cell surface by similar mechanisms (9). Major evidence for this is the homogeneity in turnover rates found in a large population of surface membrane proteins in this cell type (5). Uniform turnover rates were also noted in a study of red blood cell membranes by Morrison et al. (2), by Hubbard and Cohn using mouse L cells (3), and by Roberts and Yuan (4) in Chinese hamster ovary and fibroblast cells.

The heterogeneous turnover rates reported for surface membrane proteins in the present study suggest that many of the components of the *Tetrahymena* surface may be regulated individually. This result, by itself, is probably not adequate to rule out the possibility of the addition of interior membrane vesicles to the cell surface membrane in this cell type. If, for example, one or a few protein species entered the membrane by way of such fusion vesicles and the remainder were inserted individually, heterogeneous turnover rates would still be found. In the present study, the relatively acidic membrane proteins S7, S8, and S9 showed very similar turnover rates, and these were different from those of the other membrane proteins measured. One possibility is that the three acidic proteins enter by vesicle flow, whereas the remainder enter separately. Some morphological evidence for secretory vesicle fusion at the surface of *Tetrahymena* has, in fact, been published (31). Still, it will be necessary to obtain further information in order to determine with certainty whether one or more proteins enters the surface membrane by this means. Whether or not this occurs, the present data appear to rule out the possibility that all surface membrane proteins are coordinately assembled in *Tetrahymena*. This type of regulatory pattern, plus the ease with which surface membrane regeneration and modulation can be controlled in this cell, should make *Tetrahymena* a useful system for further studies of membrane control in eukaryotic cells.

REFERENCES

Turnover of Membrane Proteins in Tetrahymena

Studies of membrane formation in Tetrahymena. The identification of membrane proteins and turnover rates in nongrowing cells.
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