Elutriation and Coulter Counts of Tetrahymena pyriformis Grown in Peanut and Cottonseed Meal Media

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Received for publication 23 November 1970

Growth of Tetrahymena pyriformis W has been used to evaluate nutritional quality of peanut and cottonseed meals. An efficient elutriation method is described for separating cells of this organism from particulate matter left in the substrate (enriched with basal medium) after 4 days of incubation. After elutriation the cells can be counted with a Coulter counter by using calibration procedures which are presented. Elutriation and Coulter counting provide a rapid and efficient method of measuring the growth response of T. pyriformis W. Utility of the method is demonstrated by agreement between Coulter counts and visual counts of the cells and by demonstration of a linear response of cell numbers to substrate nitrogen.

A method of evaluating the effects of processing on the nutritive quality of protein products more rapid than the 28-day rat feeding test should have great utility. The growth of Tetrahymena pyriformis W has been used to assess relative nutritional quality, growth being measured by microscopic counting of the cells, and is described in recent reviews by Celliers (9), Reynolds (21), and Evans and Bandemer (12).

Microscopic cell counting to assess the growth response is very tedious, time-consuming, and subject to error particularly in the range of relatively high concentrations of the cells, food, and other particles. Several investigators have stressed the need of a quantitative method of separating the cells to allow automatic counting and to allow the criteria for protein quality to be based on parameters such as weight gain or nitrogen balance (W. R. Fernell and G. D. Rosen, Abstr. Proc. Nutr. Soc., vol. 13, p. xviii, 1954) measured in terms of protein synthesis (13, 1; W. R. Fernell and G. D. Rosen, Abstr. Proc. Nutr. Soc., vol. 13, p. xviii, 1954), dry weight (24), or total nitrogen (24) in addition to cell yield.

This is a report of a quantitative method of separating preserved T. pyriformis W cells without microscopically visible damage from particulate substrates, especially peanut and cottonseed meals, and the development of a method for electronically counting the cells of various sizes in each population using a Coulter counter.

MATERIALS AND METHODS

Assay. The procedure was essentially that previously described (28), with some exceptions.

The peanut and cottonseed meals were fed to the test organism in the same form as they were fed to animals in feeding tests and were ground to pass a 40-mesh screen. The residual oil and any fatty acids left after processing were not extracted before microbiological assay, although it is known that some free fatty acids inhibit the growth of this test organism (16).

The aqueous suspensions of the test substances were prepared to contain approximately 5 mg of nitrogen per ml after adjustment of the pH to 8.2 and were then analyzed for total nitrogen (3). The analyses of homogeneous test substances, e.g., casein, were made on duplicate subsamples of 3 ml each. The analyses of heterogeneous substances, e.g., the oilseed meals, were made on duplicate composites each prepared by transferring 1.0, 0.75, 0.50, 0.40, 0.25, and 0.1 ml of suspension with a bacteriological pipette into a macro-Kjeldahl flask. Each composite then consisted of 3 ml estimated to contain ca. 15 mg of total nitrogen. The composites were made in this manner to include sampling error similar to that possible in the assays.

The components of the basal medium were not lyophilized. The various stock solutions for this basal medium were prepared separately by using the methods of Baum and Haenel (5) with some modifications.

The stock solution of the vitamins was prepared at 10× final concentration, membrane-filtered (Millipore Corp., Bedford, Mass.), and stored frozen in sterile, screw-cap bottles. All other stock solutions (except
soluble starch) were mixed and stored frozen at 2.5× 
final concentration. A 20% solution of soluble starch
was prepared as required.

For each assay, 4 ml of solution or suspension of
test substrate of known total nitrogen content,
1 ml of the mixed vitamins, 1 ml of 20% soluble starch
(Difco), and 4 ml of mixed solution of the salts,
purines, and pyrimidines were autoclaved in a 2-oz 
bottle with a screw-cap at 120 C for 15 min.

After cooling, each bottle was inoculated with two 
drops (approximately 0.03 ml each) of actively motile 
cells of T. pyriformis W from a composite of two 3-day 
10-ml proteose peptone broth cultures. The bottles 
with loose caps were placed in special racks at a slope 
of 15° to the horizontal to insure a high surface to 
volume relationship of the culture medium. The inocu-
lated cultures were incubated for 4 days to ensure that 
comparisons were made on cultures of motile 
organisms of near maximal population (14), at 24.9 ±
0.5 C at 60 to 90% relative humidity. Sampling for 
counts was made from single cultures.

**Elutriation of the cells from the particulate substrates.**
A suitable subsample (usually 1 ml) of each culture of 
known substrate nitrogen content mixed on a shaker 
was transferred to 4 ml of potassium phosphate pre-
servative (28) in a 12- or 15-ml graduated, conical 
centrifuge tube with a screw-cap or a tightly fitting 
plastic cap. The preserved culture is later referred to as 
a subsample. The tube was shaken during addition 
of the culture and for several minutes afterwards to avoid 
clumping of the cells. The elutriation apparatus is 
shown in Fig. 1. A stirring magnet (3 by 10 mm,
Teflon-covered) was inserted in the tube of preserved 
subsample, saline (0.9% NaCl plus 0.1% formalde-
hyde) was added to bring the volume to ca. 10 ml, and 
the suspension was mixed with a magnetic stirrer. The 
suspension was viewed with a hand lens (Aspheric 
Cataract Reader, 50 mm in diameter, 20 diopters, 
Combined Optical Industries Ltd., Bath Road, Slough, 
England).

After extraneous particles settled from the upper 
part of the suspension and the cells looked clean, part 
of the suspension was drawn off through the plastic 
tube with the syringe in the continuous pipetting outfit 
(Becton, Dickinson, and Co., Rutherford, N.J.) and 
transferred through a 100-mesh and then a 200-mesh 
(sieve opening, ca. 0.074 mm) stainless-steel screen, 
each held in a stainless-steel membrane-holder (Milli-
pore Corp., Bedford, Mass.) into a volumetric flask of 
suitable size for the final dilution desired for counting. 
The dilution was usually 1:200. The elutriation process 
was repeated until the extraneous particles left in the 
centrifuge tube were washed free from cells, a clean 
tube was substituted, and the apparatus was washed 
well with saline-preservative. This saline was also 
transferred into the volumetric flask.

**Coulter counting of the cells.** For Coulter counting, 
the cells are suspended in an electrolyte. The suspen-
sion of cells is drawn under vacuum through an aper-
ture of known size in a tube which has an immersed 
electrode within it and another outside of it. These 
electrodes cause an electric current to flow through the 
aperture. Each cell passing through the aperture dis-
places an equal volume of electrolyte causing a modula-
tion (or resistance) in the electric current. This 
modulation is detected by the instrument as a signal or 
pulse. The pulses are electronically amplified and 
automatically counted. Threshold settings of the in-

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**Fig. 1. Apparatus used to elutriate Tetrahymena pyriformis W from particulate substrates.**
instrument must be determined for cells of particular volumes. An upper threshold setting is chosen to screen out counts of particles above a predetermined volume, and a lower threshold is selected to screen out counts of particles below a selected volume. Therefore, the dimensions of cells of various sizes and shapes grown in several substrates, including casein, were measured microscopically with a calibrated micrometer. The volumes of the cells were then calculated as prolate spheroids, since the predominant type was pyriform, or as spheres, even though the round types are not quite spheres.

**Cell dimensions and counter settings.** Of numerous cells measured, the smallest were almost round, approximately 15 to 20 μm in diameter. The largest cells, pyriform, were 30 by 70, 28.7 by 71.4, 28 by 70, 30 by 78, 32 by 78, and 17.5 by 80.5 μm. Dividing cells, attached pairs, were 21 by 38 and 21 by 39, 23 by 36 and 23 by 36, and 26 by 47 and 26 by 37 μm. The measurements of some of the predominant sizes, mostly pyriform, were 30 by 62, 25 by 61, 27 by 57, and 26 by 46 μm.

The ranges of the calculated volumes of the cells, assumed to be primarily prolate spheroids and secondarily spheres, are given in Table 1 with the settings of the Coulter counter (model B, aperture 140 μm) required for counting these volumes. The particles are counted according to volume and not as to shape. Even if the cells were rectangular, the volume of the largest measured would be included for counting. Each time the instrument was repaired, it was recalibrated, and the “zero” of the threshold settings was checked frequently.

A model B Coulter counter, described by Barnes et al. (4), was used with a tube with an aperture of 140 μm and without the size distribution plotter. It was calibrated with ragweed pollen, diameter predominantly 20.3 μm, to obtain a constant for determining the volumes of particles that would be counted at stated thresholds and settings of the instrument. The method of calibration is described in the manual supplied by the manufacturer of the instrument. The settings were chosen to include the range of the volumes of the smallest, the predominant size, and the largest cells measured. Two-milliter subsamples of the diluted cell suspension were counted. At least three readings were made and averaged for each of the three ranges of cell volumes. The recorded total cell count is the sum of these means.

The electrolyte used in the counting assembly was 0.9% NaCl plus 0.1% formaldehyde, filtered through a membrane (Millipore Corp.) of 4-μm porosity and counted to be sure it was substantially free from particles the same size as the particles to be counted.

The functioning of the instrument was checked periodically by testing the zero settings of the thresholds, by recalibrations with the ragweed pollen, and by counting the particles in weighed samples of pecan pollen (Hugh Graham Laboratories Division, Hollister-Stier Laboratories, Dallas, Tex.). The pollen was suspended in the electrolyte at known volume and counted at the same settings used for the *T. pyriformis* cells. The diameter of the pecan pollen was reported to be predominantly 45 to 50 μm (48,032 to 65,888 μm³) and the particles were considered to be “essentially mono-sized.” It was expected that these particles could be counted at the same instrument settings that were selected for counting the cells of the test organism. As shown in Table 2, this was true.

If blocking of the aperture occurred too frequently for one subsample, another elutriated subsample was poured through a 200-mesh stainless-steel funnel from the volumetric flask into the beaker for counting. If another subsample was not available, the one partly used for counting was filtered in the same manner into another beaker, stirred well, and counted. No corrections were made for coincidence counting.

**Growth response.** The growth response was evaluated by computing the linear regression coefficient for the regression of numbers of organisms per unit of medium, without the restriction that the line of response must pass through the origin.

**RESULTS**

**Elutriation of the cells.** During development of the elutriation method, its adequacy for quantitatively transferring the cells from the particulate substrates to another medium for counting was checked by microscopic examination of rinsings of the stainless-steel screen after elutriation of numerous samples. Few, if any, cells were found

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**Table 1. Volumes of Tetrahymena pyriformis W cells**

| Settings of counter | Volume (μm³) at |
|--------------------|----------------|
|                    | k = 18.9       | k = 23.8       | k = 24.8*     |
| 16                 | 60,480–120,960 | 76,160–153,320 | 79,616–159,232|
| 8                  | 6,048–60,480   | 7,616–76,160   | 7,962–79,616  |
| 8                  | 3,024–6,048    | 3,803–7,616    | 3,981–7,962   |

* Assumes that the predominant prolate spheroids and secondarily spheres.

* The instrument was calibrated with ragweed pollen, volume predominantly 4379.3 μm³ each.

* k = 1/amplication; C = 1/aperture current; LT = lower threshold; UT = upper threshold.

* k = Calibration constant obtained by counting ragweed pollen of stated diameter at various times and particularly after the instrument was repaired.

* Same k for two runs.
in the rinsings. The adequacy of the method was also checked to determine if extraneous particles of sizes similar to the cells were being transferred into the suspensions to be counted. Microscopic examinations of numerous cell suspensions diluted for counting showed that they were virtually free from extraneous particles that might be counted as cells. The number of cells lost during elutriation was negligible. This is shown by examples of counts of cells not elutriated and elutriated from proteose peptone broth in Table 3 and from casein in Table 4.

Six subsamples were elutriated in about 1 hr, and usually two sets of six subsamples could be partitioned in 1 day. This included setting up parts of the equipment, washing the necessary parts at the end of the runs, and diluting the elutriated cells for counting. The time for the

**Table 2. Coulter counts of weighed pecan pollen**, at instrument settings used for counting *Tetrahymena pyriformis* W cells, made at various times

| Particle size (μm³) | No. of particles/mgd |
|--------------------|----------------------|
| 76,160 to 159,232  | 315                  |
| 7,616 to 79,616    | 25,936               |
| 3,803 to 7,962     | 557                  |
| Σ 26,808           | Σ 25,688             |

* Essentially mono-sized particles, ca. 45 to 50 μm in diameter.
* See Table 1. Tube aperture of 140 μm was used.
* Particularly after the instrument was repaired.
* Average of three readings. Corrected for dilution, sample size, and weight of pollen.

**Table 3. Comparison of visual and Coulter counts of *Tetrahymena pyriformis* W cells grown in several substrates**

| Substrate              | Visual countb,c | Dilution of original culture | Cells not elutriated | Cells elutriatedd |
|------------------------|-----------------|-------------------------------|----------------------|-------------------|
|                       |                 | 0.30 ml                       | 2.0 ml               | 0.05 ml          | 0.50 ml          | 2.0 ml          |
| Proteose peptone broth| 0.360           | 1-40                          | 0.281                | 0.284            |                  |                  |
|                       | 0.750           | 1-100                         | 0.275                | 0.283            |                  |                  |
|                       | 0.780           | 1-200                         | 0.285                | 0.276            | 0.7655           | 0.6998           |
|                       | 0.990           | 1-100                         | 0.285                | 0.276            | 0.7034           | 0.6998           |
|                       |                 | 1-200                         | 0.7111               |                  | 0.6874           |                  |
|                       | 0.750           | 1-200                         | 0.7111               | 0.6874           |                  |                  |
|                       | 0.780           | 1-200                         | 0.7111               | 0.6874           |                  |                  |
|                       | 0.990           | 1-200                         | 0.7111               | 0.6874           |                  |                  |
| Peanut meal            | 0.060           | 1-200                         | 0.281                | 0.284            | 0.060            | 0.062            |
|                       | 0.160           | 1-200                         | 0.275                | 0.283            | 0.137            | 0.149            |
|                       | 0.380           | 1-200                         | 0.285                | 0.276            | 0.336            | 0.349            |
|                       | 0.600           | 1-200                         | 0.7111               | 0.6874           | 0.544            | 0.520            |
|                       | 0.780           | 1-200                         | 0.7111               | 0.6874           | 0.582            | 0.546            |
| Cottonseed meal        | 0.200           | 1-200                         | 0.368                | 0.368            | 0.258            | 0.281            |
|                       | 0.600           | 1-200                         | 0.368                | 0.368            | 0.336            | 0.349            |
|                       | 0.800           | 1-200                         | 0.544                | 0.520            | 0.544            | 0.520            |
|                       | 0.620           | 1-200                         | 0.582                | 0.546            | 0.582            | 0.546            |

* Cells not elutriated from the substrate.
* Millions per milliliter of original culture.
* Each count = sum of means of at least three counts at each of three cell-volume ranges.
* Elutriated from the particulate substrates and suspended in aqueous 0.9% NaCl plus 0.1% HCHO.
procedure could be reduced by making the apparatus automatic with solenoid valves and timing devices, by the addition of saline under pressure, and by the removal of the cell suspension under vacuum.

The volumetric flask was chosen so that the cells were diluted properly for counting without further transferring. By trial, a 1:200 dilution of the original sample was usually suitable. Cells were stable in cultures preserved in phosphate buffer with 6% formaldehyde (28) for 2 years before elutriation.

**Growth response evaluated by visual and Coulter counts.** Results of visual and Coulter counts of the test organism grown in several substrates and results of replicated automatic counts are shown in Table 3. The automatic counts tend to be lower than the visual ones at the higher test substrate nitrogen concentrations assayed but check fairly well at different dilutions and volumes of subsamples.

Previous studies of assays of various protein concentrations (28) and of numerous cottonseed meals (*unpublished data*) showed that their relative nutritive value could be determined by comparing the growth response of the organism, determined by visual counts, at various levels of nitrogen of the test substance up to about 1 mg per ml of final medium, the range in which the response was linear. This is illustrated in Fig. 2 with results obtained with a very good quality meal and a moderately good one, before and after heat damage.

In developing the elutriation and Coulter counting methods, a cottonseed meal and a peanut meal were assayed at various nitrogen concentrations up to ca. 2 mg per ml of final medium, and the growth response was determined by both types of counts. The results (Fig. 3) were linear up to at least 1 mg of nitrogen per ml. The visual counts of cells in diluted substrate were made within a few days of sampling to avoid possible evaporation of the liquid, since it was not feasible to make up the volume just before counting. For these two meals, preserved subsamples for Coulter counts were stored diluted with the phosphate buffer preservative in 25-ml volumetric flasks. Just before elutriation, each suspension was quantitatively transferred to a centrifuge tube. Therefore, for these two meals, one more transfer was required than for subsequent assays. For the regression lines shown in Fig. 3, the slope b for the cottonseed meal is 0.5769 for the visual counts and 0.4608 for the Coulter counts. For the peanut meal in this same range, b is 0.6947 for visual counts and 0.5193 for the Coulter counts. The statistics for all of the data for each of these two meals are shown in Table 5.

**Fig. 2.** Growth response of *Tetrahymena pyriformis* W to various nitrogen concentrations of two cottonseed meals. Symbols: O, a very good meal; Δ, a moderately good meal not autoclaved before the assay; and ▽, autoclaved before the assay. Solid symbols, $\bar{x}$. The probability for the correlation between the nitrogen levels and the growth response for each type of counts for both meals is > 99.9. Although for each meal b is somewhat greater for the visual than for the Coulter counts, it seems feasible that the latter could also be used in determining the nutritional index.

Two more peanut meals were assayed with the Coulter counts spot-checked by visual counts (Fig. 3).

The efficacy of the elutriation and automatic counting methods was tried on five more peanut meals before using the methods to determine the
Fig. 3. Comparison of visual and Coulter counts of Tetrahymena pyriformis W grown in various nitrogen concentrations of a cottonseed meal and three peanut meals used in developing a method to elutriate the cells from the particulate substrates. Open symbols indicate visual counts of cells in diluted substrate, and solid symbols indicate Coulter counts on cells elutriated from the substrate and then diluted (△, ▲, a cottonseed meal; ▼▼, ◊ ◊ , and □ □, peanut meals). For the two meals on the left, the lines of regression are shown for the data of both types of counts. For the two meals on the right, the line of regression is shown for the Coulter count data only. Oval symbols, x̄y.

Table 4. Effect of elutriating Tetrahymena pyriformis W cells from 4-day casein cultures evaluated by Coulter counts at the higher nitrogen levels assayed

| Amt (mg) of casein nitrogen/ml | Assay | Cells (millions/ml of original culture) | Not elutriated | Elutriated<sup>c</sup> |
|-------------------------------|-------|----------------------------------------|----------------|-----------------------|
| 0.50                          | 1-4   |                                        |                | 0.5304                |
|                               | 1-5   |                                        | 0.5588         | 0.5495                |
|                               | 3-1   |                                        | 0.5036, 0.5338 |                      |
|                               | 4-8   |                                        | 0.5544         | 0.5639                |
|                               | 5-27  |                                        | 0.6515, 0.6399 |                      |
| 0.75                          | 1-6   |                                        | 0.6557         | 0.6618                |
|                               | 3-9   |                                        | 0.6893, 0.7067, 0.7135 |        |
| 0.99                          | 1-7   |                                        | 0.7187         | 0.7079                |
|                               | 4-10  |                                        | 0.8920         | 0.7852                |
| 1.04                          | 4-7   |                                        | 0.7766         | 0.6814                |
| 1.05                          | 5-28  |                                        | 0.6746, 0.6458 |                      |
| 1.24                          | 1-8   |                                        | 0.6576         | 0.6471                |
| 1.49                          | 2-20  |                                        | 0.6988         | 0.6463                |
| 1.74                          | 1-9   |                                        | 0.6806         | 0.6690                |
| 1.99                          | 1-10  |                                        | 0.7445         | 0.5642                |

<sup>a</sup> Each count = sum of the averages of at least three counts at each of the three cell-volume ranges tested in 2 ml of cell suspension diluted 1:200 in aqueous 0.9% NaCl plus 0.1% HCHO.
<sup>b</sup> First number = run. Second number = sample.
<sup>c</sup> Elutriated from the substrate.

Table 5. Statistics for the data for cottonseed and peanut meals

| Determination       | Slope b | y Intercept |
|---------------------|---------|-------------|
|                     |         |             |
| Cottonseed meal     |         |             |
| Visual counts       | 0.6339  | 0.0792      |
| Coulter counts      | 0.4205  | 0.0336      |
| Peanut meal         |         |             |
| Visual counts       | 0.5936  | 0.0748      |
| Coulter counts      | 0.5035  | 0.0616      |

Relative nutritive value of a series of treated and untreated meals with the growth response determined by Coulter counting only. As shown in Fig. 4, the response was again linear in a meal nitrogen range of as high as ca. 1 mg per ml of final medium.

These results indicate that the growth response of T. pyriformis W to peanut and cottonseed meals at various meal nitrogen contents of at least 1 mg per ml of final medium, evaluated by Coulter counting of cells elutriated by the described method, can be used to demonstrate differences between meals. A study is to be reported on the successful use of this assay by the described procedures to predict for higher
FIG. 4. Growth response of Tetrahymena pyriformis W to various nitrogen concentrations of (○, △, □, ◊, ▽) several peanut meals evaluated by Coulter counts of cells elutriated from the particulate substrate, showing linearity of response up to at least 1 mg of test substrate nitrogen added per ml of culture medium. Solid symbols, xy.

FIG. 5. Comparison of visual (○) and Coulter counts (△) of Tetrahymena pyriformis W cells grown in various levels of casein nitrogen, showing similarities in both types of counts up to ca. 0.6 to 0.7 mg of added nitrogen/ml and higher visual counts at higher nitrogen levels. Solid symbols, xy.

animals the nutritional quality of a series of peanut meals and of cottonseed meals.

Reference standard. Casein is usually used as the reference standard for these assays to compare the results of different series of protein substances when the assay conditions change, such as use of different batches of basal medium, etc., or when different investigators assay the same test substrates. A comparison of visual and Coulter counts of the cells grown in various levels of casein nitrogen is shown in Fig. 5. Both types of counts are similar up to ca. 0.6 to 0.7 mg of added nitrogen per ml, but at higher nitrogen levels discrepancies occur. These have not yet been explained. Coulter counts of replicate subsamples of the cells grown in the higher levels of casein nitrogen assayed are shown in Table 4. These results indicate that the discrepancies may not be due to coincidence counting, for which no corrections were made, but that perhaps the visual counts may be too high. The results of the visual counts are similar to those previously reported (25, 28, W. R. Fernell and G. D. Rosen, Abstr. Proc. Nutr. Soc., vol. 13, p. xviii, 1954). Further studies are being conducted with casein and also with egg albumin and lactalbumin which may be better reference standards for this assay.

DISCUSSION

Fernell and Rosen (13) reported that they were unable to separate Tetrahymena cells from pro-
tein media by differential centrifugation in sucrose solutions or by electro-migration techniques. Walthe (30) reviewed reports of other investigators who tried to obtain undamaged, washed suspensions of protozoa from nonparticulate media for physiological studies, but no report is known of an adequate method of separating *Tetrahymena* or other protozoa from a particulate medium. In addition, no report was found giving details of Coulter counting of a population of *Tetrahymena* of various sizes.

**Elutriation of the cells from particulate substrates.** Attempts were first made to partition the cells from other particles in the media by methods similar to those suggested by Albertsson (1). Two water-soluble polymers, arabinogalactan (kindly supplied by Stein, Hall and Co., New York, N.Y.) and sodium carboxymethyl cellulose, were tried at various concentrations. Both were satisfactory in removing the relatively large meal particles, but the lightest-weight extraneous particles of various sizes remained in the phase with the cells. The most dilute concentrations of the polymers used were the best for partitioning the cells, so the potassium buffer of the basal medium and then 0.9% aqueous sodium chloride were tried. Both were satisfactory for the purpose. The latter plus 0.1% formaldehyde is suitable for use in a Coulter counter. In the buffer and in the saline, the meal particles in a mixed suspension settle faster than living or preserved cells in separatory pyriform funnels and conical centrifuge tubes but not in straight-sided vessels, such as cylinders or ordinary test tubes. The funnels of various sizes, especially 125 ml, are satisfactory for large volumes of culture.

**Volumes of the cells.** The volumes given are within the ranges of those reported for *T. pyriformis* W calculated as prolate spheroids by Cashland and Johnson (8) and Reynolds and Wragg (22). These volumes are also within the ranges of other strains whose volumes were calculated by other methods by Thompson (Ph.D. Thesis, Univ. of Alabama, 1960), Thor- sum (29), and James and Read (14), and for an unidentified strain during different phases of growth but with the method of volume calculation not reported by Summers, Bernstein, and James (26). The dimensions of width and length are within those given for strain W by Røslile (23) and for 16 strains of *T. pyriformis*, including W, by Loefer (19).

**Method of Coulter counting.** The method of using the instrument had to be devised because of the scant information found in the literature. There are a few reports of Coulter counting of *Tetrahymena* in nonparticulate media but few details of the method used are given. Using a model A, Leboy, Cline, and Conner (18) used a tube with a 200-μm orifice. The model A is similar to the model B without the automatic recording device, except that the model B has the advantage of more refined electronics, according to Barnes, Parker, and Bradley (4). Using the model B, Kovács et al. (17) counted unpreserved *T. pyriformis* G, precooled to 0°C, with "a capillary tubesize 100 μm," Byfield and Scherbaum (6, 7) counted *T. pyriformis* GL and WH-14, and Wille and Ehret (32) and Szyszko et al. (27) reported counts of *T. pyriformis* using a model B but did not give details of the method used.

**Coincidence counting.** The results were not corrected for coincidence counting. For the assays of peanut and cottonseed meals, this apparently was not necessary under the conditions of this study.

It is not feasible to determine the coincidence error per se for every type of sample counted at various cell-volume ranges and substrate nitrogen levels because of the amount of time required to assay, preserve, and elutriate enough subsamples and then make dilutions and counts to calculate the correction required. The method of averaging at least triplicate counts for each of the three cell-volume ranges and using the sum of the means for the final count should reduce this error to a minimum. Each of these nine counts of the cells grown at a particular nitrogen concentration is made on a different sample of the cell suspension of high dilution. Actually, determining the final counts at various substrate nitrogen concentrations is in effect determining the counts at various dilutions of the original sample. The results of final Coulter counts of various dilutions and sizes of subsamples of the organism grown in proteose peptone broth, peanut meal, and cottonseed meal (Table 3) and the good checks of final counts of replicate subsamples of the organism grown in the higher levels of casein during several years (Table 4) indicated that corrections for coincidence were not required.

Only a few reports were found on correction of Coulter counts of *Tetrahymena* for coincidence, and the correction was very small or negligible when the organism was grown in nonparticulate media. Macdonald (20) found that coincidence effects were negligible for *T. pyriformis* W. Byfield and Scherbaum (7) reported a coincidence of less than 1% for cells of *T. pyriformis* GL and WH-14 fixed in 4% Formalin. Wille and Ehret
(31) made counts of T. pyriformis W in duplicate with a model B instrument without the distribution plotter and found they usually agreed within 0.5% when the average counts were corrected for coincidence. These investigators did not describe the method they used for counting or for the coincidence correction.

Discrepancies between the two types of counts are probably not due to changes in temperature at the aperture (4), since the model B has built-in automatic compensation for such changes (Coulter Manual for the Model B Coulter Counter, 5th ed. 4-67, 420100201, Coulter Electronics, Inc.), or of changes in the temperature of the cell suspensions which were counted at room temperature regulated by air conditioning. Degkwitz and Selle (10) found discrepancies due to variations in ambient temperatures in a laboratory without air conditioning. Anderson, Petersen, and Tobey (2) observed anomalies in the determination of cell number by Coulter counting of Chinese hamster ovary cells due to the failure of daughter cells to separate after mitosis. However, this is not true for the T. pyriformis W assays incubated for 4 days. Only an occasional pair of dividing cells was found during visual counting of each assay of more than 50 substrates, including casein (Teunissen, unpublished data), each assayed at six or more nitrogen levels. Discrepancies would more probably be due to sedimentation of cells in the more dense suspensions during counting, but more study is required on this aspect of the problem.

The method has not yet been tried for separating living cells, but it seems reasonable to assume that it could be used for this purpose. Since T. pyriformis W is negatively geotrophic and grows best with aeration, the actively motile cells swim near the surface of stationary medium, and the less motile cells can be easily suspended by gentle shaking of the container and could also be removed from the upper portion of the diluted suspension. A method of harvesting Tetrahymena cells without the deleterious effect of centrifuging is desirable. This is especially so for cells grown in Tween (15), for cell extraction procedures, and for enzyme and cytological studies (11).

The procedure might also be used to separate other organisms from particulate substrates for similar purposes; in cell culture studies, e.g., to separate trypsinized cells from fibrous tissues; or to determine the microbial populations in food slurries, soil suspensions, washed fibers, etc., without adding nutrients or inhibitory substances from the test substrates.

Other possible uses for the elutriation procedure. In addition to cell yield, other parameters of growth response can be determined on cells elutriated from particulate substrates. For the assay of nutritional quality of oilseed meals or of other proteinaceous substances with T. pyriformis W, measurement of cell nitrogen or dry weight produced would be desirable.

ACKNOWLEDGMENTS

I express my gratitude to Mona L. Brown for instructions in the use of the counter and her assistance in developing the method of automatic cell counting, to Eva V. D'Arcangelo for the calculation of the statistical analyses by computer, and to Mildred K. Black for the nutrient analyses.

LITERATURE CITED

1. Albertsson, P. Å. 1960. Partition of cell particles and macromolecules. John Wiley & Sons, Inc., New York.
2. Anderson, E. C., D. F. Petersen, and R. A. Tobey. 1967. An effect of cell shape on apparent volume as determined with a Coulter aperture. Biophys. J. 7:975-977.
3. Association of Official Agricultural Chemists. 1960. Official methods of analysis, 9th ed., p. 12. Association of Official Agricultural Chemists, Washington, D.C.
4. Barnes, M., M. S. Parker, and T. J. Bradley. 1967. Particle size analysis and the Coulter Counter. Mfr. Chem. Aerosol News 37:47-50, 51.
5. Baum, F., and H. Haenel. 1965. Determination of the biologic value of proteins with Tetrahymena pyriformis. 1. Methodological hints and determination of the value of some foods. Nahrung 9:517-525.
6. Byfield, J. E., and O. H. Scherbaum. 1966. A rapid radioassay technique for cellulose suspensions. Anal. Biochem. 17:434-443.
7. Byfield, J. E., and O. H. Scherbaum. 1968. Amino acid control of RNA synthesis in an animal cell (Tetrahymena) and its relation to some aspects of gene expression. Exp. Cell Res. 49:202-206.
8. Cashland, B. W., and V. A. Johnson. 1957. Variations in growth characteristics of seven strains of Tetrahymena. Growth 21:11-19.
9. Celliers, P. G. 1961. Microbiological evaluation of the nutritive value of South African marine products with Tetrahymena pyriformis. S. Afr. J. Agr. Sci. 4:191-204.
10. Degkwitz, R., and H. Selle. 1966. Sources of error in the counting of leukocytes with the Coulter Counter. Bibliotheca Haematol., no. 24, part 1:32-36.
11. De Terra, N. 1969. Cytoplasmic control over the nuclear events of cell reproduction, p. 1-29. In G. H. Bourne, J. F. Danielli, and K. W. Jeon (ed.), Int. Rev. Cytol., vol. 25. Academic Press Inc., New York.
12. Evans, J. E., and S. L. Bandemer. 1967. Nutritive values of some oilseed proteins. Cereal Chem. 44:417-426.
13. Fernell, W. R., and G. D. Rosen. 1956. Microbiological evaluation of protein quality with Tetrahymena pyriformis W. 1. Characteristics of growth of the organism and determination of relative nutritive values of intact proteins. Brit. J. Nutr. 10:143-156.
14. James, T. W., and C. P. Read. 1957. The effect of temperature on the cell size of Tetrahymena pyriformis. Exp. Cell Res. 12:510-516.
15. Kidder, G. W. 1963. The nutrition of protozoa, p. 44-61. Sixth Int. Congr. Microbiol., Rome, Symp. Nutrition and Growth Factors.
16. Kidder, G. W., and V. C. Dewey. 1951. The biochemistry of ciliates in pure culture, p. 323-400. A. Lwoff (ed.), In Biochemistry and physiology of protozoa, vol. I. Academic Press Inc., New York.
17. Kovács, E., G. Kolompars, and B. Buzz. 1967. Propagation of mammalian viruses in protista. III. Change in population densities, viability and multiplication of yeasts and Tetra-
hymena experimentally infected with encephalomyocarditis virus. Life Sci. 6:2359–2371.
18. Leboy, P. S., S. G. Cline, and R. L. Conner. 1964. Phosphates, purines and pyrimidines as excretory products of Tetrahymena. J. Protozool. 11:217–222.
19. Loefer, J. B. 1952. Some observations on the size of Tetra-
ymena. J. Morphol. 94:407–414.
20. Macdonald, A. G. 1967. The effect of hydrostatic pressure on the cell division and growth of Tetrahymena pyriformis W. Exp. Cell Res. 47:569–580.
21. Reynolds, H. 1964. Potential analytical applications of Tetra-
ymena pyriformis. J. Wash. Acad. Sci. 54:99–108.
22. Reynolds, H., and J. B. Wragg. 1962. Effect of type of carbo-
hydrate on growth and protein synthesis by Tetrahymena. J. Protozool. 9:222–234.
23. Røsle, G. 1968. Undersøgelse over bestemmelse af protein-
stoffers biologiske værdi med Tetrahymena pyriformis W. Husholdningslærerind. 39:213–220.
24. Rosen, G. D. 1958. The micro-biological assay of protein quality, p. 212–219. Proc. Int. Symp. Microchem. 1958. Pergamon Press, London.
25. Rosen, G. D., and W. R. Fernell. 1956. Microbiological eval-
uation of protein quality with Tetrahymena pyriformis W. 2. Relative nutritive values of proteins in foodstuffs. Brit. J. Nutr. 10:156–169.
26. Summers, L., E. Bernstein, and T. W. James. 1957. A corre-
lation between nuclear activity and the growth phase in cultures of protozoan cells. Exp. Cell Res. 12:436–437.
27. Szyszko, A. H., B. L. Prazak, C. F. Ehret, W. J. Elster, Jr., and J. J. Wille, Jr. 1968. A multi-unit sampling system and its use in the characterization of ultradian and infradian growth in Tetrahymena. J. Protozool. 15:781–785.
28. Teunison, D. J. 1961. Microbiological assay of intact proteins using Tetrahymena pyriformis W. I. Survey of protein concent-
rates. Anal. Biochem. 2:405–420.
29. Thormar, H. 1962. Cell size of Tetrahymena pyriformis incub-
ated at various temperatures. Exp. Cell Res. 27:585–586.
30. Waithe, W. I. 1964. Glucose utilization by suspensions of Tetra-
ymena pyriformis. Exp. Cell Res. 35:100–107.
31. Wille, J. L., and C. F. Ehret. 1968. Light synchronization of an endogenous circadian rhythm of cell division in Tetra-
ymena. J. Protozool. 15:785–789.