Serum obtained prior to primary immunization (control) and 14 days thereafter neither inhibited PCC activity nor caused precipitin lines to form against normal liver extract on Ouchterlony plates. However, sera obtained 8 days after the 2nd immunization (22 days after primary immunization) gave clear indication of anti-human carboxylase antibody formation. Added to normal liver extracts, the serum inhibited carboxylase activities and formed a precipitable complex that was readily removed from solution by centrifugation at 5000 × g for 5 min; non-precipitated complexes retained residual enzyme activity. The anti-carboxylase action was completely eliminated when the serum was passed through a carboxylase-avidin-sepharose column, but not by passage through a avidin-sepharose column. In addition, the antiserum caused a broad precipitin region to form against normal liver extracts on Ouchterlony double-diffusion studies in agarose. The precipitin region retained fluorescamine-tagged avidin, a response indicating the presence of biotin-containing protein. No precipitin arcs or fluorescamine staining regions were detected when pre-immune serum was diffused against normal liver extract or against solutions of avidin.

The specificity of the antiserum was demonstrated by its inhibition only of the mitochondrial biotin-containing enzymes, whereas the activity of the cytosolic enzyme, ACC, that appeared to be removed during the acetone precipitation, was not inhibited. The activity of GDH, another mitochondrial enzyme that does not contain biotin, was also not inhibited by the antibody (table).

These heterologous antibody preparations can then be used to purify individually the 3 human carboxylases on antibody-sepharose affinity columns, to prepare monoclonal antibodies to the individual enzymes, to study biotin-dependent intermediary metabolism or to investigate further the various carboxylase deficiencies.

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A new rapid method for collection and preparation of cell suspensions for electron microscopy

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Summary. We describe a new rapid and simple method for collection and preparation of cell suspensions for electron microscopy; the cells are prefixed with glutaraldehyde in their culture medium, and are then compacted on a filter disc. Post-fixation in osmium, staining and dehydration are performed by transferring the filter disc and the cell pellet from one solution to the next. The pellet is easily separated from the filter disc just before treatment in propylene oxide. This method preserves the fine structure as well as the classical technique. Advantages are that numerous cells have the same orientation in the sections and that many samples can be taken in a very short time.

A number of methods have been described for collecting suspended cells into a compact pellet suitable for ultrastructural studies. Centrifugation requires numerous manipulations and is not well adapted to viscous embedding media. Fibrinogen[2], fresh cock plasma[3], nucleohistones[4] and agar[5] have been proposed. The use of these techniques is delicate and often requires several centrifugations, and the results are not always satisfactory, especially when one has to process numerous samples during a short period of time. During the course of our work on *Tetrahymena pyriformis*, we have developed a new method which seems to be well adapted to the study of cell suspensions.

Material and methods. Tetrahymena pyriformis GL was grown and synchronized according to Zeuthen[6].

1. *Prefixation:* 1 ml of culture was fixed with 1 ml of 1% glutaraldehyde in 0.1 M phosphate buffer (pH: 7.4) for 20 min.

2. *Filtration* (operating system: fig. 1): A filter disc (F in fig. 1, B) cut from a Millipore filter type RA (1.2 µm pore size) was damped with phosphate buffer and adjusted on the top of a glass tube (p in fig. 1 A, external diameter 6 mm, internal diameter 1 mm) and kept in place by vacuum. Prefixed cells were gently drawn up into a Pasteur pipette (Pp in fig. 1, A) with a peristaltic pump (pp in fig. 1,

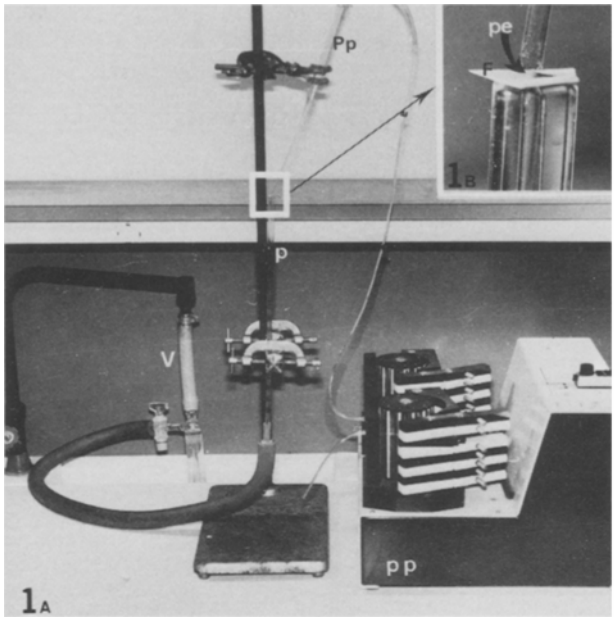


Figure 1. *A* Operating system. p, pipette (0.2 ml, 1/100); pp, peristaltic pump; Pp, Pasteur pipette; V. vacuum. *B* Pellet after filtration. F, Filter disc; pe, pellet.

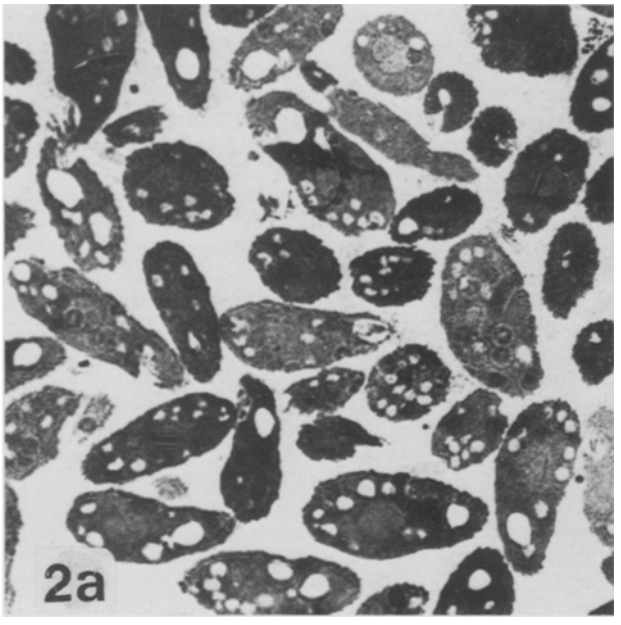
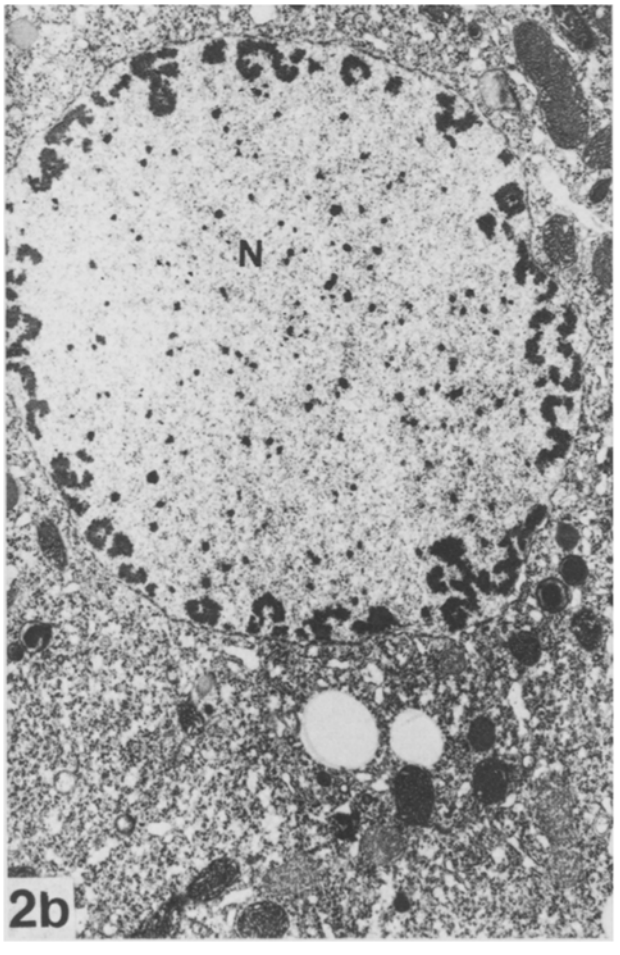
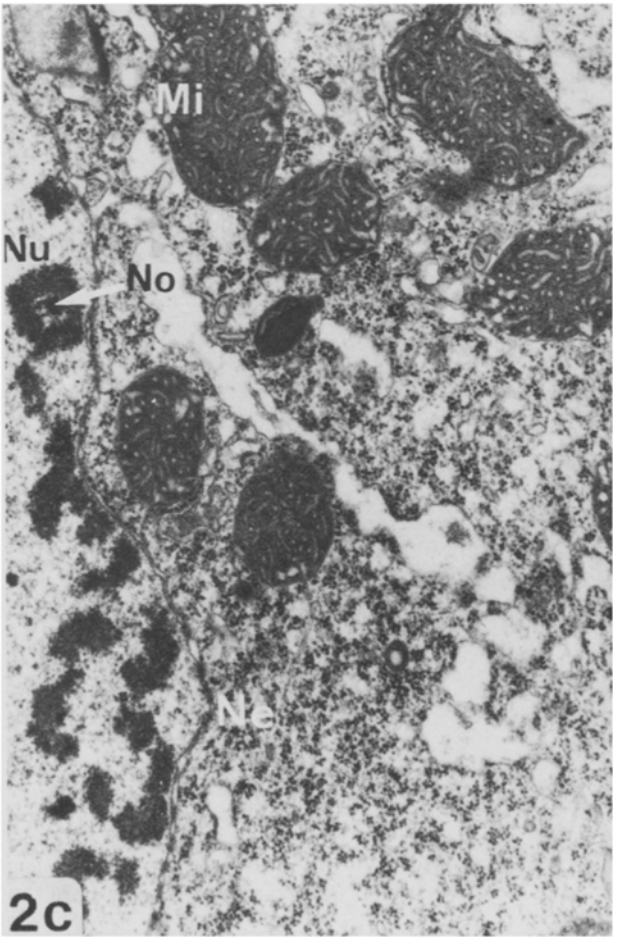


Figure 2. *a* Optic micrograph of semi-thin section (× 500). *b* and *c* Electron micrographs of equatorial section (B, × 9400; C, 16,000). Mi, Mitochondria; N, nucleus; Ne, nuclear envelope; No, nucleolar organizer; Nu, nucleolus.





A). The tip of the pipette was then placed at about 1 mm above the filter disc facing the hole of the thick glass tube (fig. 1, B). Then, regulating the flow of the mixture with the peristaltic pump according to the suction, ciliates were packed into a pellet (pe in fig. 1, B). The filter disc was immediately immersed in a washing solution (phosphate buffer 0.1 M; pH: 7.4).

3. *Post-fixation and embedding:* After 3 changes of buffer, the filter disc with its adhering pellet was immersed into a post-fixation mixture (OsO_4 1% in phosphate buffer 0.1 M; pH: 7.4) for 1 h. Staining with uranyl acetate 1% in alcohol 70 °C and dehydratation were performed using classical methods by transferring the filter disc and the cell pellet from one solution to the next. During the 2nd absolute alcohol bath, the cell pellet was separated from the filter disc by a gentle bending of the latter. Embedding was performed with Araldite, Epon or Spurr. Sections were stained with uranyl acetate and lead citrate[7] and were examined with a Hitachi HU 11 E microscope.

Results and discussion. The use of this method leads to very compact pellets which do not disintegrate during treatment. Semi-thin sections (fig. 2, A) demonstrate the density of the pellet. Numerous cells have the same orientation within the pellet; the antero-posterior axis is parallel to the filter, so it is possible to obtain many sections of cells identically oriented. Examination of cells (fig. 2, B and C) demonstrates that their fine structure is well preserved, though cells in direct contact with the filter may show slight shape distortion if the suction has been too strong.

This technique greatly facilitates manipulation of the biological materials during fixation and embedding; it is very useful when many samples are to be processed during a short time. It can be used for any type of suspended cells and does not only apply to ciliates.

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