

Synchrony of division in mass cultures of *Paramecium tetraurelia* by a selective density-labelling technique*

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SUMMARY

A technique has been developed for achieving synchrony of division by selection for immediate pre- and post-fission cells of *Paramecium tetraurelia*. Cell populations are fed granular tantalum, followed by density-gradient centrifugation. Dividing cells, which do not feed, are separated from feeding interphase cells. The selected cells are division-synchronous for at least two generations when resuspended in fresh culture fluid. This technique provides a rapid means of obtaining large numbers of synchronously dividing paramecia for cell-cycle studies and has general application for selective enrichment of non-feeding sub-populations of cells.

1. INTRODUCTION

Techniques for achieving synchrony of mass cultures of paramecia have not been developed. This is due partly to an insensitivity of *Paramecium* to the heat shock techniques used for *Tetrahymena* synchronization (Sonneborn, 1970). In general, the only technique described for obtaining synchronous populations of paramecia has been manual selection of dividers (Sonneborn, 1970). This technique, although useful for obtaining small synchronous samples, is impractical for large cultures. In this paper, a technique is reported for mass selection of immediate pre- and post-fission cells of *Paramecium tetraurelia*, which are synchronous for subsequent cell divisions. This technique is based, with only minor modifications, on the density-labelling synchronization method developed for *Tetrahymena* by Wolfe (1973).

2. MATERIALS AND METHODS

Paramecium tetraurelia (Sonneborn, 1975), strain 51s, mating type O, from the Sonneborn stock collection (Sonneborn, 1974), was used. Cultures were started from single cell isolates which were 5-20 generations from the previous autogamy, and were grown at 27 °C on baked lettuce medium (BL) inoculated with *Klebsiella pneumoniae* as a food organism (Sonneborn, 1970). The tube cultures were fed sufficient fresh BL daily to give 4-5 fissions per day (fpd) (Preer, unpubl., as reported in Sonneborn, 1970).

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After 3 days' growth at 4–5 fpd, the cultures were pooled (100 ml total), centrifuged at 90 g for 3 min, and then resuspended in Dryl's solution (Sonneborn, 1970) to a volume of 15 ml. Granular, metallic tantalum (average particle size, 2 μm) was added to the cell suspension to a concentration of 2–3 mg/ml. The cells were exposed to tantalum for 9–10 min at 22 °C, with agitation every 2 min to keep the metal in suspension. The cell suspension was then layered over a step gradient made of 4 ml each of 20 and 10% (w/w) Ficoll in Dryl's solution, in 15 ml conical centrifuge tubes, and was spun at 300 g for 5 min. Cells heavily labelled with tantalum were pelleted, while unlabelled and lightly labelled cells were found at the 10/20% interface, and were collected.

For mass culture studies, the collected cells were washed once by centrifugation at 100 g in Dryl's solution and were resuspended in fresh BL to a concentration of approximately 20/ml. Cultures were incubated at 27 °C. Samples of 0.5 ml volume were withdrawn at timed intervals, killed with formaldehyde, and the number of cells in each sample counted. The cell concentration was calculated from the average of the samples at each time.

Individual isolation cultures omitted the final wash. The collected cells were isolated with a micropipette, with as little selection as possible, and were individually resuspended in drop cultures of BL in depression slides. At timed intervals, counts were made of the total number of cells in a collection of drop cultures. Incubation and counting were done at 27 °C.

One control was treated as above, including all centrifugation and incubation steps, except for the deletion of tantalum from the feeding suspension. Another control omitted all centrifugation and incubation: cells were picked from the growth tubes and were placed in drop cultures of fresh BL with no other treatment.

Dividers were cells with a discernable fission furrow under $\times 15$ magnification. At 27 °C, approximately 20 min are required to complete the process of furrowing and cytokinesis (Sonneborn, 1974).

3. RESULTS

Before harvesting, the cell concentration in the culture tubes was approximately 100–150/ml. The suspensions contained less than 2% dividers (Table 1) and, in two separate observations comprising a total sample of 204 cells stained with the Dippell (1955) autogamy stain, no autogamous forms were observed. The proportion of dividers in the population suggests that the cultures were in late log or early stationary phase when collected.

The cell concentration in the feeding suspension was approximately 1000/ml. Cellular uptake of tantalum with time is portrayed in Fig. 1, and appears to be similar to that for *Tetrahymena* (Wolfe, 1973). Roughly 95% of the population has at least one tantalum-filled vacuole after 20 min exposure.

After centrifugation, the cells collected from the 10/20% Ficoll gradient interface were mostly unlabelled, although a few (less than 10%) cells were observed to contain one or two small tantalum-filled vacuoles. The fraction of dividers in a tantalum fed and centrifuged sample is significantly higher than in the untreated

population (Table 1). Conversely, centrifuged but tantalum unfed controls demonstrated no change in the fraction of dividers. The basis for selection of dividing cells in the Ficoll gradient therefore appears to depend upon cellular uptake of tantalum. Young post-fission cells also appeared to be selected by the tantalum procedure, although it was difficult to assess their frequency with precision.

Table 1. *Fraction of dividing cells in populations with different treatments*

Cells	Total cells observed	Number of dividers	% dividers
Untreated	2478	34	1.4
Tantalum fed, centrifuged	379	97	25.6
Tantalum unfed, centrifuged	681	9	1.3

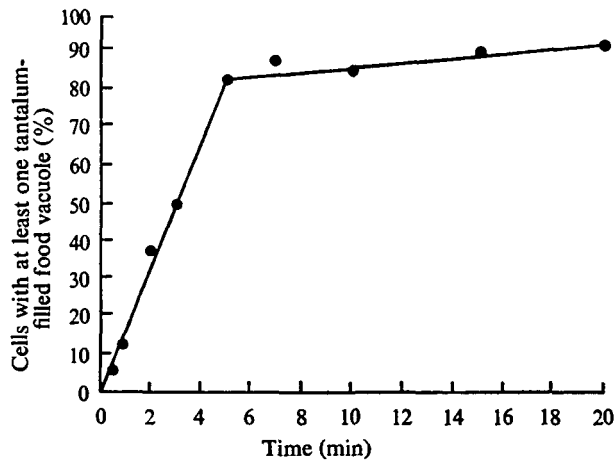


Fig. 1. Uptake of tantalum into cells. Cell concentration, 1360/ml; tantalum concentration, 2 mg/ml; temperature, 22 °C.

Mass culture growth characteristics of tantalum fed populations and unfed controls are shown in Fig. 2. Each curve represents the average of three parallel experiments. When resuspended in excess fresh BL, cell populations selected by tantalum feeding and centrifugation display a rapid doubling beginning between 6.5 and 7 h after resuspension (Fig. 2*a*). Conversely, tantalum unfed controls displayed no such concentrated population increase (Fig. 2*b*).

Singly isolated cells in drop cultures were observed at timed intervals in order to obtain more precise information about the kinetics of population growth. Since the same cells, or their descendents, were re-examined at timed intervals, the sample variations inherent in mass culture measurements were eliminated. The times of cell divisions of single isolates in drop cultures are shown in Fig. 3. Control A contained cells which were centrifuged in Ficoll, but not fed tantalum. Control B contained cells taken from culture tubes and resuspended in fresh BL with no other treatment. All data in Fig. 3 are the total of three repeats for each class.

Tantalum fed and centrifuged cells display an increase in numbers 0.5 h after

resuspension. This is to be expected since many cells in the sample are dividing when isolated (Table 1). Only a few divisions occur between 0.5 and 5 h after resuspension. Between 5.5 and 7.5 h, however, the population rapidly doubles, with the greatest

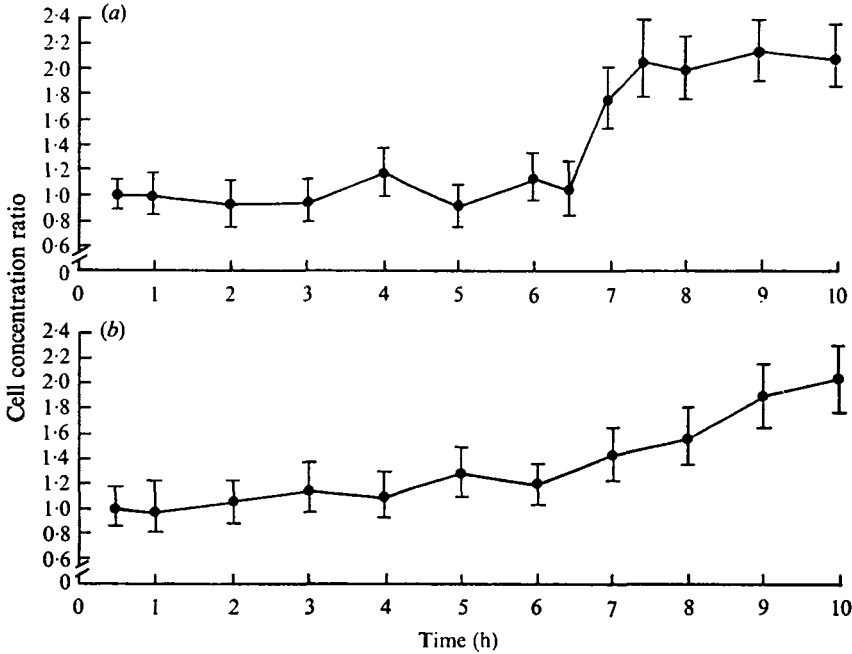


Fig. 2. Growth characteristics of mass cultures. Ordinate, concentration at time t over concentration 0.5 h after resuspension in fresh BL. (a) Tantalum fed, centrifuged; (b) control, tantalum unfed, centrifuged. Bars represent 95% confidence limits, calculated assuming Poisson distribution of population counts.

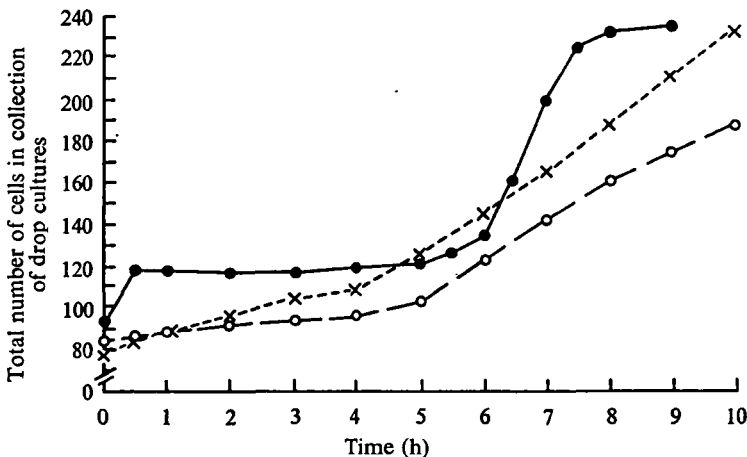


Fig. 3. Growth characteristics of collections of individual isolated cells. ●—●, tantalum fed and centrifuged; ○—○, control A, tantalum unfed, centrifuged; ×---×, control B, untreated.

number of divisions (35 %) occurring from 6.5 to 7 h after resuspension. After 8 h, the population has approximately doubled and growth temporarily ceases. Of the total amount of divisions after 0.5 h, approximately 80 % occurred in a 2 h period, from 5.5 to 7.5 h.

Controls A and B, without tantalum feeding and without treatment, respectively, displayed little division activity until approximately 4 h after resuspension. Although a 'burst' of divisions occurs, it is not as intense as that of the tantalum fed population. Controls A and B have completely doubled in 9 and 7 h, respectively. After doubling, the control populations continue to increase. In both controls, 80 % of the divisions are distributed over a 5 h period: from 3 to 8 h after resuspension for control A and from 2 to 7 h after resuspension for control B.

The data in Fig. 3 suggest that the growth patterns of the control populations after resuspension in fresh BL are not random, but neither do they appear to be synchronous. However, the tantalum fed and Ficoll centrifuged population is unlike the controls and appears to be synchronous.

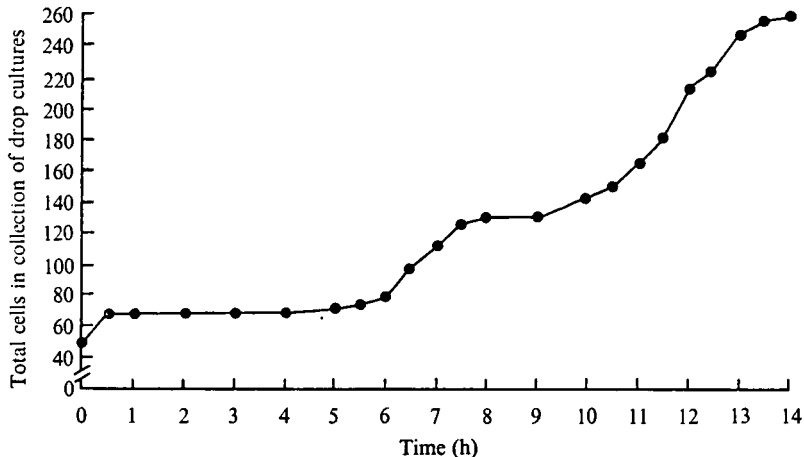


Fig. 4. Two generations growth of cells from tantalum fed and centrifuged population.

A single experiment, recording two generations of individual cells isolated from a tantalum fed and centrifuged population is presented in Fig. 4. As might be expected, the second generation is less synchronous than the first.

4. DISCUSSION

Paramecia are known to eat such diverse materials as india ink, carmine and latex spheres (Preer, 1975). Therefore, *paramecia* could be fed a density label, such as tantalum, provided that the particles were of appropriate size. Also, *P. tetraurelia* ceases feeding approximately 10–15 min before fission, and does not resume feeding until approximately 10 minutes after fission (Preer, 1975; Dryl & Preer, 1967). This phenomenon has recently been correlated with the morphogenetic events in the oral apparatus (Jones, 1976). During the total 20–25 min non-feeding period, the

proter is incapable of feeding because of disorganization of the cellular oral structures and the opisthe has not completed formation of the food vacuole-forming region of its new oral apparatus.

The synchronization procedure reported here density-labels all but non-feeding cells by presenting granular tantalum to the population. The unlabelled sub-population is then separated from the density-labelled population by density gradient centrifugation. This selected sub-population contains a significant fraction of dividing and recent post-fission cells, and will produce a division-synchronous culture when resuspended in fresh culture fluid. Since the procedure is selective, rather than inductive, it seems likely that the population is also cycle-synchronous, assuming a fair degree of coupling between division and other cell cycle events (James, 1966).

Several factors are critical in this technique. The cultures used must be free from scum and detritus, and should be in log or early stationary phase when collected. A 10 min feeding period was considered maximal. Beyond this time, many dividers would reach the end of their non-feeding period and would then become density labelled. The concentration of Ficoll in the 20% portion of the step gradient is quite critical. Concentrations of less than 18% or more than 22% (w/w) were found to give unsatisfactory results.

The technique reported here appears to be a rapid and repeatable means of producing large division-synchronous populations of paramecia, and has applications for the study of cell cycle events in mass cultures of paramecia, including the study of cycle related morphogenesis. An additional potential use would be for screening and enriching for temperature sensitive non-feeding mutations, as has been reported for *Tetrahymena* (Orias & Pollock, 1975). Other potential applications include enrichment for autogamous forms, conjugating pairs or amacronucleate cells, all of which are non-feeding cell types (Preer, 1975; Dryl & Preer, 1967; Sonneborn, 1974). In general, it would appear that the density labelling technique reported here can be applied in any instance where the cells to be selected are in a non-feeding state. Any or all of these applications could provide significant assistance in the advance of *Paramecium* cytology and genetics.

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