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Division Competence in *Tetrahymena:* Determination of Minimum Cell Volume and Rate of Nutrient Uptake

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Cell volume and doubling time have been determined for exponentially growing *Tetrahymena pyriformis* cells in broth medium with and without glucose and in media made from these media by dilution with water. The cells tolerate media with dry weights from 105 down to 0.06 g/L. In the diluted media the cells have small volumes and the doubling time is increased.

When the cell volume increase per time per cell in a given medium is expressed as a function of the cell volume in this same medium, a direct proportionality is found. From this equation the minimum cell volume of division competence (MVDC) can be found. It is 2,100 μm^3 for *T. pyriformis* at 28°C.

The lag period resulting from an upshift of exponentially growing cells from diluted media to more concentrated media is a function of the initial and resulting cell volumes and MVDC.

The increase in cell volume per unit of time for a given cell depends on the dry weight of the medium. This parameter can be transformed to mass increase per cell surface area per time, which represents rate of nutrient uptake. When plotted against the dry weight of the media, a Michaelis-Menten-like curve is obtained with two K_m values of 3.8 and 0.08 g/L with corresponding V_{max} values of 20 and 4 ng/cm^2 · s.

The low K_m value (0.08 g/L) indicates that *Tetrahymena* is able to take up nutrients from highly diluted media. The high value of V_{max} (20 ng/cm^2 · s) increases the ability of growth in more concentrated media. Thus, the adaptability of *Tetrahymena* to regulate its growth rate on media with considerable differences in nutrient supply is partly explained.

Key words: cell size, cell division, growth media, Ciliates, enkaryotic cells

Recently [1] we showed that *T. pyriformis,* GL cells grown under different conditions have different cell-size and doubling times. For cells grown in broth medium or in aqueous dilutions of broth medium we found that the cell size decreases and the doubling time increases when the growth medium is diluted. Whereas starving cells get smaller and smaller towards a non-viable state, this is not the case with cells in strongly diluted media. Here we show that exponentially growing cells in broth media (with or without glucose) diluted several hundredfold all have about the same cell size and that

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TABLE I. Cell Volume, Doubling Time, and Nutrient Transport of *T. pyriformis* Cells Grown Exponentially in Different Media at 28°C*

Medium	Dry weight of medium (g/L)	Cell volume (μm^3)	Doubling time (min)	dvol/dt (μm^3/min)	Nutrient transport (ng/cm^2 · s)
1/100 × PY without glucose	0.15	2,810	830	3.4	3.1
1/25 × PY without glucose	0.6	2,830	400	7.1	6.4
1/10 × PY without glucose	1.5	3,160	300	10.5	8.7
1/3 × PY without glucose	5	4,080	190	21.5	16.0
PY without glucose	15	6,220	160	39.6	21.0
1/500 × PY	0.06	2,350	1,200	2.0	2.0
1/100 × PY	0.3	2,570	480	5.4	5.1
1/25 × PY	1.2	3,320	300	11.1	8.9
1/10 × PY	3	4,190	240	17.5	12.0
1/3 × PY	10	5,580	180	31.0	17.7
PY	30	6,800	160	42.0	21.0
2 × PY	60	11,500	180	63.9	22.5
2½ × PY	75	13,400	230		
3 × PY	90	15,600	260		
3½ × PY	105	17,200	400		

*Figures for dvol/dt are derived from dividing cell volume with doubling time. Nutrient transport is calculated by using 1 μm^3 = 0.52 pg [1] and the relevant cell surface areas.

there is a minimum cell volume of division competence (called MVDC). The mathematical equation used for calculation of MVDC can also be applied to the lag phase problems: What determines the length of the lag phase preceding cell division at a shift-up? Finally, the data suggest that *Tetrahymena* uses two different mechanisms for uptake of molecules in particle-free media. One mechanism works mainly in highly diluted media; the other one dominates in normal growth media.

MATERIALS AND METHODS

Cell Culture

Tetrahymena pyriformis, strain GL (an amicronucleate and thus asexual cell) was grown in a number of different media (Table I), the most concentrated being 3½ × PY, the most diluted being 1/500 × PY. PY was 0.75% (w/v) proteose peptone + 0.75% (w/v) yeast extract + 1.5% (w/v) glucose + 1 mM $MgSO_4$ + 50 μM $CaCl_2$ + 100 μM ferric citrate [2]. PY medium without glucose was also used. All media were autoclaved at 2 atm, 30 min. In some experiments the media were sterile filtrated to test if such particle-free media behaved differently. This was not the case. The cultures were shaken gyratorically (60 rev/min), but cells grown in diluted media were left without shaking in flasks so that the height of the medium was less than 2 mm, thus ensuring sufficient aeration [2].

Cell Volume and Doubling Time

At the various growth conditions the cell volume and the doubling time were carefully determined by use of a Coulter Multisizer as described [1]. The cell volume estimation for a given medium showed a standard deviation of 7%. The doubling times were estimated over a period corresponding to about 2–3 doublings, ensuring no lag-period at the beginning of the experiment. Furthermore, the final cell concentrations

did not exceed one-third of the maximum obtainable cell concentration in a given medium and were always below $2.5 \cdot 10^5$ cells $\cdot$ ml^{-1} (to avoid oxygen limitation) [3]. The standard deviation on doubling time estimates was 13%.

Determination of the Minimum Volume of Division Competence

On the basis of the cell volume and the doubling time, a volume increase velocity, dvol/dt, can be calculated as dvol/dt = volume/time (μm^3/min) per cell. The standard deviation was 11%. The minimum volume of division competence (MVDC) is then given by the expression

$$\frac{V_{obs} - MVDC}{dvol/dt} = k$$

where V_{obs} is the observed cell volume and k is a constant, which depends on the growth temperature and the composition of the medium. Dimensions for k: min^{-1}. If dvol/dt is plotted against the observed volume, a straight line is obtained; the intersection on the x-axis gives the value of MVDC (Fig. 4). The slope of the curve (Fig. 4) is k^{-1}.

Determination of the Lag Period

The lag period (t_{lag}) which a starved cell or a cell in exponential growth needs in order to adjust its volume during a nutritional up-shift can be calculated on the basis of the expression above:

$dt = (dvol/(V_{obs} - MVDC)) \cdot k$, which by integration leads to $t + c = \ln (V_{obs} - MVDC) \cdot k$

For $t = 0$

$c = \ln (V_o - MVDC) \cdot k$

If inserted this gives

$t + \ln (V_o - MVDC) \cdot k = \ln (V_t - MVDC) \cdot k$,

where V_t is the volume at time t. Thus,

$$t_{lag} = k \cdot \ln \frac{V_t - MVDC}{V_o - MVDC}$$

where V_o and V_t are the initial and resulting cell volumes.

Determination of Nutrient Uptake

The values for dvol/dt for a given medium were transformed into mass increase per cell surface area per time. This is a parameter for nutrient uptake. The cell volumes (Table I) were multiplied by 0.52, which is the dry weight (pg) per cell volume (μm^3) for cells on medium with a dry weight of 3–60 g/L [1]. This value is also applied here to cells in very diluted media.

Estimate of K_m and V_{max}

The values plotted in Figure 5 were used to create a non-linear fit based on the least sum of squares. The dotted line indicates the curve obtained under the assumption that one set of K_m and V_{max} describes the curve. The solid line is a fit based on two sets of values for K_m and V_{max}.

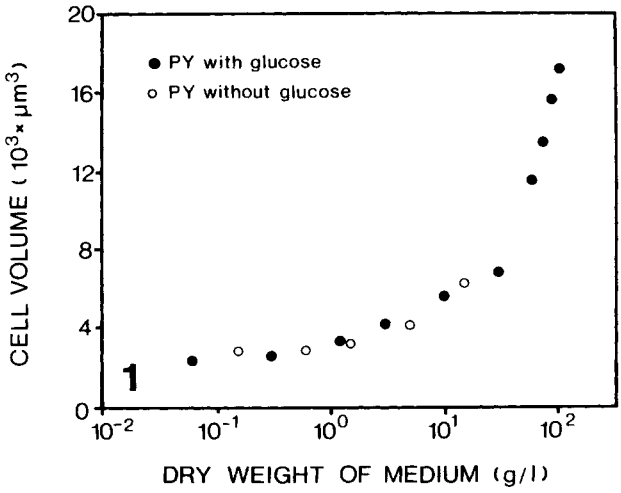

Fig. 1. Cell volume of exponentially growing *Tetrahymena* cells cultured in different media as a function of the dry weight of the media used. The media were either related to PY (0.75% PP + 0.75% YE + 1.5% glucose) (●) or to PY without glucose (O). All cells were cultured at 28°C.

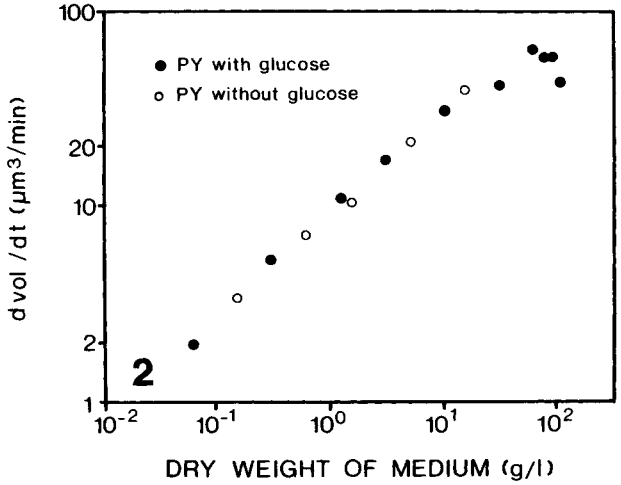

Fig. 2. Cell volume increase per time (dvol/dt) as a function of the dry weight of the media used. Symbols as in Figure 1. Data sets from the 15 different media (Table I) are presented.

RESULTS

The growth media used and their dry weights are shown in Table I. The dry weight of PY, for example, is composed of 7.5 g/L each of proteose peptone and yeast extract and 15 g/L of glucose. The table shows that the cell volume decreases by dilution of the media regardless of its content of glucose (Fig. 1). The doubling time increases by dilution of the media. The doubling times range from about 20 h for 1/500 × PY to 160 min for PY medium. Especially at the extreme conditions, the cells need to adapt to

growth for several weeks before a reproducible doubling time is reached. The lag period induced by an upshift is much smaller and can be estimated by the equation given in Materials and Methods. The medium 3½ × PY is the most concentrated medium which supports maintained exponential growth at 28°C. The observed cell volumes, the corresponding cell surface areas, and the calculated doubling times form the basis for the figures.

Figure 1 shows the cell volume of *Tetrahymena* cells grown in different growth media with or without glucose. The cell volume is plotted against the dry weight (log scale) of the 15 different media (Table I) ranging from 1/500 × PY to 3½ × PY. The figure shows that the cell volume increases considerably in concentrated media. Furthermore, the cell volume in strongly diluted media approaches a certain minimum value about 2000 μm^3.

The average rate of increase in cellular volume in a given growth medium called dvol/dt (Table I) is now plotted (log scale) against the dry weight of the medium (log scale) (Fig. 2). As can be seen, the values from media with and without glucose apparently fit the same graph. The lower values seem to follow a straight-line dependency, whereas the concentrated media 2, 2½, 3, and 3½ × PY do not result in an increase in dvol/dt. The concentrated media might cause cellular stress due to osmosis. If the data in Figure 2 are plotted with dvol/dt in a linear scale (not shown), it is possible to make a regression analysis on the data with the lower five coordinates and estimate the value of X for dvol/dt = 0. This value (0.013 g/L) represents the medium dry weight concentration which results in no size increase. In other words, PY medium diluted 2,300 times should be sufficient to support the basal metabolism but not growth.

The relation between doubling time and the cell volume is shown in Figure 3. The medium PY has a doubling time of about 2.7 h, whereas the cells in media made by dilution of PY or PY without glucose have increased doubling times. Figure 3 also indicates—as did Figure 1—that there exists a minimum size for dividing cells. This becomes more evident when dvol/dt (see Table I) is plotted against the cell volume (Fig. 4). A straight line is obtained and the value of X for Y = 0 is 2,113 μm^3. This figure is the minimum mean cell volume of division-competent *T. pyriformis,* GL cells grown in broth medium with or without glucose at 28°C. The graph shown (Fig. 4) is a regression line through data obtained with cells grown in media with *and* without glucose. If the data are kept apart, PY with glucose gives MVDC = 2,080 μm^3, whereas PY without glucose gives MVDC = 2,130 μm^3. The closed circle in the upper-right corner represents PY medium. The value for 1/10 × PY is the filled circle at x = 4.2, whereas the value for 1/10 × PY without glucose is the open circle at x = 3.2. In order to test the generality of the conclusions drawn, we made a series of media as those in Table I, but including 0.1% (v/v) ethanol. For PY with ethanol a dvol/dt value of 29 μm^3/min and a volume of 6,300 μm^3 was obtained. A plot (not shown) of these "ethanol" values is consistent with a straight line (as in Fig. 4) but with a smaller slope. Interestingly, it gives a value for MVDC of 1,820 μm^3, probably not significantly different from the MVDC values mentioned above. A plot (not shown) with an even smaller slope than that of the ethanol values was obtained when data from 15°C were plotted. For PY, PY without glucose, and 1/10 × PY, the following values of dvol/dt were obtained: 19.8, 16.7, and 7.9, respectively. The cell volumes were 8,600, 7,300, and 5,200 μm^3, respectively. A regression line based on these data gives an MVDC at 15°C of 2,800 μm^3 and at 8°C of 5,000 μm^3.

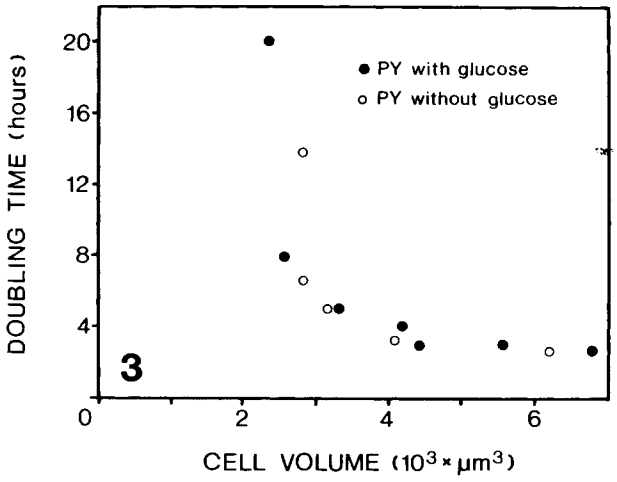

Fig. 3. Doubling time of cells as a function of the cell volume. Symbols as in Figure 1. Data are from the 15 media except for the four most concentrated media.

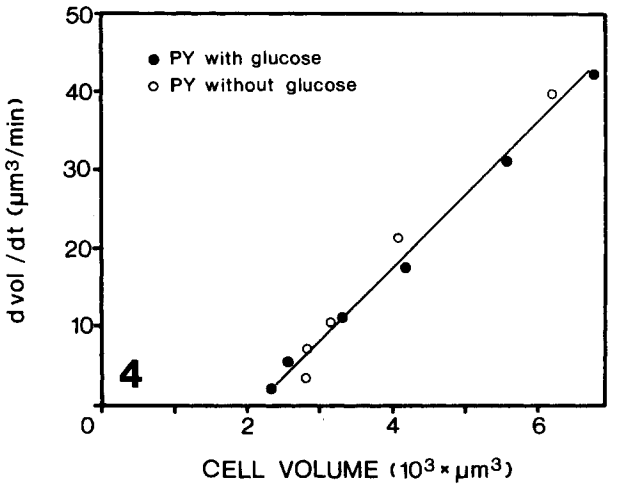

Fig. 4. dvol/dt as a function of the cell volume. Data sets as in Figure 3. The line is based on regression analysis (see text).

If dvol/dt is multiplied with 0.52, which is the figure for dry weight (pg) per volume (μm^3) (Table I), and then divided by cell surface area in the given medium, all dvol/dt values can be transformed into figures for nutrient transport, excluding transport for the basal metabolism. In Figure 5 these figures are plotted against the dry weight of the media. Clearly, a Michaelis-Menten curve is obtained. The dotted line corresponds to an assumed V_{max} of about 22.5 ng/cm^2 · s and K_m = 2.0 g/L. When Lineweaver-Burk plots were made, it became clear that the data were inconsistent with a single value for V_{max} and K_m (not shown). The fit gave a least sum of squares of 16.3. This is also evident from the insert in Figure 5, which shows that the data obtained with strongly diluted

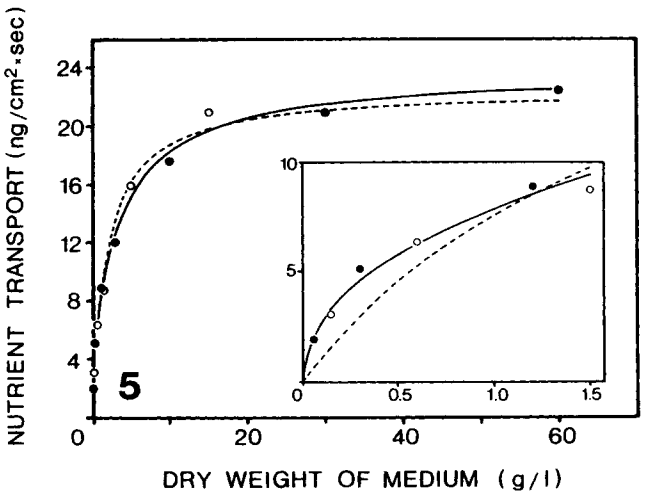

Fig. 5. Nutrient uptake as a function of the dry weight of the media used. Symbols as in Figure 1. For details see Table I. The curve drawn with dotted line has a $V_{max} = 22.5 \pm 0.9$ ng/cm^2 · s and a $K_m = 2.0 \pm 0.3$ g/L. The curve drawn in full has two sets of parameters: $V_{max} = 19.7 \pm 1.4$ and $K_m = 3.8 \pm 0.9$ and for the low values $V_{max} = 4.1 \pm 1.7$ and $K_m = 0.08 \pm 0.09$. **Inset:** The fit of the curves at low values of medium concentration.

media are not in agreement with the dotted line. However, if we assume that the uptake is characterized by two sets of values for V_{max} and K_m, namely, $V_{max} = 4.1$ and $K_m = 0.08$ and $V_{max} = 19.7$ and $K_m = 3.8$, a very good match of the data and the solid curve is obtained with a least sum of squares of 4.2 (Fig. 5).

DISCUSSION

The doubling times depend on the growth medium [4], the temperature [4–6], and the degree of aeration [2–4]. There is also a considerable period of adaptation before optimal growth rates are obtained. For a downshift, for example, from 1/10 × PY to 1/500 × PY several weeks are necessary. Few reports have studied the cell volumes in highly diluted media. With *T. pyriformis,* GL the minimum doubling time was 160 minutes (PY medium with or without glucose) (Table I). The dry weight of the media determines the doubling time (Table I). In Figure 4 it can be seen that data from media with and without glucose follow the same graph. By varying the composition of the broth media we could demonstrate that the total dry weight of the medium is by far the most important parameter determining the doubling time. We specifically checked whether glucose in the highly concentrated media could be substituted with PY or YE. A medium with 4% PP, 4% YE, and 3% glucose (dry weight 110 g/L) gives a doubling time of 400 min (like 3½ × PY) and a cell volume of 17,000 μm^3 (like 3½ × PY).

The determinations of cell volume were performed on a Multisizer (Coulter) by a technique described recently [1]. It is important to note that the volumes registered represent cell mass. In other words, this study deals with regulation of the cellular synthesis and deposition of macromolecules, etc., and not with osmosis.

When cells are transferred to inorganic medium they get smaller rapidly—after 24 h, about 2,600 μm^3; after 48 h, 1,500 μm^3. This is in agreement with the value of MVDC

of 2,100 μm^3, which means that the cells at the time of transfer from PY (with a volume of about 7,000 μm^3) divide once or twice until the mean volume of the cell population is less than 2,100 μm^3. After 5–6 days small living cells are still seen, but heterogeneity in size occurs [1]. The cultures in diluted media seem to keep a minimum size of about 2,000 μm^3 (Figs. 1, 4). It could be interesting to study the ultrastructure of these cells and of the strongly starved cells. Preliminary data on exponentially growing cells indicate that the protein, RNA, and glycogen content is kept constant regardless of the medium, in percent of dry weight, 43%, 10%, and 10%, respectively (unpublished data, P.H.-L. and A.P.A.).

The calculation of dvol/dt values for different media (Table I, Figs. 2, 4) clearly illustrates that the cells take up more and more nutrients from the media and reach a saturation level in PY medium. Furthermore, it is possible to obtain an estimate of the strength of the medium, which gives dvol/dt = 0. This figure is 0.013 g/L. It remains to be seen whether it is possible to culture GL cells in PY medium diluted 2,300 times (dry weight: 0.013 g/L), for example, in excess of medium inside a Millicell [2].

As explained in Materials and Methods, the straight-line dependency in Figure 5 is based on the equation $dvol/dt = k \times V_{obs} - k \times MVDC$. Thus for $dvol/dt = 0$, $V_{obs} = MVDC$. A value of about 2,100 μm^3 is found for GL cells in PY medium and dilutions thereof at 28°C. As mentioned in Results, the value seems to hold for other media but is increased at lower temperatures. At 4°C the cells do not multiply, but increase in volume and dry weight.

From the equation given above the value of k (the slope of the curve) can be calculated. With this value the lag period at an upshift can be estimated:

$$t_{lag} = k \cdot \ln \frac{V_t - MVDC}{V_o - MVDC}$$

An example: Cells are transferred from 1/25 × PY ($V = V_o = 3{,}320\ \mu m^3$) to PY ($V = V_t = 6{,}670\ \mu m^3$). MVDC = 2,100 μm^3. k is 113 min^{-1}. The value for t_{lag} can then be calculated as 148 min. This theoretical value has been confirmed by experiments and seems to hold for shift-up from Tris-starved cells as well [1].

The conversion of dvol/dt values to mass uptake per area per time (Fig. 5) allows a plot of these values against the dry weight of the medium. This plot is based on particle-free medium, where food vacuole formation is at minimum. On the basis of the data from diluted media (insert) we ignore the plot (dotted line) based on one set of $K_m - V_{max}$ values, and we think that the cell has two different type of uptake mechanisms each characterized by a specific set of $K_m - V_{max}$ values. This has been further checked by making Lineweaver-Burk plots and by testing with least sum of square statistics.

In the standard medium, PY, the rate of nutrient transport is close to maximum, namely, 22.5 $ng/cm^2 \cdot s$ (not corrected for basal metabolism). If corrected for the transport rate which is found in a diluted PY medium with a dry weight of 0.013 g/L, a medium where the volume increase is zero, the K_m values given earlier are changed to 0.06 and 3.7 g/L and the V_{max} values to 4.8 and 19.8 $ng/cm^2 \cdot s$. This implies that 10^6 cells use about 5% of the dry weight of the PY medium in 1 h. This figure relates to exponentially growing cells with close to maximal uptake. Preliminary experiments have been performed on conditioning of media where we have registered specific changes in

the media where the uptake of the cells has been kept low so that the composition of the medium has been essentially unchanged.

The present study tells nothing about *how* the nutrients enter the cells. This question has been addressed in other studies [7,8]. It has been shown that small peptides can pass the cell membrane [8] and that the cells produce proteolytic enzymes [9,10], which probably cleave the long peptides in the medium. The fact that our data with defined medium are in agreement with those using broth media indicates that we are mainly dealing with uptake across the cell membrane, which follows Michaelis-Menten kinetics.

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