On a collision frequency restricting the growth and population density of *Tetrahymena pyriformis*

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Summary. Tetrahymena multiplies very rapidly up to a certain limit of population density, but growth then ceases and the number of cells becomes stationary. We report here the results of experiments using hollow spherical glass beads with a size and weight similar to those of the cells. Growth inhibition may be a result of cell-to-cell and cell-to-bead collision but not due to production of waste materials nor to exhaustion of nutrients in the medium.

How a cell population grows and what factors control its growth are fundamental biological problems. We and other workers have investigated growth-promoting and -inhibiting factors for the protozoan *Tetrahymena*[1–3]. When *Tetrahymena* cells are cultured in a fresh medium, the cells produce promoting and inhibiting factors during the logarithmic and the stationary phase, respectively, and the cell population reaches a saturation density. This saturation density is almost the same as that obtained by cell growth after inoculation in a conditioned medium already containing the promoting and inhibiting factors. Therefore, in addition to the promoting and inhibiting chemical factors already found by us and other workers, other unknown factors must be involved. *Tetrahymena pyriformis* strain W[4] was used in an attempt to identify essential factors which control and 'recognize' the cell population density. The growth curve of *T. pyriformis* in a culture medium can be generally divided into 3 parts or phases; A) the period called the lag phase, during which the cells themselves become larger but their number remains constant; B) the period called the logarithmic growth phase, during which the cells multiply rapidly; C) the period called the stationary phase, during which the number of cells becomes stabilized.

A 'crowding' effect involved in population growth of *Tetrahymena* was investigated by the use of hollow spherical glass beads of 0.043–0.060 mm diameter. When glass beads (purchased from Nippon Rikagaku Kikai Co.) were placed in distilled water, most of the glass beads sank. However, some were hollow and remained floating in the distilled water. These floating glass beads were gathered with a pipette and were used as dummy *Tetrahymena* cells in this experiment. Thus, the growth response of *T. pyriformis* in the presence of hollow spherical glass beads was compared with that in their absence. *T. pyriformis* cells were grown axenically in capped, upright 15 × 125 mm Pyrex culture tubes containing 0.5 ml of a peptone basal medium, supplemented with 0.5 mM Mg^{2+} and 0.05 mM Ca^{2+} and adjusted to pH 6.8 with Tris-maleate buffer. The culture tubes were placed in an incubator with shaking (Taiyo M-100 incubator) at 27 °C. The frequency and the amplitude of shaking were 1 Hz and 4 cm, respectively.

We measured growth curves of *T. pyriformis* after inoculation in media containing various numbers of floating glass beads. Stock cells of *T. pyriformis* were inoculated into fresh medium containing the hollow glass beads, and then grown by the shaking culture method. As shown in figure 1, the growth curve of *T. pyriformis* in the fresh medium gave a saturation density of 500,000 cells/ml and a lag time of 15 h. When the fresh medium contained 320,000 hollow beads/ml or 400,000 hollow beads/ml, the saturation densities of *T. pyriformis* decreased by about 300,000 cells/ml and 400,000 cells/ml, respectively. The lag phase in the presence of the hollow glass beads was longer than that in their absence. When more than 600,000 hollow beads/ml were suspended in the fresh medium, no growth of *T. pyriformis* was observed. It is evident, however, that a collision with the glass beads did not cause death of the inoculated *T. pyriformis* (50–100 cells/ml), because the inoculated cells appeared to be intact when observed through a microscope. Moreover, when a significant amount of the floating glass beads was removed from such a culture medium, the inoculated *Tetrahymena* cells grew very well.

A cell in a culture medium, subjected to the shaking culture method used here, must collide with hollow glass beads and with other cells present in the medium. It is, therefore,

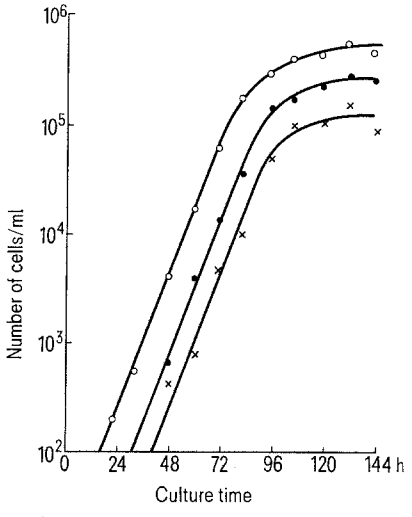

Figure 1. Growth curves of *T. pyriformis* after inoculation into fresh media containing various numbers of glass beads. Growth curves of *T. pyriformis* after inoculation: ○, fresh medium without beads; ●, fresh medium containing 320,000 hollow beads/ml; ×, fresh medium containing 400,000 hollow beads/ml.

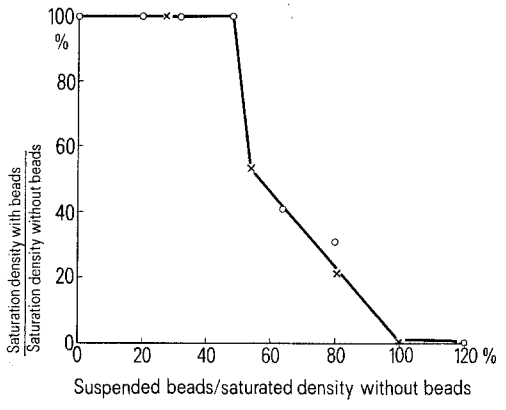

Figure 2. Saturation densities of *T. pyriformis* after inoculation into fresh media containing various numbers of floating beads (○) and into the media conditioned for 240 h and containing various numbers of floating beads (×). The saturation density of *T. pyriformis* in the absence of beads in fresh medium is 500,000 cells/ml and that in the absence of beads in the conditioned medium is 350,000 cells/ml.

conceivable that the saturation density of *T. pyriformis* is determined by this collision effect.

A medium from which previously grown cells had been removed by filtration was designated as conditioned medium and was used for the following experiments. The growth curve of *T. pyriformis* after inoculation into a medium conditioned for 240 h, gave a lag time of 22 h and a saturation density of 350,000 cells/ml. When 160,000 hollow glass beads/ml and 240,000 hollow glass beads/ml were suspended in the medium conditioned for 240 h, the saturation densities decreased by about 150,000 cells/ml and 250,000 cells/ml, respectively.

When *Tetrahymena* cells were inoculated into media containing a significant amount of the floating glass beads, as previously shown in figure 1, the lag time before cell division was initiated was significantly lengthened. Namely, the higher the amount of glass beads in the medium was, the longer was the lag time of cell division. Furthermore, the higher the amount of glass beads in the medium was, the lower was the saturation density of *Tetrahymena,* until the point at which growth no longer occurred. However, the growth rate of *Tetrahymena* during the logarithmic growth phase was not dependent upon the amount of hollow glass beads present. The relationship between the saturation density and the number of floating glass beads is shown in figure 2. This plot suggests that saturation density is determined by the frequency of collisions between cells and glass beads or between the cells themselves. When the ratio of the number of hollow beads, in the presence of the hollow beads, to the saturation density in the absence of beads in fresh medium, or in conditioned medium, was less than 50%, no decrease in the saturation density was observed. The growth-promoting and growth-inhibiting substances are already present in a conditioned medium. Thus, it is possible that the influence of the growth-inhibiting factors in the conditioned medium is more effective than that of growth-promoting factors and that this results in a slight reduction of the saturation density (70%) in the conditioned medium (see the legend of fig. 2).

The saturation density of protozoa such as *Tetrahymena* is generally less than the saturation density of bacteria. It is known that the saturation density of bacteria is determined by the concentrations of oxygen, nutrients and end-products in the medium. In contrast, we have shown here that, in the case of *T. pyriformis,* the maximal cell density which can be reached in a *Tetrahymena* culture seems to be regulated, at least in part, by the frequency of collisions and not by nutrient effects.

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Visualization of centromeric spots in the chromosomes by UV-light exposure

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Summary. When exposed to UV-light in the presence of Hoechst 33258, chromosomes of *Allium cepa* were progressively photolyzed with increasing length of exposure; they retained their delineated contours, centromeric spots and sometimes secondary constriction bands.

Visible- or UV-light exposure following BUdR incorporation and Hoechst 33258 treatment has been successfully used for differential staining of sister chromatids[1–4]. Goto et al.[3] and Sugiyama et al.[5] proposed that differential staining produced by this technique depended on dye concentration and light-exposure time, and suggested that preferential photolysis of BUdR-substituted DNA was responsible for differential staining. It is reported that no degradation has been demonstrated with BUdR-unsubstituted DNA bound to Hoechst 33258 and exposed to UV-light[6,7]. These data suggest that an interaction of BUdR and Hoechst 33258 is related to photolysis of the chromosomes. However, the present authors have investigated the effects of UV-light on BUdR-unsubstituted chromosomes to study the organization and chemical properties of chromosomes, and found that UV-light could drastically photolyze the chromosomes under appropriate conditions without BUdR labeling. The present paper introduces a new procedure for photolysis of the chromosomes and describes chromosome architectures produced by this technique.

Material and methods. Actively growing roots of *Allium cepa* L. were excised and pretreated in aqueous 0.025% colchicine at 18 °C for 3.5 h and fixed overnight in 1:3 glacial acetic acid – 99.5% ethanol at 5 °C. The chromosomes were prepared by the enzyme maceration and flame-drying technique[8]. The chromosomes on the glass slides were soaked in McIlvain buffer (pH 8.0) for 5 min. Then, the slides were drained, blotted with paper towels and immediately stained for 5 min with freshly-prepared 50 µg/ml Hoechst 33258 (Wako Pure Chem. Ind. Ltd, Japan) dissolved in the same buffer. After a brief rinse in the buffer, the chromosomes were mounted in the buffer and sealed with paraffin. UV-light was provided by a fluorescence microscope, equipped with an 40 times objective lens (Nikon, UV-F) and a Nikon epiilluminator (Osram HBO 50 W mercury vapor lamp, Germany). The filter combination used for Hoechst 33258 fluorescence yields UV-light with a predominant wave length of 365 nm. The exposure was carried out in the dark room, focusing on the metaphase chromosomes for various times at 25–28 °C. After UV-light exposure, the coverslips were gently removed from the slides with a razor blade. Immediately, the slides were restained for about 20 min in 15–20% Giemsa solution, made up in 1/15 M phosphate buffer (pH 7.0).

Results and discussion. The effects of UV-light on the

Effect of pH of the buffer on chromosome extraction

pH	Exposure time (min) 10	30	60	90
8.0	d	b	b	a
7.0	d	c	b	a
4.0	d	d	d	d

a, remarkable; b, medium; c, slight; d, no extraction.