Growth-controlling factors produced during the logarithmic and stationary phases of *Tetrahymena pyriformis*

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Summary. Tetrahymena pyriformis grown to logarithmic phase and then inoculated into either fresh or conditioned medium exhibited a shorter lag phase than ciliates taken from stationary phase cultures. The growth inhibiting substance(s), which is probably produced by the growing ciliates, is thermostable and can be adsorbed to activated charcoal.

Tetrahymena pyriformis strain W was grown axenically at 27 °C in capped, upright 15 × 125 mm Pyrex culture tubes containing 5 ml of a peptone basal medium supplemented with 0.5 mM Mg^{2+} and 0.05 mM Ca^{2+}; these ions are required for effective growth, as described in our previous paper[1].

For some time after inoculation into fresh medium, the number of ciliates remains constant (no division occurs). The length of this induction period, termed the lag time, was measured in the above medium adjusted to 7 different pH values (from 4.0 to 10.0). The pH of the medium was adjusted with concentrated NaOH or HCl. Growth at pH 10.0 was extremely slow. No growth was detectable at pH 4.0. The ciliates in the culture medium adjusted to pH 6.0 showed a lag time of about 10 h, and a generation time of about 3 h (figure 1, B). The stationary growth phase was reached about 4 days after inoculation.

Tetrahymena pyriformis multiplies very rapidly and produces pronounced changes in culture media in a short period of time. Under favorable conditions, a single cell of *T. pyriformis* divides into 2 about every 3 h. However, this rate of multiplication is not maintained indefinitely. The reproduction rate begins to slow down, the generation time increases, and finally the number of *T. pyriformis* cells becomes stabilized during the stationary phase. As a first step to explore the reason for this, the present experiments were undertaken to determine the characteristics of growth-promoting or -inhibiting factor(s) produced by *Tetrahymena*.

Medium in which *T. pyriformis* has been grown affects the growth of subsequent ciliates newly inoculated into the same medium. Medium from which the previous ciliates had been removed by filtration (Toyo Roshi Co., Tokyo) was autoclaved for 15 min at 120 °C and 2 at. This medium was designated as biologically conditioned medium. Figure 1, A, shows the growth responses of ciliates inoculated simultaneously into individual batches of conditioned media. The optimum growth of *T. pyriformis* was in medium conditioned for 48–72 h, and the growth in medium conditioned for 96 h was inhibited; it seemed likely that the culture media from the logarithmic growth phase and from the stationary growth phase contain a growth-promoting substance and a growth-inhibiting substance, respectively. Kidder[2] reported in his aseptic experiment that both the growth-promoting and the growth-inhibiting substances in a medium which had not previously been autoclaved were completely destroyed by heating at 100 °C for 5 min. However, in our case the growth-promoting and growth-inhibiting substances were not destroyed by autoclaving for 15 min at 120 °C and 2 at. If ciliates from the logarithmic phase in the same medium were inoculated, rather than an older culture in the stationary phase or a stock culture, there was little or no lag before the onset of rapid division (figure 2).

The ciliates inoculated into medium conditioned for 48 h, containing the growth-promoting substance(s) which are not present in fresh medium, showed the best growth (curve II, figure 2), Irrespective of the conditioning-time of conditioned media, this means that the optimum growth of *T. pyriformis* was obtained by the use of ciliates grown in the logarithmic phase in the same medium as an inoculum. The ciliates in the logarithmic phase presumably have a full complement of metabolic machinery, enzyme systems, etc.

In order to investigate the characteristics of the inhibitory substances, medium conditioned for 240 h was used (figure 1, B). Curve II in figure 1 B, shows the growth response of ciliates inoculated into this conditioned medium. The ciliates inoculated into a medium conditioned for 240 h after treatment with active carbon exhibited a long generation time and a lower maximum yield, but no lag phase, as shown by curve III. These results suggest that the inhibitory substance(s) and also some

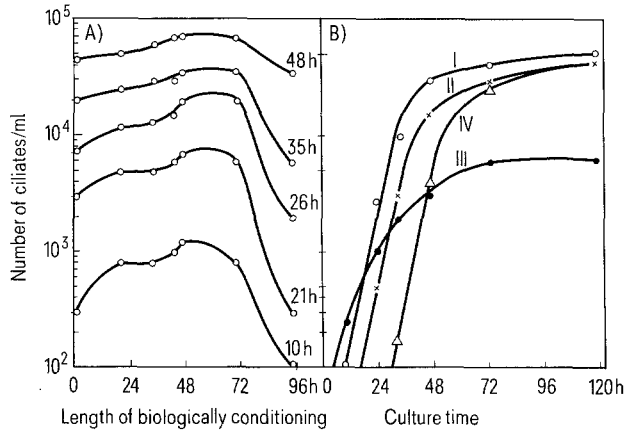

Fig. 1. Evidence showing growth-controlling factors of *T. pyriformis*. *A* Growth responses at various times after the inoculation of ciliates into various conditioned media. *B* Growth responses of ciliates inoculated into various media. I, fresh medium; II, 240-h conditioned medium; III, 240-h conditioned medium after treatment with active carbon; IV, 240-h conditioned medium after dialysis against distilled water at high internal pressure.

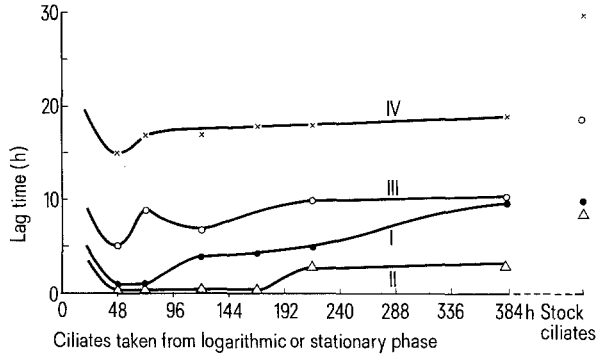

Fig. 2. Lag times in the growth of ciliates taken from cultures at various stages of growth and inoculated into variously conditioned media. I, fresh medium; II, 48-h conditioned medium; III, 96-h conditioned medium; IV, 384-h conditioned medium.

nutrients had been adsorbed by the active carbon from the medium conditioned for 240 h. Furthermore, medium conditioned for 240 h was dialyzed in membrane tubing No 132678 (Spectrum Medical Industries, Inc., Los Angeles) against distilled water at high internal pressure. After dialysis, the medium exhibited a considerably longer lag phase, as shown by curve IV. This suggests that the inhibitor was nondialyzable, having a mol. wt higher than about 14,000. It was also found to be thermostable, since after autoclaving the 240-h conditioned medium for 1 h at 120 °C and 2 at, the growth response of ciliates inoculated into the autoclaved medium was essentially unchanged from that with unautoclaved medium.

In our recent work, the growth response of *T. pyriformis* in the presence of glass beads similar in size and weight to ciliates was compared with that in the absence of glass beads. The growth response of the ciliates inoculated into fresh medium containing suspended glass beads was almost the same as that in the absence of the beads. On the other hand, when the ciliates were inoculated into 240-h conditioned medium containing the glass beads, the growth response was considerably inhibited. It is possible that inhibitory substance(s) were produced as a result of collisions between the ciliates and the glass beads. Furthermore, collision of a ciliate possessing an inhibitory substance with other ciliates or glass beads may suppress further multiplication of the ciliates. As mentioned previously, when stock ciliates are inoculated into fresh medium, they show a lag time. This may represent the period required for diluting or removing inhibitory substances bound to the inoculated ciliates. The results will be reported elsewhere.

1 T. Saito and H. Asai, J. Protozool. *26*, 286 (1979).
2 G. W. Kidder, Physiol. Zool. *14*, 209 (1941).

The effect of insulin on the electrophoretic mobility of rat hepatocytes

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Summary. The addition of insulin (10 μU) to a suspension of isolated hepatocytes in Krebs-Ringer bicarbonate solution, causes an increase in the negative electrophoretic mobility of the cells from - 1.68 μm sec^{-1} V^{-1} cm to 2.26 μm sec^{-1} V^{-1} cm. This observation supports the findings by other workers that the binding of insulin to its receptor leads to a marked change in the membrane.

The initial step in insulin action is the binding of the hormone to receptors in the cell membrane of its target cells[2]. This triggers, by an unknown mechanism, the metabolic changes in the cells that typify insulin action. Many of these changes are intracellular, but there are also several changes in the functions and properties of the cell membrane itself. Functional changes include the stimulation of the passage of substrates into cells such as the transport of glucose into adipocytes and muscle cells[3-5], and the transport of amino acids into a variety of cell types[6,7]. Insulin also modifies ion transport, promoting Ca^{2+} efflux from adipocytes and muscle cells[8]. It stimulates the transport of K^+ ions into adipocytes[9] and Na^+ efflux from frog skeletal muscle[10], probably as a result of stimulation of the membrane $Na^+ + K^+$-activated adenosine triphosphatase of the sodium pump[11]. These changes in ionic fluxes may also relate to the hyperpolarisation of adipocytes[12] and muscle cells[13] which occurs following treatment with the hormone.

Other observations imply that the physical properties of the membrane may be changed when insulin binds to its receptor. For example, the insulin receptor has been shown to be mobile in the plane of the membrane of cultured fibroblasts, and the receptors appear to aggregate and subsequently be internalized after binding the hormone[14,15]. Electron microscopic examination of freeze-fractured plasma membranes from adipocytes has shown an increase in the number of intramembranous particles following treatment with insulin[16]. Insulin also affects the cooperativity shown by the membrane-bound enzymes, acetylcholinesterase and the $Na^+ + K^+$-activated adenosine triphosphatase, in erythrocytes from rats fed a diet high in corn oil[17,18], indicating a possible decrease in membrane fluidity.

The wide range of these observations implies that a marked change may occur in the structure and physical properties of cell membranes following interaction of insulin with its receptor. Support for such a change has come from studies involving fluorescent probe molecules[19] but the validity of the results has been challenged by some authors[20,21] and such experiments have not always been successful[5]. However, a recent paper showed that insulin markedly decreased the translational diffusion coefficient of a probe molecule in plasma membranes isolated from rat liver[22] which further supports the idea that the binding of insulin to its receptor leads to a change in the membrane. In this present study we have used cell microelectrophoresis as a means to examine possible changes in the surface charge on hepatocytes following treatment of these cells with insulin in vitro. Microelectrophoresis is a technique that has been successfully used to examine perturbations in the ionizable groups present on the surface of a range of cells and organelles[23,24]. It can detect changes in the surface following minimal chemical and physical manipulation of the membrane and would thus seem to be a useful technique for examining the possible effects of insulin at the membrane level. The rationale for these experiments has been strengthened by the report that exceptionally low concentrations of conca-

Changes in the electrophoretic mobility of isolated hepatocytes following the addition of insulin and concanavalin A. The results show the average mobility value ± SEM from measurements made on 30 cells

Treatment	Electrophoretic mobility (μm sec^{-1} V^{-1} cm)	
	Control cells	Test cells
Insulin (100 μU/ml)	−1.76±0.09	−2.40±0.15
Insulin (10 μU/ml)	−1.68±0.09	−2.26±0.12
Concanavalin A (15 μg/ml)	−1.65±0.08	−2.37±0.11