HeLa Cells: Effects of Temperature on the Life Cycle

Abstract. *After a shift in temperature, the population kinetics of HeLa cells does not immediately reach a steady state characteristic of the new temperature. During the transition period the duration of each period of the life cycle (period before DNA synthesis, of DNA synthesis, after DNA synthesis, and the period of mitosis) increases toward its steady-state value. Of the four periods, the period before DNA synthesis reaches its steady-state value fastest. Exponential growth can be maintained only from 33°C through 40°C. The period of mitosis is the most temperature-sensitive period of the life cycle of the cell. At subnormal temperatures (26°C through 31°C) there is an accumulation of cells in mitosis, and mitotic indices as high as 0.44 can be obtained. The duration of mitosis is a function both of the temperature and of the time which the cell has spent at this temperature.*

We have systematically investigated the effects of temperature on the kinetics of proliferation in HeLa cell cultures, as related to synchronization of mammalian cell lines by the use of temperature shocks (*1*).

HeLa cells (*2*) in suspension culture were routinely maintained in exponential growth at 37°C in Eagle's minimum essential medium supplemented with Eagle's nonessential amino acids, pyruvate, and 5 percent calf serum. A *p*H of 7.4 was achieved by passing air containing 2.7 percent CO_2 through culture flasks. Most measurements were made during the transient period immediately after a sudden shift of temperature from 37°C to a new temperature. Life-cycle analyses, however, were performed on cultures in the steady state.

Measurements of growth rates during the transient period were obtained from the rate of mitotic accumulation in cultures treated with colcemide (0.25 μg/ml). After a shift to temperatures in the range 33° to 40°C the culture did not reach an immediate steady state (Table 1). Instead, an immediate change in generation time occurred which was characteristic of the new temperature. The generation time then increased with time as it approached a steady-state value at the new temperature—the greater the temperature shift from 37°C the longer the duration of the transient increase in generation time.

The cells could be grown in the steady state (as exponential cultures) only at temperatures ranging from 33° through 40°C. Growth rates (reciprocals of generation times) were calculated from the slopes of semilogarithmic plots of cell number (hemocytometer counts) versus time. The growth rates shown in Fig. 1 represent an average over a 4-day period during the steady state. After a shift to 32°C the cells divided at least once over a period of 72 hours after which time no measurable increase in cell number was observed. At 41°C there was no cell division, but there was an increase in mitotic index.

An Arrhenius plot of growth rate as a function of temperature obtained by Johnson and Lewin for *E. coli* falls in a straight line on each side of the maximum growth rate (*3*). A similar plot for *Tetrahymena pyriformis* is not linear (*4*). An Arrhenius plot of our data likewise failed to fall in a straight line. A simple plot of growth rate versus temperature was quite linear on each side of the maximum growth rate (Fig. 1).

In exponentially growing cultures at 37°C the mitotic index averaged 0.03, and mitosis occupied about 4 percent of the generation time. The shortest duration for mitosis (0.8 hour) occurred at 38°C. When cells were grown above and below 38°C, the duration of mitosis increased as did the mitotic index (Fig. 1). Growth rate varied inversely with mitotic index, the growth rate being maximum when the mitotic index was minimum.

Measurements of the durations of S (the period of DNA synthesis), G1, G2 (pre- and post-synthetic periods, respectively), and M (mitosis) were carried out on cells grown for at least 5 days at given temperatures. When cell growth reached a steady state, colcemide (0.25 μg/ml) as a mitotic block, and H^3-thymidine (0.05 μc/ml, 0.36 c/mM, New England Nuclear Corp.) as a label for DNA, were added and cell samples taken at regular intervals. The cells were prepared for autoradiography, stained with toluidine blue (*5*), and scored for labeled and nonlabeled interphases and mitoses. Durations of G1, S, G2, and M were derived from these data by the method of Puck and Steffen (*6*).

The variation of G1, S, G2, and M with temperature from 33°C through 40°C is shown in Fig. 2. All four phases of the mitotic cycle tend to increase in duration above and below 38°C. Of the four phases, M shows the most striking increase. Thus, the fraction of the life cycle which the HeLa cell spends in mitosis increases as the growth temperature increasingly deviates from 38°C (see Fig. 1).

The results shown in Fig. 2 are for the steady-state. When similar measurements were made during the transient period—that is, during the first cell-doubling period after the temperature shift, the increase in the duration of G1 was great compared to that of S and G2, since G1 approached its steady-state value much faster than did S and G2. The disproportionate increase of G1 following a sudden temperature change was noted previously in other cell lines by Sisken (*7*).

In the range 26°C through 31°C the cells could not be grown exponentially; however, in this range the cells manifested an interesting transient period during which the rate of cell division approached zero while the mitotic index increased with time.

When a HeLa cell culture containing no colcemide was transferred from 37° to 29°C, the mitotic index remained constant for about 12 hours after the temperature shift and then increased with time in a linear fashion. A maximum mitotic index of 0.36 was

Table 1. A comparison of generation times for HeLa cells grown at 37°C after a temperature shift.

State of the culture	Generation time (hours)					
	33°C	34°C	36°C	37°C	38°C	40°C
Immediately after the temperature shift	36.0	32.3	23.5		18.4	24.0
Steady state	73.6	44.8	25.8	21.8	19.2	33.7

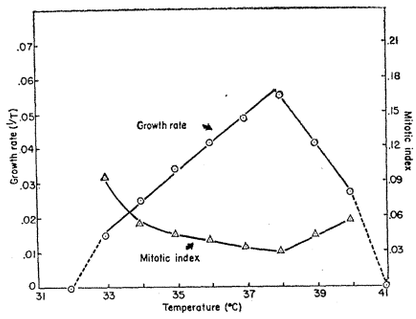

Fig. 1. Growth rate and mitotic index versus temperature for the HeLa cell in steady-state (exponential) growth (33° to 40°C).

reached at about 60 hours, after which time the mitotic index gradually declined. When colcemide was added to a culture at the time of the temperature shift there was an immediate linear increase in mitotic index through a maximum of 0.65 at 60 hours. Thereafter the cells in mitosis started disintegrating. The lag in the control signifies that during this period the number of cells entering mitosis equals the number leaving. Subsequently, the mitotic index increases with time when the rate of entry into mitosis exceeds the rate of division.

Similar studies were made over the range 26°C through 31°C. In this temperature range the lag period in the control varied from 11 to 24 hours. The maximum mitotic index (without colcemide treatment) at each temperature was reached at about 60 hours. The mitotic accumulation at 60 hours is plotted as a function of temperature in Fig. 3. The duration of mitosis and, consequently, the mitotic index was influenced by *p*H. A record high of 44 mitoses per 100 cells was observed after 60 hours in a culture at 29°C and *p*H 7.7.

One of the reasons for the progressive accumulation of cells in metaphase at low temperatures (cold-blocked cells) can be understood in terms of Fig. 2. Because M is more sensitive to temperature than G1, S, and G2, the increase in the duration of M is significantly greater at low temperatures than the increase in the duration of the rest of the mitotic cycle. This results in more cells entering, but few being able to complete, mitosis; thus the mitotic index increases. Similar mitotic accumulation in the newt, as a result of cold treatment, was observed by Barber and Callan (*8*).

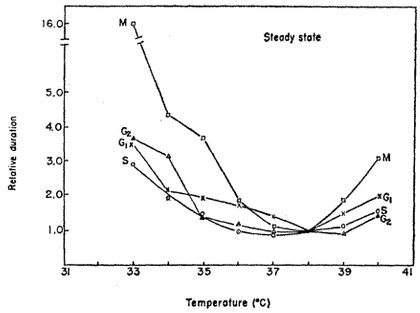

Fig. 2. The effect of temperature on the steady-state durations of the growth periods G1, S, G2, and M of the HeLa cell.

Cells in mitosis at these subnormal temperatures had a normal appearance, manifesting intact spindles and well-defined and aligned chromosomes. This was in contrast to colcemide-treated cells in which spindles were disorganized and the chromosomes scattered. Lysis of cold-blocked cells with 0.1*M* citric acid (*9*) yielded intact mitotic figures. Our study reveals the possibility of using subnormal temperatures as a means of obtaining large numbers of mitotic figures for use in analytical studies on the mitotic apparatus of mammalian cells. Unfortunately, mitoses accumulated in the cold did not divide normally when returned to 37°C.

The physiological basis of this cold-induced mitotic accumulation was sought in synchronized cells. Cells synchronized at 37°C in G1, S, or G2 by the excess thymidine technique (*10*) were transferred to 29°C and their rate of cell division followed. Rough estimates for the duration of mitosis for cells which were in G1, S, and G2 at the time of the temperature shift were obtained from a plot of mitotic index versus time for colcemide-treated and control cultures. The estimate was taken to be the time elapsed between the appearance of the first mitoses and the point where the curves for colcemide-treated and control cultures diverged. This divergence occurred when the cells in the control culture started dividing. The durations of mitosis were of the order of 3, 6, and 28 hours for cells which were, respectively, in G2, S, and G1 at the time of the temperature shift. The great difference in the duration of mitosis of G1 cells, as compared to G2 and S cells, appears to be indicative of an early interphase event which is critical for the onset of anaphase and the completion of mitosis. Mitotic events were normal for cells in G2

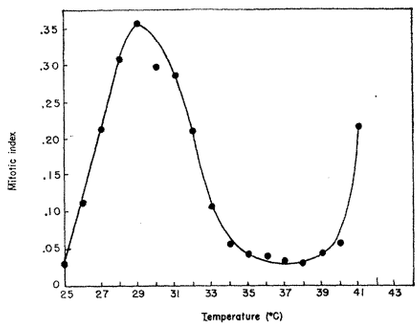

Fig. 3. Maximum mitotic index as a function of temperature. From 33°C through 40°C the curve gives the mitotic index for steady-state growth.

and S at 29°C, but those in G1 passed through an abnormal anaphase.

An interesting fact which has emerged from these studies is that the duration of mitosis (M) does not depend on the temperature alone but also on the time which the cell has spent at the new temperature. For example, the time required for mitosis immediately after a temperature shift to 33°C is only one-fifth of that required for mitosis after the culture reaches the steady state (exponential growth) at this temperature. The effect of low temperature on the duration of mitosis in the steady state is, therefore, not merely a simple effect of temperature on chemical reaction rates involved in mitosis. Rather, the effect also reflects the previous history of the cell's life cycle at the lower temperature.

Of the many temperature-time patterns these HeLa cultures were subjected to, none yielded an appreciable synchrony of cell division when the cells were returned to 37°C.

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References and Notes

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11. We express our appreciation to Dr. Harry Eagle and Dr. Jacob Maizel, for supplying a starter culture of HeLa cells and for kind help and advice. We thank Mrs. Nora Mitchell, Mrs. Mattie Mitchell, and Mr. Christopher Landrum for their valuable technical assistance. We are indebted to Dr. Jesse E. Sisken for making available to us unpublished data on the effects of temperature on mammalian cells. This investigation was aided by PHS grant NIH-CA-06835-01, 02 from the National Cancer Institute and by research grant E-303 from the American Cancer Society.

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Scenedesmus obliquus Sexuality

Abstract. *The flagellated cells of two mixed strains of* Scenedesmus obliquus *can initiate a sexual cycle when grown at 15°C in a defined medium lacking a nitrogen source. Clumps of five to ten cells, paired gametes, plasmogamy, and quadriflagellated zygotes were observed.*

Reproduction in *Scenedesmus*, particularly *S. obliquus*, a widely distributed fresh-water alga, was thought to occur by the development of nonmotile spores, which become arranged in a definte pattern within the parent cell before release as a colony. However, motility was observed in cultures of *S. obliquus* and *S. dimorphus* (*1*). Although we tried different media and conditions of growth, we were not able to keep the motile cells alive. Because of this difficulty, as well as the fact that the motile cells were produced under conditions which stimulate a sexual cycle in other algae (*2*), we thought it possible that the motile cells were obligate gametes.

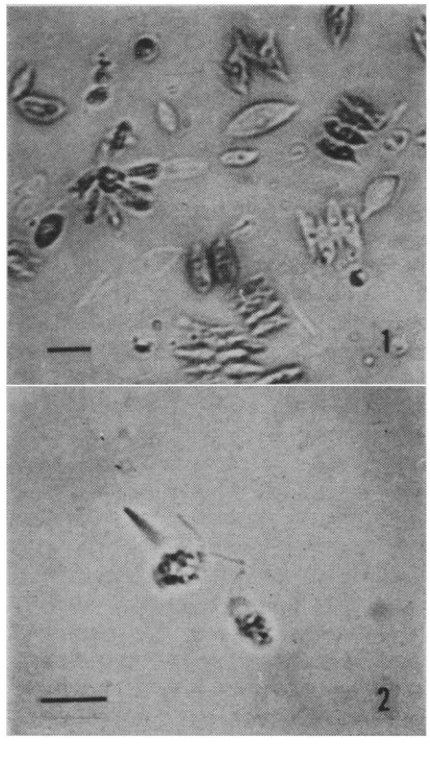
In culture studies dealing with pleomorphism in *Scenedesmus*, forms resembling *Dactylococcus*, *Chlorella*, *Oocystis*, and *Ankistrodesmus* are commonly observed (*3*). Among the spine-bearing species, certain pleomorphic strains can produce several different coenobial types that resemble other stable species, which in turn can produce just one coenobial type in culture (*4*). Although variability may result from a nutritional deficiency (*5*), existence of sexuality in *Scenedesmus* would provide a basis for other explanations.

Numerous clones of *S. obliquus* which produce motile cells have been isolated into axenic culture. To induce motility, a dense suspension of an actively growing culture in glucose-supplemented medium was transferred to a medium lacking a nitrogen source. A 3-ml sample in a sterile flask was incubated under continuous illumination at 15°C for 48 hours. When motile cells of one of our clones (*6*) were mixed with the WH-50 strain of *S. obliquus* (*7*), there was immediate clumping of gametes (Fig. 1). Usually there were five to ten cells per clump, and the clumps were numerous. When pairs broke away from the clumps, the flagella tips were joined (Fig. 2). The pairs swam actively for several minutes, but plasmogamy soon followed. Within 30 seconds the gametes had fused laterally, and a quadriflagellated zygote resulted (Fig. 3). Zygotes remained motile for many hours, but showed neither a phototactic response nor a tendency to become quiescent near the edge of a hanging-drop preparation. It was not possible to determine their fate.

Previously we could not follow motile cells through a division after quiescence; presumably they all died (*1*). During the present experiments many of the unpaired gametes also disintegrated. Cells that had recently conjugated might live after transfer to a medium containing a nitrogen source, for example, our complete medium (*5*). Or, if gametogenesis occurs in a medium providing some nitrogen, the cycle might be completed.

Fritsch (*8*) placed *Scenedesmus* in the order Chlorococcales and family Coelastraceae, a family in which no member has a motile stage or sexual cycle. Among the strictly colonial or coenobial algae in this order, those with zoospores or sexuality are placed in the Hydrodictyaceae. With the demonstration of sexual reproduction in *Scenedesmus*, we now agree with Fritsch that at least this member of the Coelastraceae could be transferred to the Hydrodictyaceae without any great alteration of the diagnosis. Furthermore, he might indeed be correct in questioning the feasibility of maintaining these two families.

Numerous strains of *Scenedesmus* have been used in many laboratories in photosynthetic and nutritional studies, not only because they can be easily grown and manipulated, but also because one avoids some of the complications which might result with any organism possessing a sexual cycle. If sexuality is found to be common in the



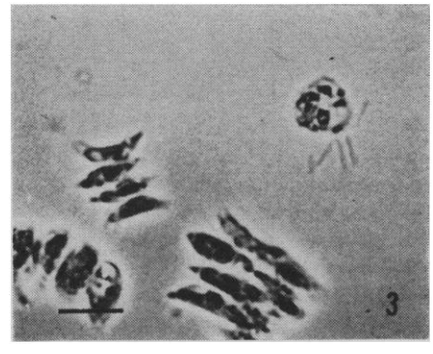


Fig. 1. A clump of approximately eight gametes. Some colonies visible. Living specimens. Fig. 2. Tips of flagella of two gametes joined. Material killed with iodine potassium iodide. Fig. 3. Quadriflagellated zygote and colonies. Material killed with iodine potassium iodide. The scale on all figures is 10 microns.