SYNCHRONIZATION OF CHINESE HAMSTER CELL CULTURES WITH COLCEMID

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A method of synchronizing cultures of Chinese hamster cells with colcemid is described. The duration of the periods of interphase (tg_1 = 4-5 h, t_S = 6-7 h, tg_2 = 1-1.5 h) and the degree of synchronization of the passage through the various periods of interphase by the cells were determined with the aid of thymidine-H^3. Synchronization was most marked as the cells passed through the g_1-period and the beginning of the S-period. At the end of interphase desynchronization began as the result of variation in the duration of the individual periods of the cell cycle. The size of the proliferative pool in this cell model was 30-40%. Calculation of the degree of synchronization during the period of the wave of mitoses by Engelberg' coefficient points to a high level of synchronization in this population. On the basis of these results colcemid can be recommended as a synchronizing agent for the investigation of the metabolic basis of the cell cycle.

A culture of Chinese hamster cells was synchronized with colcemid using a slightly modified version of the basic conditions of this method [12]. To assess the advantages and disadvantages of the modifications introduced, the degree of synchronization of passage of the cells through the various periods of interphase and their starting upon the next division was investigated.

EXPERIMENTAL METHOD

A monolayer fibroblast-like culture of Chinese hamster (strain B11 dii FAF-28, line 237) cells was used. The stem line of these cells was grown in a mixture of 90% Eagle's medium and 10% serum. The cells were seeded on large, flat flasks in a concentration of 200,000 cells/ml. In the exponential stage of growth, a few hours before addition of the colcemid to the flasks, the Eagle's medium was changed for a mixture of 90% medium No. 199 with 10% serum. The colchicine derivative colcemid, which is less toxic, was used for synchronization [8]. Colcemid blocks cells in the metaphase stage of mitosis by its harmful action on the division spindle. Compared with other cell lines Chinese hamster cells are highly resistant to this alkaloid [11].

Colcemid was used in the experiments in a concentration of 0.05 μg/ml for a period of 2 h. After incubation with colcemid, the cells were shaken in a fresh portion of medium No. 199 for several minutes. Metaphase cells present in the suspension after shaking were gathered by centrifugation for 5 min at 100 g. The state of the gathered cells was verified cytologically. The duration of "harvesting" of the metaphase cells usually did not exceed 1 h, during which time the cells did not emerge from the colcemid block. The harvested cells were then seeded in penicillin flasks with a density of 100,000-150,000 cells/ml and incubated at 37°C. To investigate the degree of synchronization of the passage of the cells through the various stages of the cell cycle thymidine-H^3 was added (4 μCi/ml; 3.7 Ci/mmole) in two series of experiments every hour to 3-4 flasks throughout the period of interphase. After incubation for 1 h the cells were fixed in Carnoy's fluid (10 min). The index of labeling (per 1000 cells) was determined in each preparation, and

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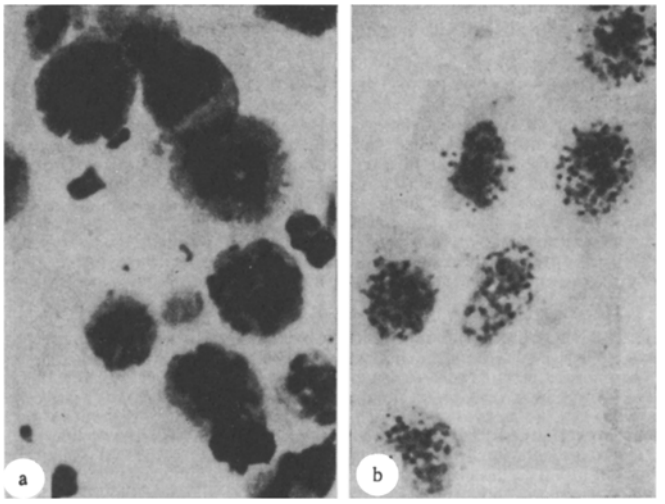

Fig. 1

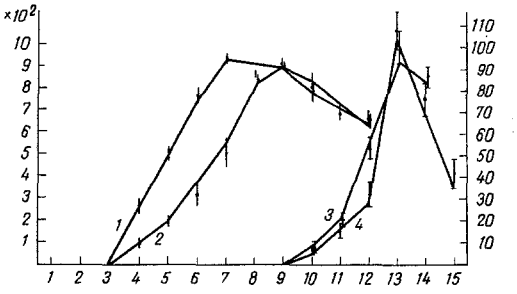

Fig. 2

Fig. 1. Cells of the synchronized culture: 1) metaphase cells in cytological preparation, Carazzi's hematoxylin 600 ×; b) cells in the S-period labeled with thymidine-H^3. Carazzi's hematoxylin, 700 ×.

Fig. 2. Changes in number of cells labeled with thymidine-H^3 (1, 2) and mitotic activity (3, 4) during cell cycle of synchronized Chinese hamster cells. Abscissa, time (in h) after escape of cells from colcemid block; ordinate: on the left – number of cells labeled with thymidine-H^3 (per 1000 cells) on the right – mitotic activity (in ‰).

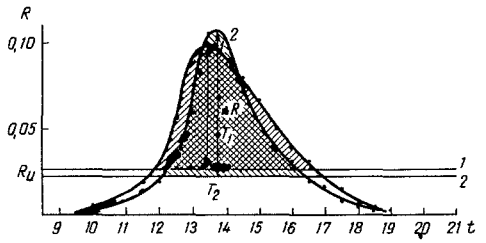

Fig. 3. Changes in normal coefficient of division R in a synchronized population. Degree of synchronization S is ratio between area T_1 (shaded area) and $T_1 + T_2$ [the area bounded by the curve R(t) and the abscissa]; Ru is the normal coefficient of division of an experimentally growing culture; abscissa, time (in h) after escape of cells from colcemid block; ordinate, normal coefficient of division R.

the mitotic activity calculated (in promille) in two other experiments. The numerical results were subjected to statistical analysis. To estimate the degree of synchronization during the period of the wave of mitoses by Engelberg's method [3, 4, 6] normal coefficients of division of the test culture R(t) and of the experimentally growing culture (Ru) were plotted on one graph.

$$R(t) = \frac{1}{n}\frac{dn}{dt},$$

where n is the relative number of cells (before the beginning of the wave of mitoses, equal to 1),

$$Ru = \frac{\ln N}{T} = \text{const},$$

where N is the relative number of cells at the end of the wave of mitoses; it shows by how many times the number of cells increased during the cycle; T represents the duration of the cycle. The value of n(t) was calculated by the equation

$$n(t) = M(t-\tau)\cdot n(t-\tau),$$

where M is the mitotic index (per cell); τ the duration of mitosis (equal to 1 h in the test culture).

The degree of synchronization was calculated by the ratio $(R_{max} - Ru)/Ru = \Delta R/Ru$ and the coefficient of synchronization S.

EXPERIMENTAL RESULTS

Analysis of the cytological preparations showed that 93-95% of the harvested population consisted of metaphase cells (Fig. 1a). The concentration of colcemid used to synchronize this particular cell line was satisfactory. It ensured that the cells were kept for a long time in the metaphase stage at a normal temperature and, in particular, at a low temperature without any appreciable effect on their metabolism, and

during incubation at 37°C it ensured the rapid escape of the cells from the colcemid block (in the course of 1 h).

The mean duration of the periods of interphase, variations in their duration, and the degree of synchronization of the passage of the Chinese hamster cells through them were determined by experiments with thymidine-H^3 (Fig. 2). The duration of interphase of the cells of this line was 13-14 h (tg_1 = 4-5 h, t_S = 6-7 h, tg_2 = 1-1.5 h). According to data in the literature, colcemid does not change the duration of the individual periods of interphase [10]. The degree of synchronization was highest for the passage of the cells through the g_1-period and the beginning of the S-period (Figs. 1 and 2). Later, as a result of variation in the duration of the individual periods of the mitotic cycle desynchronization of the cell population began, so that at the end of interphase the population consisted of cells in the S-, g_2-, and g_1-periods of the next cell cycle. These results indicate considerable variability in the g_1-period of interphase (its duration varied from 4 to 7 h). It is difficult to judge the degree of variation of the other periods of interphase from these measurements.

The first mitoses in the synchronized cell population appeared 10 h after escape of the cells from the colcemid block. The maximal mitotic activity was approximately 100% compared with 40% in the unsynchronized population. The size of the proliferative pool was judged, not from the number of cells incorporating thymidine-H^3 [1, 2], but from the increase in the cell population during the wave of mitosis. Calculation of the proliferative pool showed that it was 30-40%. The value obtained for the proliferative pool was probably underestimated, because the mitotic index was recorded only during one cell cycle, so that cells which passed through the cycle slowly may not have been taken into account.

Engelberg's coefficient S which characterizes the degree of synchronization during the period of the wave of mitoses, varies from 0 (exponential culture) to 1 (completely synchronized culture), and $\Delta R/Ru$ varies correspondingly from 0 to ∞. In the present experiment the coefficient of synchronization S was 0.53 and 0.51 and the ratio $\Delta R/Ru$ was 3.7 and 2.8 (Fig. 3). (The population is regarded as asynchronous when $\Delta R/Ru \leq 0.1$).

The results for the coefficient of synchronization S and the ratio $\Delta R/Ru$ thus confirm the high degree of synchronization and its very slight variation from experiment to experiment in the cell model tested. Comparison of the coefficient of synchronization obtained in this investigation with data in the literature [14] shows that the modification of the colcemid method as used in this case provides for an adequately high degree of synchronization of passage of the cells through the cell cycle. It can be concluded from these results and from others described in the literature [8, 9, 13, 15] that although colcemid has some toxic properties [5, 7], as a synchronizing agent it has many advantages over others (the simultaneous "start" of the cells, their rapid escape from the block at 37°C, the low percentage of pathological mitoses, the high degree of synchronization of passage of the cells through the stages of interphase, the absence of any marked effect on cell metabolism). Colcemid can therefore be recommended for the synchronization of cultures in experiments to study the characteristics of the various periods of the cell cycle.

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