Micronuclei Formation in Tissue Cultured Cells Treated with Colchicine.* (27757)

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(Introduced by M. Pollard)
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The origin of micronuclei in X-irradiated or colchicine-treated cells is uncertain. Two explanations have been offered: a) Schleich *et al.*(1) and Stroud and Brues(2) have evidence that such structures may arise by fragmentation of nuclei in interphase, and b) Bloch(3), Hsu and Kellogg(4), and others indicate that micronuclei are produced by chromatid breakage and subsequent development of these fragments to the completed forms. We have used fluorescence microscopy to measure, with respect to time of colchicine treatment, the number of mitotic figures and micronuclei produced in the McCoy cell line. Our results favor the second hypothesis.

Materials and methods. McCoy cells were propagated on cover slips in Leighton tubes at 37°C. Culture medium (1 ml per tube) consisted of Mixture 199 plus 0.5% lactalbumin hydrolysate, 5% heat-inactivated calf serum, 100 units/ml penicillin, and 0.1 mg/ml streptomycin. It was adjusted to pH 7.4 with sterile sodium bicarbonate. A large number of tubes seeded initially from the same pool of cells were drained. One ml of medium per tube was replaced and contained, in addition, one of the following concentrations of colchicine:‖ $1:10^8$, $1:10^7$, $1:10^6$, $1:10^5$, (wt/vol). Control preparations without colchicine were included. At regular intervals 2-3 tubes were removed from each group. Cover slips were fixed, stained with acridine orange, and examined by fluorescence microscopy as described by Starr *et al.*(5). Approximately 500 cells per coverslip were counted (800 × magnification) and placed into the following categories: mitotic figures, micronucleates, and interphases. Polyploid giant cells were included in the interphase group.

Results. Since the cells were reproducing asynchronously, a fixed proportion would come into mitosis at any one time. Fig. 1 shows this to be the case for control preparations. These data indicate, also, that after a lag period of approximately 4 hours mitotic figures increased in the colchicine-treated groups. This increase continued for 12 hours after which a rapid decline was noted which was correlated with the appearance of micronucleated cells (Fig. 2). Presumably, an interval of approximately 12 hours is required before a detectable number of cells in mitosis can undergo developmental stages to micronucleated forms. After 12 hours, the transition of cells in mitosis to those with micronuclei occurred faster than the appearance of colchicine-induced mitotic figures which originated from the pool of interphase cells. This brought about a reduction in the proportion of cells with mitotic figures to approximately that value observed in control populations and was noted about 15 hours after colchicine treatment (Fig. 1). At this point, a second lag period of approximately 4 hours occurred while colchicine-induced mitotic figures accumulated, perhaps from the poorer genomes of the cell population which had been delayed in coming into mitosis. During this time, the proportion of cells with micronuclei remained somewhat stationary as indicated by the plateau effects shown in Fig. 2 between the 15th and 19th hour after colchicine treatment. This indicated that the mitotic figures observed between the 12th and 15th hour must have been advanced in their development toward micronucleated forms. The colchicine-induced mitotic figures increased syste-

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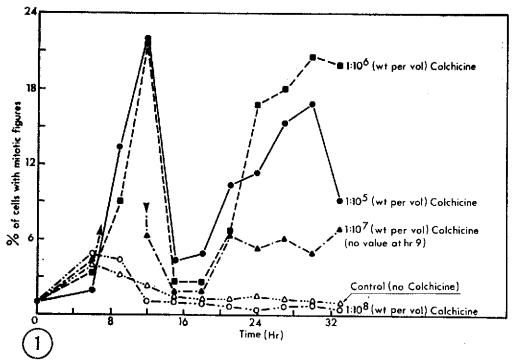

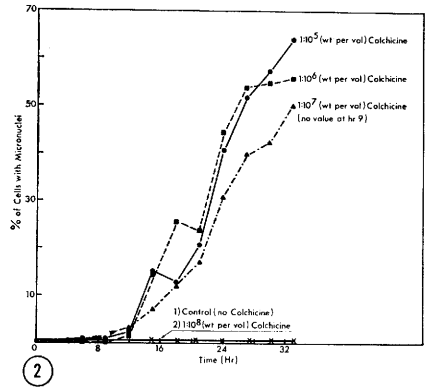

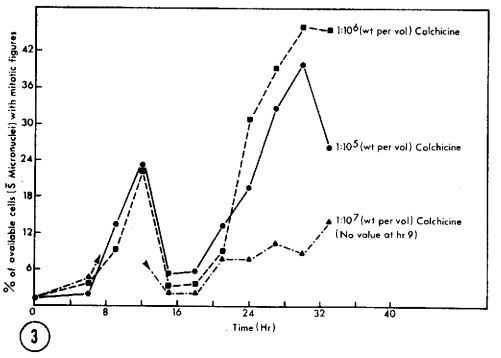

FIG. 1. Percentage of cells counted with mitotic figures.

FIG. 2. Percentage of cells counted with micronuclei.

FIG. 3. Percentage of available cells counted with mitotic figures. Cells with micronuclei were excluded.

matically between the 19th to the 30th hour. This increase was correlated with a proportional increase in the micronucleate fraction (Fig. 2). It could be inferred that the developmental time for the evolution of a micronucleated cell from one in mitosis is constant through this 19- to 30-hour interval, and furthermore, that this period is longer than that required for a colchicine-induced mitosis to occur. If this is true, the data presented in Fig. 1 indicate that the rate of mitotic figure formation is increased during this time interval. Support for this statement can be obtained by considering the micronucleated form as an irreversible stage and replotting the data of Fig. 1 in terms of corrected values to exclude this fraction. When this is done (Fig. 3), it can be seen that the proportion of cells with mitotic figures (19th to 30th hour), which originated from the interphase pool, is increasing; although a fixed number are continually being shunted toward the irreversible micronucleated state. In this regard, Harrington(6) has presented evidence that colchicine treatment can, indeed, bring about an increase in the rate at which certain cells enter mitosis.

With regard to effects noted with different colchicine concentrations, it was observed that concentrations of $1:10^8$ (wt/vol), or less, were incapable of producing colchicine-induced mitosis. A concentration of $1:10^7$ had limited efficiency and did induce mitotic figure formation and subsequent micronucleated forms but in smaller number than was observed in preparations treated with higher colchicine concentrations. However, by the end of the second lag period (approximately 19 hours after treatment) neutralization or binding of residual colchicine, apparently had reached a point where insufficient amounts were available to stimulate the enhanced rate of mitotic figure formation. Higher concentrations ($1:10^5$ and $1:10^6$ wt/vol) acted similarly until roughly the plateau point in the curve of micronuclei formation (about 30 hours after treatment). It was evident from microscopic examination that even before this period the higher concentration of colchicine was inducing toxic degeneration of the culture. Persumably these cells had been "poisoned" and mitosis was thereby retarded.

Three additional experiments were carried out in a similar manner to that described. Although some variation in the magnitude of the effects was encountered, the data were reproduced in each case in their essential form. It was also shown that as time of colchicine treatment ($1:10^6$ wt/vol) was prolonged, the

proportion of cells with mitotic figures decreased gradually to the point where at approximately 50 hours post-treatment their numbers equalled those of the control cultures. By 72 hours, virtually none were found. The curve for micronucleated cells plateaued at about 36 to 42 hours post-treatment. However, their numbers increased slowly after this point; at 72 hours about 90% of the cells were micronucleated and the remainder were polyploid giant cells. Prolonged incubation of such cultures for 1 week or longer did not alter this proportion. Cultures obtained during the 72-hour period, trypsinized, and transplanted into medium without colchicine rather quickly established a near-normal cell morphology. Some few micronucleates and giant cells persisted, however. Further work is needed to establish which cell types are responsible for growth and reproduction under these conditions(4) and whether this phenomenon might serve as a means to achieve a synchronously growing cell population.

Discussion. The capacity of both colchicine and X-irradiation to induce micronuclei formation in certain cell lines has been discussed by a number of investigators interested in mechanism of action. Harrington(6) reported a lag period of approximately 4 hours before colchicine induced a significant increase in mitosis of strain U-12 fibroblast. The initial peak of mitosis occurred at about 10 hours after treatment. Bloch(3) described the effect of colchicine on rat fibroblasts as an initial rise in mitotic activity over a 24-hour period followed by a decline to nearly control values. A progressive increase in multinucleated cells was noted after 21 hours. Mizutani *et al.*(7) found that the increase of multinucleated giant cells in X-irradiated human amnion cultures was linear from the 2nd to 5th post-irradiation day. Murray *et al.* (8) demonstrated toxicity to fibroblast cultures from rats when the alkaloid was used at a concentration of 2×10^{-5} M (*i.e.*, $1:1.27 \times 10^5$ wt/vol). Concentrations of 2×10^{-7} M resulted in little, if any, mitotic arrest.

Summary. Formation of mitotic figures and micronuclei in a colchicine-treated human synovial cell line (McCoy) were quantitated at different times after treatment. After a lag period of 4 hours, mitotic figures increased in a roughly linear manner until the 12th hour. A rapid drop to control values by the 15th hour was correlated with the appearance of micronucleated cells. A second lag period of approximately 4 hours was followed by an apparently enhanced rate of formation of mitotic figures to the 30th hour after treatment. After this point their numbers gradually decreased so that at 72 hours virtually none was seen. Except for shifts induced by this second lag period, micronucleated cells continued to increase after the 12th hour at a more-or-less constant rate through the 30th hour. The micronucleated cells were terminal forms resulting from colchicine treatment except for a nearly constant fraction (10%) of polyploid giant cells which remained unchanged.

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