AUTORADIOGRAPHIC STUDY OF DURATION OF THE MITOTIC CYCLE IN NORMAL AND IRRADIATED CELLS OF HUMAN DIPLOID STRAIN WI-38

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The duration of the mitotic cycle of cells of human diploid strain WI-38 is shown to be 22 h. Irradiation with x rays in a dose of 50 R increases its duration to 39-46 h as a result of prolongation of all periods of interphase.

Cells of human diploid strains are a most convenient object for cytogenetic research, including the study of the action of radiation on human cells.

Since the radiosensitivity of living cells is known to vary considerably in the course of the mitotic cycle, such investigations must make allowance for these changes. To do this, it is essential to have precise information concerning the duration of the mitotic cycle and of its individual periods in the cells studied. Investigations have shown that irradiation prolongs the mitotic cycle in the affected cells [1, 2, 5, 15, 16]. However, no attempt has yet been made to study the effect of irradiation on the mitotic cycle of cells of human diploid strains. The present investigation was accordingly carried out for this purpose.

EXPERIMENTAL

Cells of human diploid strain WI-38, from the 17th subculture, were used in the investigation 48 h after seeding. The cells were grown on cover slips in tubes. The seeding dose was 10^5 cells/ml. The culture medium was a mixture of Eagle's medium (70%) and a 0.5% solution of lactalbumin hydrolysate (30%), with the addition of 10% calf serum to the total volume of medium. To study the mitotic cycle by means of the curve of labeled mitoses [3, 15], the cells were incubated in medium with thymidine-H^3 in a dose of 1 μCi/ml (specific activity 1.3 Ci/mmole) for 15 min, then washed three times in warm Hanks' solution containing unlabeled thymidine in a concentration of 10 μg/ml, and added to the culture medium.

To study the mitotic cycle relative to the increase in number of labeled cells [8], the cells were incubated continuously in medium with thymidine-H^3 in a dose of 0.25 μCi/ml. Irradiation began immediately after the addition of thymidine-H^3 on the RUP-200 apparatus in a dose of 50 R. The cells were fixed in a mixture of alcohol with acetic acid (3 : 1) at definite intervals during the period of 48 h after the beginning of the experiment.

To remove unincorporated thymidine-H^3 in the continuous incubation experiments, cover slips with cells were treated with cold 5% perchloric acid for 30 min, washed in distilled water and then in alcohol and ether, and dried. The specimens were coated with type R (State Motion Picture Research Institute) liquid nuclear emulsion. The exposure was 4-5 days. After development of the specimens they were stained

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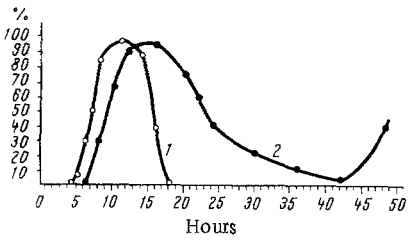

Fig. 1

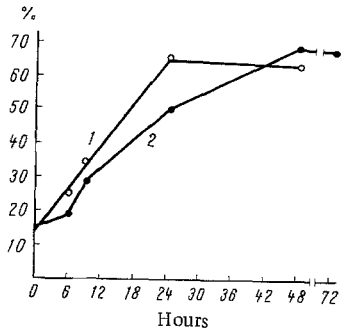

Fig. 2

Fig. 1. Changes in number of labeled mitoses in normal and irradiated cells of diploid strain WI-38, and of the 17th subculture, after brief incubation with thymidine-H^3. 1) Normal cells; 2) irradiated cells. Abscissa, time after addition of thymidine-H^3 (in h); ordinate, number of labeled mitoses (in %).

Fig. 2. Changes in number of labeled cells during continuous incubation in medium with thymidine-H^3. Ordinate, number of labeled cells (in %); remainder of legend as in Fig. 1.

with Mayer's hematoxylin. To determine each point of the curve of labeled mitoses, 150 mitoses were counted, and to determine the index of labeled cells, 3000 cells were examined in each case.

EXPERIMENTAL RESULTS

The results of determination of the duration of individual periods of the mitotic cycle from the curve of labeled mitoses are given in Fig. 1. After brief incubation of the cells with thymidine-H^3, the first labeled mitoses in the control culture were found in 5 h, and in 7 h they reached the 50% level, whereas in the irradiated culture the first labeled mitoses did not appear until after 7 h, and the 50% level was reached after 9 h. The mean duration of the postsynthetic (πG_2) period in the control culture, determined from the time of addition of thymidine-H^3 until the time of reaching 50% of labeled mitoses, was thus 7 h, compared with 9 h for the irradiated culture, i.e., irradiation caused delay of entry of the cells into mitosis. Later, in both the control and irradiated cultures, the curve of labeled mitoses reached the 100% level. Starting from 14 h after addition of the isotope, a fall in the level of labeled mitoses was observed in the control culture, and by 18 h it had reached 12%. In the irradiated culture, the fall in the level of labeled mitoses was more gradual, and by 24 h after addition of the isotope their number was still about 40%. Later it also continued to fall, to reach 5% after 42 h. A second rise in the number of labeled mitoses was observed 48 h after addition of thymidine-H^3 and irradiation, to reach a value of 42%. This rise enabled the total duration of the mitotic cycle in the irradiated culture to be determined graphically, as the distance between two corresponding points on the first and second waves of labeled mitoses [3]. This time interval was 39 h.

The mean duration of the period of DNA synthesis in the control culture, determined graphically as the distance between two points on the curve of labeled mitoses at the 50% level [3], was 8.5 h, whereas in the irradiated culture the mean duration of the S-period, determined by the same method, was 14 h, i.e., under the influence of irradiation the duration of DNA synthesis in the cells was substantially increased.

Incubation of the cells in the constant presence of thymidine-H^3 was used to determine the time when they started on the period of DNA synthesis, and also to determine the duration of the S-period from the increase in number of labeled cells [8], and to compare it with the results obtained from the curve of labeled mitoses.

It will be seen in Fig. 2 that the increase in number of labeled cells in the irradiated culture during the first 6 h after irradiation remained below the increase in their number in the unirradiated culture, reflecting delay of entry of the cells into the S-period. The duration of the S-period on this part of the curve was 19 h. By 9 h after irradiation the number of labeled cells increased sharply compared with the preceding period.

The duration of the S-period on the part of the curve between 6 and 9 h was 6.6 h. This evidently did not correspond exactly to the true duration of DNA synthesis in the irradiated cells, but rather reflected the synchronous arrival of a large number of cells at that time in the S-period after their delay in the presynthetic (G_1) period. Later, the increase in number of labeled cells in the irradiated culture followed a straight line, but its rate of increase was smaller than in the control. The mean duration of the period of RNA synthesis was 12 h.

In the control culture the cells started on the stage of DNA synthesis regularly throughout the period of observation. The duration of the S-period, determined from the curve of increase in number of labeled cells, was 6.2 h (Fig. 2).

Using the data thus obtained for the duration of the S-period in the control and irradiated cells, the duration of the total mitotic cycle was calculated, allowing for the proliferative pool [12]. Whereas the duration of the mitotic cycle for the irradiated cells, determined from the curve of labeled mitoses, was 39 h, according to the calculations (duration of the period of synthesis 12 h), the mitotic cycle lasted 46 h.

The duration of the mitotic cycle of the control culture (duration of period of synthesis 6.2 h) was 22 h.

Under the influence of irradiation, the duration of the presynthetic period also was considerably increased. Whereas the G_1-period in the control cells was 9 h, in the irradiated cells it was 16-25 h, i.e., it was more than doubled.

The results of these investigations into the duration of the mitotic cycle and its individual periods in normal cells of diploid strain WI-38, at the 17th subculture, are in agreement with data obtained by other workers [12, 13, 14].

No autoradiographic study of the duration of the mitotic cycle in irradiated cells of human diploid strains has previously beeen carried out.

After a study of changes in the type of chromosomal aberrations in cells of human diploid strains following irradiation with x rays in a dose of 50 R, it was postulated that the mitotic cycle in these cells is prolonged to 67 h [10].

Similar results were obtained by the study of changes in the types of chromosomal aberrations in cells of a primary culture of human embryonic tissue after x-ray irradiation in the same dose [4].

The disagreement between the results of these workers and those of the present investigation can be attributed to the use of different methods of investigation and different strains of human cells.

The authors cited [4, 10] investigated cells of a primary culture of human embryonic tissue and not cells of human diploid strains in early subcultures, when these cells are closest to cells of a primary culture of human embryonic tissue from which they originate.

The duration of the mitotic cycle in normal cells of a primary culture of human embryonic tissue is 30-40 h [6, 7], so that it would be expected that the duration of the mitotic cycle in these cells after irradiation would also be longer than in cells of human diploid strains in the second phase of growth, which were studied in the present investigation. The writers observed an increase in duration of the mitotic cycle in irradiated cells of human diploid strain WI-38 in the second phase of growth to 39-46 h, compared with the duration of the mitotic cycle in normal cells, which is 22 h. This increase took place on account of an increase in the duration of all periods of interphase. The most marked delay was found in transition of the cells from phase G_1 into phase S, in agreement with results obtained with other cells systems [2, 5, 9, 15]. At the same time, marked inhibition of DNA synthesis was observed, and this has also been demonstrated by other workers studying mammalian cells [5, 15]. The duration of the mitotic cycle in irradiated cells of human diploid strains is thus increased to 39-46 h because of an increase in the duration of all periods of interphase.

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