CELL CYCLE KINETICS AND RADIATION-INDUCED CHROMOSOMAL ABERRATIONS STUDIED WITH C^{14} AND H^3 LABELS

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ABSTRACT Chinese hamster cells in vitro were double labeled with C^{14}TdR and H^3TdR. At the time of irradiation with Co^{60} gamma rays (600 rad), the cells in the G_2 phase were labeled only with C^{14}, whereas cells in the late and middle S phases were labeled with both C^{14} and H^3. The cells in early S phase were labeled only with H^3 and the G_1 cells were unlabeled. Samples were fixed at various time intervals following irradiation and the metaphases were analyzed for chromosomal damage. The phase in which the cell was located at the time of irradiation was determined by counting grains in the first and second layers of autoradiographic film. In both control and irradiated cells some G_1 cells divided prior to some of the cells which were in the S phase denoting mixing of the populations. The G_2 phase sustained three times more chromosomal damage than the S phase. Little difference in chromosomal damage was found between the G_1 and S phases or among the different parts of the S phase. Cells in G_2 sustained a mitotic delay of 4 hr, while the other phases sustained a delay of 2 to 3 hr. Chromatid and chromosome (dicentrics) exchanges were induced in G_1 cells but only chromatid exchanges were induced in S and G_2 cells; this is consistent with the hypothesis that the chromosome consists of two subunits which separate either slightly before or immediately as the cell enters the S phase.

INTRODUCTION

Previous studies with Chinese hamster cells in vitro (1) have shown that in terms of chromosomal damage the DNA postsynthetic phase (G_2) is more radiosensitive than both the DNA synthetic (S) and DNA presynthetic (G_1) phases of the cell cycle. It was also shown that both chromosome exchanges (both sister chromatids broken at the same locus) and chromatid exchanges (only one of the sister chromatids broken at a particular locus) where induced during the G_1 phase and that restitution of chromosomal aberrations induced during the G_1 phase occurred during a

5 to 10 min period (2). In these experiments the cells which were in the S phase at the time of irradiation were pulse labeled with tritiated thymidine (H^3TdR) and thus distinguished from the unlabeled cells which were in either the G_1 or G_2 phase at the time of irradiation. The cells in the G_1 phase were distinguished from those in the G_2 phase only by the time interval between irradiation and the time at which the cells entered mitosis. In order to more clearly differentiate between the G_1 and G_2 phases and between early S and late S, the cells were double labeled, first with $C^{14}TdR$ for 30 min and then 3 hr later with H^3TdR (3). With autoradiographic techniques it was possible to study the radiation response of these separate phases as well as the movement of the separate populations of cells through the cell cycle.

METHODS

Culture Conditions and Labeling Methods. Chinese hamster cells, strain CH-24, which was 96% diploid with 21 to 23 chromosomes (supplied by Dr. Ernest Chu, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee) were cultured in McCoy's 5a medium (4) supplemented with 15% fetal calf serum. T-30 flasks, each containing 1×10^6 cells in 8.0 ml of medium were incubated in 6% CO_2 for 48 hr at 37°C. At this time the "conditioned medium" was poured off and saved and the cells were labeled for 30 min at 37°C with 3.0 ml of medium containing $C^{14}TdR$[1] at a concentration of 0.25 μc/ml (0.025 c/mM or 2.4 μg/ml). The C^{14} medium was discarded and the cells were washed once with 5 to 8 ml of fresh medium. The conditioned medium (5 ml with no stable TdR added) was returned to the flasks and incubation continued for 3 hr. The cells were then pulse labeled for 10 min at 37°C with 4.0 ml of 0.1 μc/ml of H^3TdR[1] (1.9 c/mM), washed once with 5.0 ml of fresh medium containing 10 μg/ml TdR, and 5.0 ml of conditioned medium containing 10 μg/ml TdR was returned to the flasks. All washes were done with warmed medium. Immediately after labeling with H^3, the flasks were placed in a water bath at 37°C and were irradiated with 600 rad of Co^{60} gamma rays at a dose rate of 520 rad per minute (5, 2). To serve as controls some cultures were labeled only with H^3TdR for 10 min or $C^{14}TdR$ for 30 min. The flasks were returned to the CO_2 incubator, and the cells were incubated and fixed over an 18 hr interval. Colcemide (0.06 μg/ml) was added to each of the flasks 1 hr prior to fixation to arrest cells in metaphase and the cells were squashed by the hypotonic method (1). At the time of irradiation, the cells in the G_2 phase were labeled only with C^{14}, whereas cells in the late and middle S phases were labeled with both C^{14} and H^3. The cells in the early S phase were labeled only with H^3 and the G_1 cells were unlabeled (see Fig. 1).

Scoring Chromosomal Aberrations and Identification of H^3 and C^{14} Labels by Autoradiography. A modification of the double stripping film technique described by Dawson, Field, and Stevens (6) was used in which H^3 was detected only in the first layer and C^{14} in both layers. The first layer of Kodak AR-10 stripping film was applied to the slides and was developed after 6 days of exposure. The metaphases were then scored for chromosomal damage (2) using phase contrast optics with 1000 × magnifica-

[1] $C^{14}TdR$ and H^3TdR were supplied by New England Nuclear Corporation, Boston and Schwarz Bio Research Inc., Orangeburg, New York, respectively.

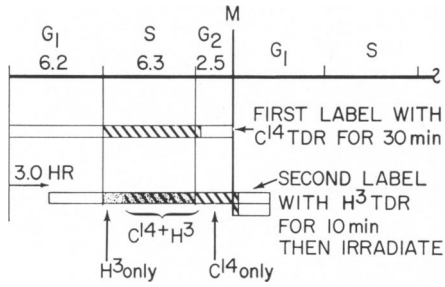

FIGURE 1 Position of CH-24 Chinese hamster cells in the cell cycle (*M* designates mitosis) at the time of irradiation; i.e., immediately after labeling with H^3TdR. The cells were labeled first with C^{14}TdR for 30 min and then 3 hr later with H^3TdR for 10 min. Both labeled cells (H^3 only, C^{14} and H^3, and C^{14} only) and unlabeled cells (open bars) are indicated in relation to the average length of the separate phases. Because of asynchrony which is illustrated in Fig. 4, a few of the H^3 only cells, which were in the S period for less than 3 hr, may have proceeded further in the process of DNA synthesis than a few of the C^{14} and H^3 cells, which were in S for more than 3 hr. As discussed in the text, C^{14} labeling continued at a reduced rate for about 1.5 hr after the 30 min period; therefore, it was possible to distinguish late S phase cells from middle S phase cells by C^{14} grain counts.

tion. Chromatid breaks including isolocus breaks and both interchanges and intrachanges of the chromatid and chromosome (mostly rings and dicentrics) types were scored. Since idiograms were not prepared it was not possible to recognize all of the isolocus breaks and symmetrical chromosome exchanges. A second layer of film was applied over the first layer and was exposed for 30 days. The method of applying the stripping film and the developing procedures have been described previously (5). The number of grains in each layer of film was then counted over each metaphase cell which had been scored previously for damage. In the first layer of film both the number of grains over the nucleus and the number of grains over the chromosomes were determined.

To establish criteria for distinguishing between C^{14} and H^3 labels in the metaphase cells grain counts were made over 25 cells from each of 2 samples labeled either with H^3 or C^{14}. The metaphase cells labeled only with H^3 produced grains only in the first layer with an average of 100 grains per cell (background of 3) with 95% of the grains appearing to lie over or to touch the chromosomes. The cells labeled only with C^{14} produced an average of 200 grains per cell in the second layer (background of 10 to 15 in the 50 μ square enclosed by a reticle) and 87 grains per cell in the first layer; 44% of the grains lay over the chromosomes (see Fig. 2). From this data the following criteria were established for the identification of the C^{14} and H^3 labels:

(*a*) For cells labeled only with C^{14}:

$$\text{Ratio A (1st layer only)} = \frac{\text{grains over chromosomes}}{\text{grains over cell}} = 0.44 \pm 0.06 \text{ (SD)}$$

$$\text{Ratio B} = \frac{\text{grains in second layer}}{\text{grains over cell in first layer}} = 2.3 \pm 0.4$$

(*b*) For cells labeled both with H^3 and C^{14}:

$$\text{Ratio A} > 0.50 \text{ and}$$
$$\text{Ratio B} < 1.9$$

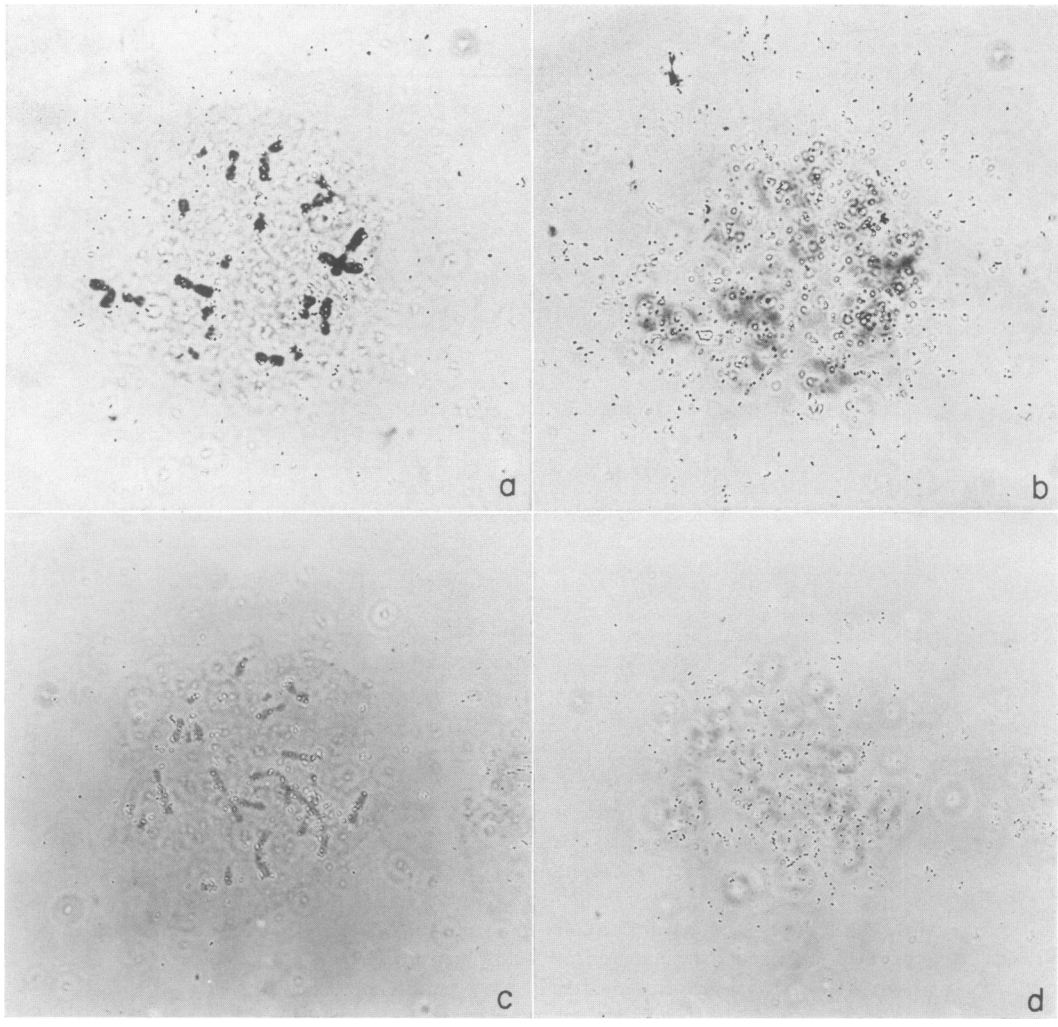

FIGURE 2 Photomicrographs were taken of cells with two layers of autoradiographic stripping film placed over them; the first layer detected both C^{14} and H^3, and the second layer detected only C^{14}. The cells were scored for chromosomal damage after the first layer had been applied; therefore, the chromosomes could be seen more clearly than is illustrated in this figure. A 100 X dark phase oil immersion lens and a Leitz Wetzlar camera attachment were used. (*a*) The grain distribution is shown in the first layer of autoradiographic film placed over a cell labeled only with C^{14}TdR; 52 grains or 0.4 of the total (130) in the cell lay over the chromosomes. (*b*) The grain distribution (339 or 2.6 times the number in the first layer) is shown in the second layer of film placed over the same cell as that shown in *a* above. (*c*) Grains in the first layer (a total of 120 with 0.6 of them over the chromosomes) are shown for a cell labeled with both C^{14}TdR and H^3TdR; note that in many regions the grains are directly over the chromosomes. (*d*) Grains (185 or 1.5 times the number in the first layer) are shown in the second layer placed over the same cell as that shown in *c* above.

(*c*) For cells labeled only with H^3:

>5 grains in first layer over the chromosomes, and
<15 grains in the second layer.

These criteria, especially ratio B, apply only to this experiment and should be determined from control cells labeled either with H^3 or C^{14} each time the stripping film is applied to a series of slides.

Examples of cells labeled with C^{14} and H^3 are shown in Fig. 2. Note that for the cell labeled with only C^{14} many of the grains lie outside of the chromosomes; for the cell labeled with both H^3 and C^{14}, however, certain portions of the chromosomes have most of the grains over them. Thus, in many cells it was possible to visually determine that a cell labeled with C^{14}, as shown by the second layer, was also labeled with H^3. The final decision that a cell which was labeled with C^{14} was also labeled with H^3, depended on both ratio A >0.50 and ratio B <1.9.

From the disintegration rate per cell determined by liquid scintillation counting of the doubly labeled cells (7), it was found that the ratio of H^3 to C^{14} in the cells was 3.0. The film efficiencies (number of grains for each disintegration) for H^3 and C^{14}, respectively, were 7 and 33% in the first layer and 0 and 15% in the second layer. For the cells scored at metaphase the average grain counts were as follows: 126 grains in the second layer for C^{14}, 55 grains in the first layer for C^{14}, and 32 grains in the first layer for H^3.

RESULTS

Movement of H^3-Labeled Cells through Mitosis (M). As described under Methods, the cells were identified as unlabeled, labeled with H^3, labeled with H^3 and C^{14}, or labeled with C^{14} only. In order to show that it was possible to identify cells labeled with H^3, although they were also labeled with C^{14}, the movement through mitosis of cells labeled with H^3 was studied as a function of time after H^3 labeling. In Fig. 3, a double labeling experiment is compared with a single labeling experiment in which there was no difficulty in detecting the H^3 label. The validity of the double labeling method is established by the coincidence of the single and double labeling points. With methods described previously (5), the average generation time of this cell line was established as 15 hr with a G_1 phase of 6.2 hr, an S phase of 6.3 hr, and a G_2 phase of 2.5 hr. As indicated in Fig. 3 by the shift of 2 to 3 hr in the curve for irradiated cells relative to the curve for control cells, the 600 rad dose induced a mitotic delay of about 3 hr in the H^3-labeled cells which were in the S phase at the time of irradiation.

Movement of Individual Populations of Cells through Mitosis. The time intervals during which the different populations of cells divided is shown in Fig. 4. The G_2 cells (C^{14} only) divided during the first 4 hr after the H^3 labeling, and it is extremely unlikely that this group of cells labeled only with C^{14} contained any G_1 cells which would have been required to proceed through S and G_2 in less than 4 hr. The cells in the late and middle portions of the S phase (both C^{14} and H^3) started dividing prior to 4 hr and completed their division in about 10 hr. Note that the cells both in the early portion of the S phase (H^3 only) and in the G_1 phase (unlabeled) divided between 6 and 16 hr following the H^3 label. In fact, some of the cells in G_1

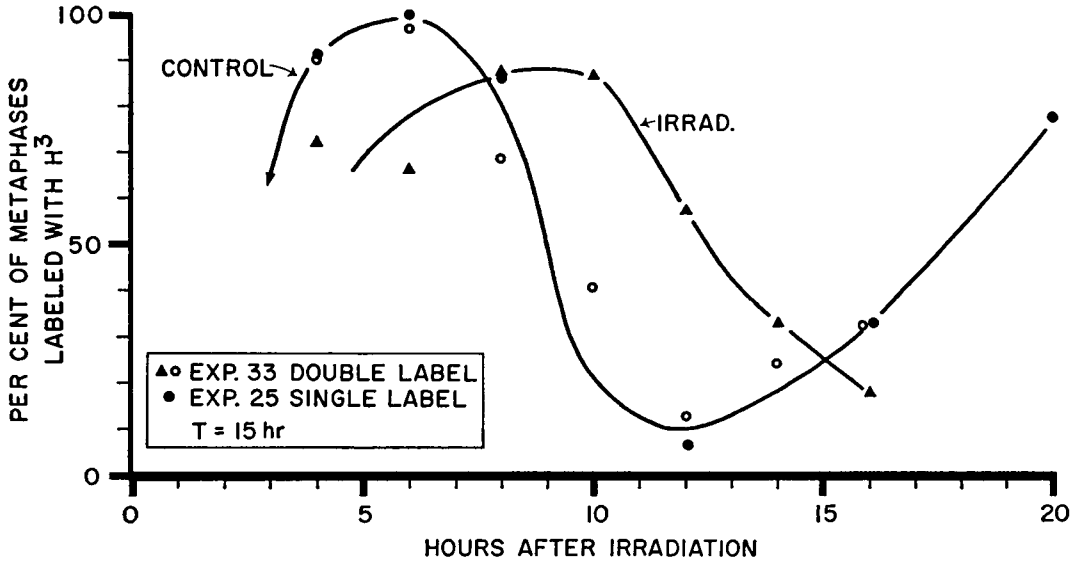

FIGURE 3 This figure illustrates that H^3 can be detected in cells also labeled with C^{14}. The per cent of metaphases labeled with H^3TdR (includes those also labeled with C^{14}TdR) is plotted as a function of time after H^3 labeling and irradiation. For the double labeling experiment, the cells were labeled first with C^{14}TdR and then with H^3TdR as indicated in Fig. 1. To identify the H^3 label in cells also labeled with C^{14}, the number of grains was determined in each of two layers of autoradiographic stripping film (see text). For the single labeling experiment, the cells were pulse labeled only with H^3TdR. The irradiated cells received 600 rad of CO^{60} gamma rays immediately after the H^3 pulse label.

at the time of labeling reached mitosis before some of the cells which were in the S phase at the time of labeling.[2] (The lack of any unlabeled metaphases in the 4 and 6 hr samples indicated that the unlabeled cells which reached division by 8 hr were not cells which had been delayed abnormally in the G_2 period. Instead, these unlabeled cells must have moved rapidly in about 8 hr from G_1 through S and G_2.) This indicates a considerable degree of mixing of the various populations; i.e., all cells did not move through the cycle at the same rate. The second rise in the curves for C^{14} only and for both C^{14} and H^3 at 12 and 13 hr, respectively, must be caused by a few cells entering division for the second time.

The C^{14} grain count in the second layer was greater for the cells in G_2 and in the latter part of S than for cells in the middle part of S (Fig. 4). In the cells dividing at 4 hr the grain count was about 200 and decreased to about 55 as the cells finished dividing at 10 hr; the average grain count was 126. Normally, the

[2] During the 15 hr interval after H^3 labeling (18 hr interval after C^{14} labeling) about 48 and 20 H^3 and C^{14} disintegrations, respectively, occurred in the nucleus. It has been shown that this amount of H^3 labeling produces negligible chromosomal damage and mitotic delay (3, 7). The

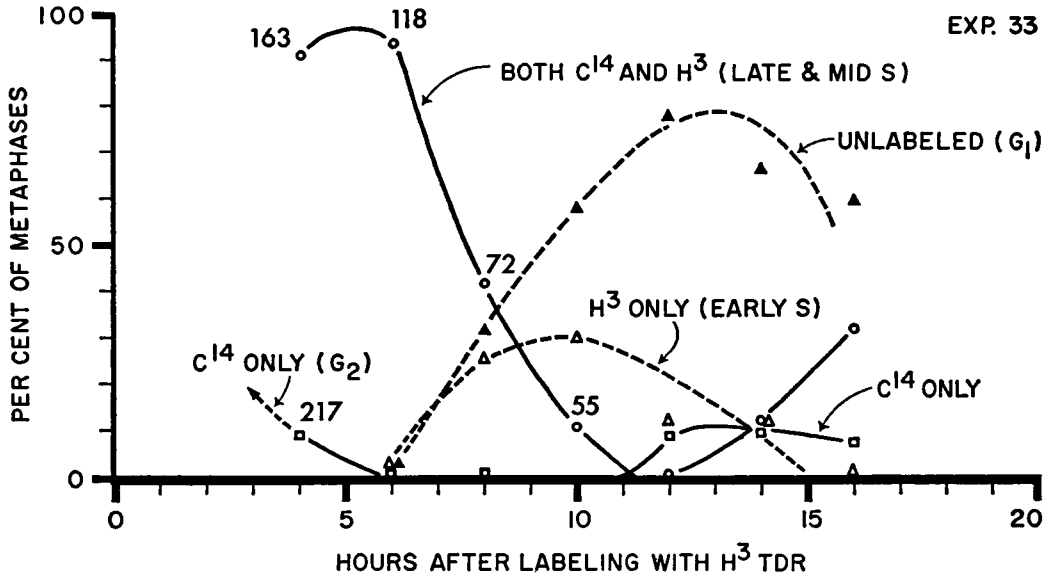

FIGURE 4 The time at which the different populations of cells (G_2, late and middle S, early S, and G_1) entered mitosis is shown; the cells were double labeled, first with C^{14}TdR and then with H^3TdR, as indicated in Fig. 1. The C^{14} grain counts in the second layer (indicated by the numbers) decreased with time; this indicates that the cells in the latter part of S were more heavily labeled than those in the middle part of the S phase. (The average grain count was 126). Note that although the various populations were separated at the time of labeling (Fig. 1), they became mixed by the time they reached mitosis.

grain count is higher in cells labeled in middle S phase than those labeled in early or late S phase (8, 9), and if samples had been taken at 0, 1, and 2 hr, the grain counts probably would have been less than 200 in these samples. In the present experiment the decrease in grain count was caused to a large extent by a continuation in labeling at a reduced rate for a period of about 1.5 hr beyond the 30 min pulse with 2.4 μg/ml TdR; this occurred because stable TdR was not added to the medium.[3] Thus, cells which entered S phase after pulse labeling with C^{14} where more lightly labeled. From the areas under the curves in Fig. 4, it was calculated that 76% of the cells labeled with H^3 were also labeled with C^{14}; if labeling stopped after the 30 min pulse with C^{14}, about 53% of the H^3-labeled cells would have been labeled with C^{14} (refer to Fig. 1). For an S phase of 6.3 hr these doubly labeled

coincidence of the points in Fig. 3 for cells labeled either with H^3 or with both H^3 and C^{14} indicates that the additional radiation from C^{14} did not delay the progression of the cells through the cycle. Also, the frequency of aberrations in unlabeled control cells (0.06) was the same as that in control cells labeled with both C^{14} and H^3.

[3] In a subsequent experiment, cells were labeled for 30 min with 2.4 μg/ml of H^3TdR and washed immediately thereafter. Conditioned medium was returned to the cells, and 10 to 30

cells, at the time of the H^3 pulse, should have been located in the last 4.8 hr (0.76 × 6.3) of the S phase. Most of the late S phase cells heavily labeled with C^{14}(>126 grains) by the 30 min pulse should have been located in the last 3.3 hr (6.3 to 3.0) of the S phase, and most of the middle S phase cells lightly labeled (<126 grains) with C^{14} should have been located between 1.5 and 3.0 hr after the beginning of the S phase. The early S phase cells labeled with H^3 only should have been located in the first 1.5 hr of the S phase.

The division of the various populations of cells following irradiation (Fig. 5) is similar to that shown for the control cells (Fig. 4), although it is apparent that following irradiation there is even more mixing of the separate populations than was seen in the controls.

By comparing the times at which the different populations of irradiated cells reach division (Fig. 5) with the times at which the same populations of control

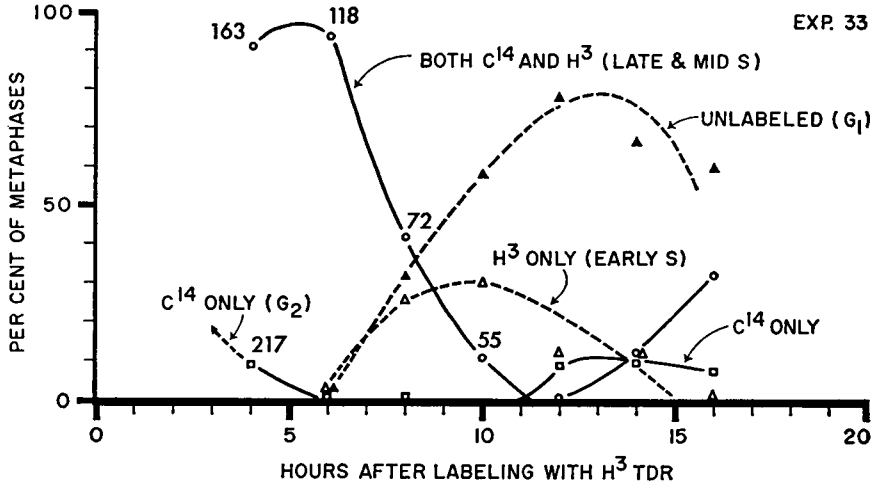

FIGURE 5 The time at which the different populations of cells entered mitosis is shown (see Fig. 4 for more details). The cells received 600 rads of Co^{60} gamma rays immediately after pulse labeling with H^3TdR (see Fig. 1). By comparing the curves for the irradiated cells in this figure with the curves for the control cells in Fig. 4, it is apparent that following irradiation the various populations became mixed even more than for the control cells.

min later it contained 0.4% (equivalent to 0.01 μg/ml H^3TdR) of the radioactivity originally used for labeling. This conditioned medium was added to other cells, and 1 hr later these cells continued 17% as much radioactivity as the cells labeled with the original medium. Under the conditions of the present experiment, it is estimated that the concentration of C^{14}TdR in the conditioned medium was about 0.02 μg/ml. It has been found that this radioactivity in the conditioned medium results primarily from radioactivity coming from the labeled cells during the first 30 min after the labeling medium is removed. The amount of radioactivity coming from the cells increases with the concentration of TdR in the labeling medium (up to about 0.2 μg/ml), and is approximately equal to the amount of perchloric acid soluble radioactivity found in the cells immediately after the labeling medium is removed.

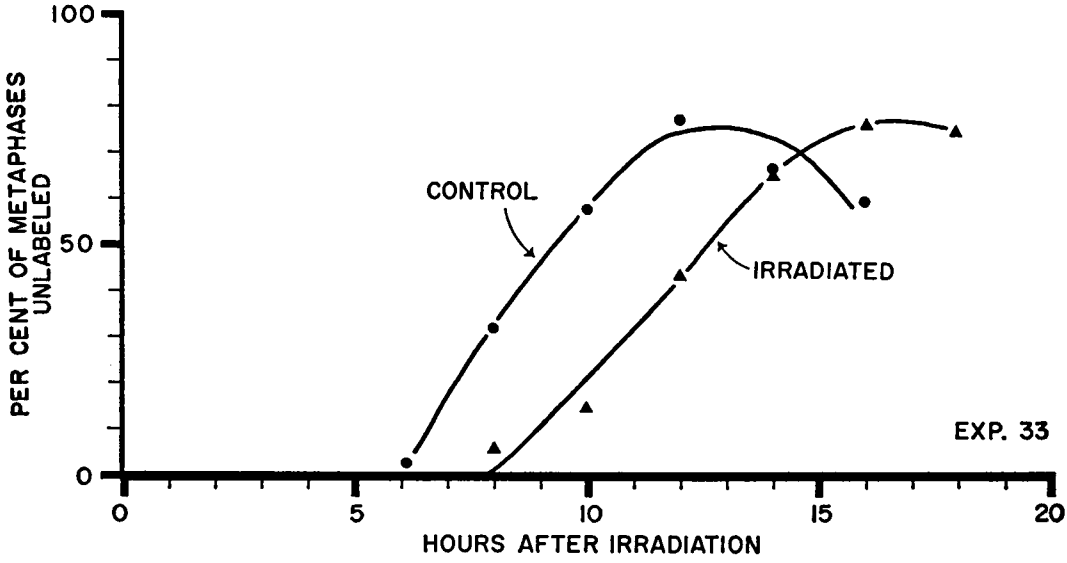

FIGURE 6 The time at which the unlabeled cells in G_1 entered mitosis is shown; the control curve was obtained from Fig. 4, and the irradiated curve was obtained from Fig. 5. The shift between the curves indicates that radiation induced a mitotic delay of about 3 hr in cells irradiated in the G_1 phase. Mitotic delay for the other phases was determined in a similar manner, and the results are shown in Table I.

cells reach division (Fig. 4), the average mitotic delay can be established separately for the different populations of cells. For example, in Fig. 6 the unlabeled control and irradiated cells which were in the G_1 phase at the time of irradiation are compared, and it is seen that irradiation produced a 3 hr lag in the G_1 cells. The mitotic lag for the G_2 cells was about 4 hr, and for the S phase cells the delay was 2 to 3 hr (Table I). Within the different populations there was no consistent trend indicating that the cells which were cytologically abnormal sustained more mitotic delay than

TABLE I

RADIATION-INDUCED MITOTIC DELAY*

Phase irradiated	Delay
	hr
G_2	4
Late and middle S	3
Early S	2
G_1	3

* Obtained by comparing Figs. 4 and 5. An example for G_1 cells is shown in Fig. 6. No significant difference in delay was seen between cells which were cytologically abnormal and those which were cytologically normal.

the normal cells; e.g. in the early S phase cells 57% were abnormal at 8 and 10 hr, and 59% were abnormal at 12, 14, and 16 hr.

Chromosomal Aberrations Induced in Different Phases of the Cell Cycle. Results of the chromosomal analysis at metaphase (Table II) indicate that cells

TABLE II

CHROMOSOMAL ABERRATIONS IN CH-24 HAMSTER CELLS IRRADIATED WITH 600 RADS OF Co^{60} GAMMA RAYS

Phase irradiated	No. of cells	No. of breaks	No. of chromatid exchanges	No. of chromosome exchanges	Total aberrations per cell*	Due to exchanges	Cells abnormal
						%	%
G_2	29	38	32	0	3.5	62	93
Late and middle S	155	72	34	0	0.90	49	49
Early S	66	27	20	1	1.0	61	56
G_1‡	221	57	16	46	0.82	68	43
Late S§ cells > 126 grains/cell	74	44	11	0	0.89	33	—
Middle S§ cells < 126 grains/cell	81	28	23	0	0.91	62	—
Control	51	3	0	0	0.06	0	6

* Each exchange was counted as two aberrations.

‡ In the 221 G_1 cells, 14 (or 0.063) had chromatid exchanges, 45 (or 0.20) had chromosome exchanges, and 2 of the 45 (or 0.045) had both chromosome and chromatid exchanges.

§ As shown in Fig. 4, the cells in late S were more heavily labeled with C^{14} than those in middle S. As discussed in the text, the S phase was divided as follows: early S, 0 to 1.5 hr; middle S, 1.5 to 3.0 hr; and late S, 3.0 to 6.3 hr.

irradiated during the G_2 phase sustained about 3.5 times more damage than cells irradiated during the S phase.[2] This increase in damage appeared as an increase in the frequencies of both breaks and exchanges. For cells irradiated in the G_2 phase or S phase, 16% of the breaks were of the isolocus type, and for the G_1 phase the percentage increased to 50. Little difference in the amount of chromosomal damage was found between the G_1 and S phases, as well as between the different parts of the S phase, although the early S phase cells may have sustained 10 to 20% more damage than the G_1 and late S phase cells. It is most important to note that *both* chromatid and chromosome (dicentrics) exchanges were induced in the G_1 cells, whereas, *only* chromatid exchanges were induced in S and G_2 cells.

DISCUSSION

Double Labeling Method. It was possible to distinguish between cells in the different parts of the cell cycle (G_1, early S, late S, and G_2) by double labeling

the cells, first with C^{14}TdR and then with H^3TdR. With 2 layers of autoradiographic film it is easy to differentiate between a cell labeled only with H^3 (average energy of 5 kev) and a cell labeled with C^{14} (average energy of 50 kev) (6, 10–14). However, it is more difficult to show that a cell which is labeled with C^{14} is also labeled with H^3. In the present study, it was possible to identify the H^3 label in a cell also labeled with C^{14} by relating the number of grains over the chromosomes to the number of grains over the cell, and by comparing the grain count in the first layer (detects H^3 and C^{14}) with that in the second layer (detects C^{14} only).

It is believed that improvements can be made in the techniques presently described. In our experiment the ratio of the number of H^3 disintegrations to the number of C^{14} disintegrations was 3.0 on the average; in the first layer there were averages of 55 grains from C^{14} and 32 from H^3, and in the second layer there were 126 grains from C^{14}. To facilitate the identification of H^3 in cells also labeled with C^{14}, the ratio of H^3 to C^{14} should be increased to 10 to 15. Then, with about 22 grains from C^{14} in the first layer (from 6 days' exposure) there would be about 60 grains from H^3 which could be detected easily, and in the second layer following a 30 day exposure there would be about 50 grains from C^{14} which is adequate for identification of the C^{14}. The method could also be improved by using a thinner emulsion for the first layer. Our first layer consisted of an emulsion 2.5μ in thickness covered with a gelatin layer 8μ in thickness. By reducing the emulsion thickness to about 1μ with a 4μ gelatin layer over the emulsion, the H^3 beta particles would be detected quite efficiently in the first layer but would be unable to reach the second layer, whereas, the C^{14} beta particles would be detected even less efficiently in the first layer and more efficiently in the second layer. Therefore, a thinner first layer would improve the ratio of the number of C^{14} produced grains in the second layer to the number of C^{14} produced grains in the first layer. However, in order to maintain the same efficiency for detecting the C^{14} betas from all of the cells, the thicknesses of the emulsion and gelatin must not vary appreciably from one part of the slide to another.

Modifications in the experimental technique can be used to great advantage. By doubling labeling cells, first with C^{14}TdR and then with H^3TdR, it was very easy to distinguish cells in early S (H^3 only) from cells which were in G_1 (unlabeled) and from cells which were in late S or G_2 (C^{14} labeled). By reversing the labels, i.e. labeling with H^3TdR first, it would be very easy to distinguish G_2 cells (H^3 only) from both G_1 cells (unlabeled) and S cells (C^{14}).

The double labeling method offers both advantages and disadvantages over other methods used for studying radiation response during the cell cycle. The primary disadvantage of the double labeling technique is that the analysis is tedious and time consuming and would be difficult to use for studying cell lethality. However, for studying chromosomal damage the double labeling technique is superior in many ways. By lightly labeling the cells, a large number of cells can be accurately

identified in relation to different phases of the cell cycle (G_1, early S, late S, and G_2) and studied without distributing their normal growth and metabolic patterns. Complications which may occur in synchronization procedures can be avoided. These complications are: (*a*) With the mitotic synchrony method (15, 16) it is difficult to obtain a large number of mitotic cells and the cells may sustain some trauma during the trypsinization and replating procedure. (*b*) With synchrony induced by metabolic inhibitors such as FUdR (17–19) there is always a question as to whether the cells are behaving as normal cells; e.g., it has been shown (20, 21) that FUdR treatment induces chromosomal aberrations. (*c*) With both the metabolic inhibition and mitotic synchrony methods, less than 100% of the cells are usually in a particular phase of the cell cycle at a given time. This percentage will decrease with time, especially as the cells pass into G_2 because of the decay in synchrony associated with the variation in generation time [discussed below and by Till et al. (19)]. (*d*) With the H^3TdR suicide technique of obtaining synchrony (22), the initial degree of synchrony may be very good, but decay of synchrony is still a problem.

Progression of Cells through the Cycle. The studies of cell progression through the cycle (Figs. 4 and 5) indicate that there is considerable variation in the generation time of individual cells; i.e., a cell in the process of synthesizing DNA will not necessarily reach mitosis prior to a cell in the G_1 phase. Large variations in generation times also have been reported by others (23–26); as summarized by Dawson, et al. (25), the standard deviations were observed to be from 9 to 26% of the mean generation times. In HeLa cells which had a mean generation time of 30.5 hr, Hsu (24) observed, even in sister cells, that the generation times differed by an average of 3.5 hr.

As shown previously (5, 15, 27, 28), ionizing radiation induced more mitotic lag in the G_2 phase than in the other phases (Figs. 4 and 5 and Table I). The greater lag in the G_2 cells correlates qualitatively with the greater amount of chromosomal damage in the G_2 cells (Table II). However, such a correlation may not be too meaningful, because it was shown previously (5) and observed in the present study that within a given phase of the cell cycle, irradiated cells with chromosomal damage sustained no more mitotic delay than cells which appeared to be cytologically normal.

Induction of Chromosomal Aberrations. In contrast to other studies (1, 2, 3, and 5 in which several references are included), the double labeling method used in the present study provided for clearly distinguishing cells irradiated in the G_1 phase from cells irradiated in the G_2 phase. As generally reported, the G_2 phase sustained the greatest number of chromosomal aberrations. The most important finding was the definite confirmation that *both* chromatid and chromosome exchangers are induced in the G_1 phase (even in the same G_1 cell) (1, 2, 29), while only chromatid exchanges are induced in the S phase. Similar observations have

been reported for plants (30, 31), and implications involving chromosome structure have been discussed (1, 2, 8, 30–32). Essentially, our results are consistent with the hypothesis that the chromosome consists of two subunits which separate either slightly before or immediately as the cell enters the S phase. If it is assumed that all subunits separate at the same time and that radiation produces chromosome exchanges only before the two subunits have separated, then, since radiation produces both chromatid and chromosome exchanges in the same G_1 cell, it must also be possible to produce chromatid exchanges before the two subunits have separated. In fact, it was shown previously (2) that the frequency (0.183) of chromatid exchanges in those G_1 cells which contained chromosome exchanges was about the same as the frequency (0.198) of chromatid exchanges in the whole population of G_1 cells; this makes it unlikely that the G_1 cells containing chromatid exchanges were actually cells synthesizing DNA at a reduced rate. Thus, even in the G_1 phase when the two subunits are close together, it appears that it is possible for only one of the two subunits to have an open break or lesion at a particular locus at the time when the exchange occurs. An alternative hypothesis is that during either the entire G_1 phase or a part of it, the subunits in some chromosomes exist in the "closed" condition while concurrently in the same cell the subunits in other chromosomes or parts of chromosomes exist in the "separated" condition.

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