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Author(s): L. G. Lajtha, R. Oliver, T. Kumatori and F. Ellis

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RADIATION RESEARCH 8, 1–16 (1958)

On the Mechanism of Radiation Effect on DNA Synthesis[1]

L. G. LAJTHA, R. OLIVER, T. KUMATORI, AND F. ELLIS

Radiobiology Laboratory, Department of Radiotherapy, Oxford, England

INTRODUCTION

A previous publication has reported investigations of the cell cycle and the effects of large doses (5000 rads) of X-radiation on the synthesis of deoxyribonucleic acid (DNA) in human bone marrow cells *in vitro* (*1*). The present paper is an investigation of the mechanism by which inhibition of DNA synthesis by X-rays is produced.

METHODS

Bone marrow culture and autoradiography. The technique for culturing cell suspensions of human bone marrow *in vitro* and for a high-resolution autoradiography on smear preparations from such cultures has been described previously (*2*, *3*).

Irradiations. The radiation employed was in the range of 140 to 300 kv, with dose rates to the cells of 250 to 400 rads/min. The nonuniformity of the thickness of the walls of the culture bottles made radiations of 250–300 kv preferable, but none of the doses delivered to the cells is expected to be in error by more than $\pm 10\%$. All radiations were performed at room temperature within 1 to 3 hours after the cultures were set up. Preliminary experiments did not indicate any difference whether the radiation was delivered at room temperature or at 37° C, immediately after setting up of the cultures or after 3 to 4 hours of standing at room temperature.

Grain counting. Grain counts were performed on stained autoradiographs (Fig. 1). Unless otherwise specified, 50 myelocytes and promyelocytes were counted, and the results are expressed as average grain counts per cell. The background usually was less than 1 grain per cell, but for ease of counting and to avoid errors induced by random grouping of background grains no cell was counted "positive" which showed a grain count below 10 grains. Where grain counts were performed on nucleated red cells (basophilic and early polychromatic normoblasts, or megaloblasts) this is specified. Grouping of nucleated red cells and myelocytes together in counts was avoided because of the difference in the cycle time of these two series of cells. Grain counts of over 50 cells of the same type in a preparation were found to

[1] This work is part of a project supported by a full grant from the British Empire Cancer Campaign.

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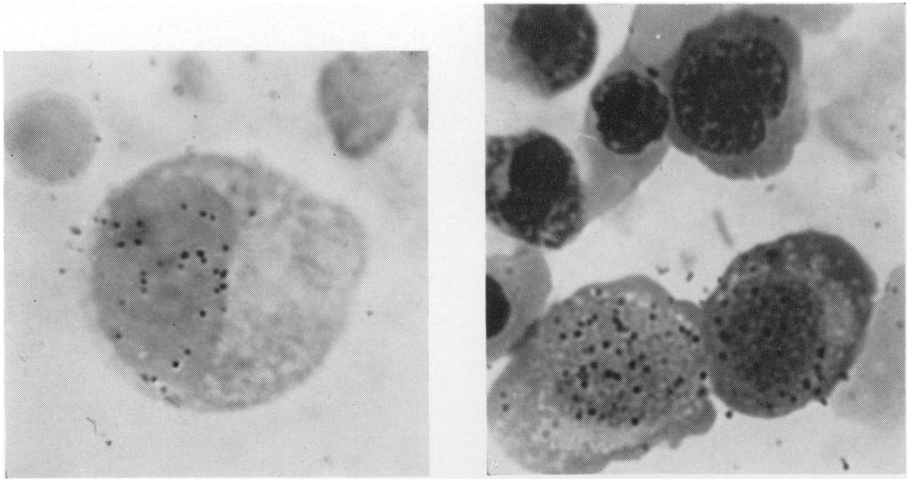

FIG. 1. Stained autoradiographs (×2000) of human bone marrow cells cultured *in vitro* with formate C^{14}. Note the nuclear localization and the lack of labeling in the late normoblasts.

be repeatable within ±10% by different observers. The autoradiographs were exposed to give average grain counts of the order of 25 to 35 grains per cell. Significantly lower or higher grain counts considerably increase the error of counting.

Proportion of labeled cells (%+). Initially this was determined by counting 250 cells of the same type in a random fashion and expressing the proportion of "positive cells" (cells with 10 or more grains over them). Since myeloid cells after the myelocyte stage and nucleated red cells after the early polychromatic normoblast do not synthesize DNA, such cells have to be excluded from the differential counts. Also, because of the shorter cycle time of nucleated red cells, the myelocytes and the basophilic and polychromatic normoblasts have to be counted separately. This involves a simultaneous differential count with its relatively high error range. The method nevertheless is sufficient to show gross differences, but for the more accurate determinations the planchette counting procedure, described below, has been adopted.

Planchette counting (*cpm*). When the cultures were opened the supernatant medium was discarded and smears were prepared for grain counting. The remaining cells were washed once in 15 ml of physiological saline, centrifuged for 10 minutes at 3000 rpm, then washed in 15 ml of 2% acetic acid, centrifuged again, and finally resuspended in 2 ml of 2% acetic acid. These procedures were performed at 4° to 7° C to avoid loss of DNA from the cells and resulted in removal of all free radioactivity originally present in the culture medium and most of the hemoglobin, thus enabling samples to be prepared with very small self-absorption. Accurate nucleated cell counts were performed on the 2-ml suspensions (usually giving values between

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4 and 7 $\times$ 10^3/mm^3), and three 0.5-ml aliquots from each culture bottle were placed into planchettes 2 cm in diameter. In all the experiments duplicate culture bottles were used. The planchettes were dried overnight at 60° C and then counted in a windowless methane flow proportional counter (Harwell Type 1364 A). The results in the tables are expressed as the average counts per minute for the six planchettes; since each planchette contained of the order of 2 $\times$ 10^6 cells, the counts thus expressed take into consideration over 12 $\times$ 10^6 cells from duplicate bottles. The variation between bottles was found to be within $\pm 10\%$ (largely due to error in pipetting).

Labeling of DNA. DNA synthesis was measured by the incorporation of formate C^{14}. In bone marrow cultures *in vitro* over 95% of the formate C^{14} is utilized for the 5-methyl group of DNA thymine, thus giving a satisfactory specific DNA labeling (*4*, *5*).[2] Sodium formate C^{14} (specific activity 4.8 mc/mM) was added to the cultures, 1.25 μc/ml of medium.

Investigations of different parts of the cell cycle. Bone marrow cells do not represent a population with a synchronized division; nevertheless, with suitably designed experiments the radiosensitivity of the DNA synthetic stage (*S* period) and the long resting stage (G_1 period) can be measured separately. Figure 2 indicates the cell cycle for the average myelocyte. Labeling occurs only in the *S* period. Therefore, if formate C^{14} is added to the cultures only after 17 hours of incubation, then those cells which get labeled between 17 and 22 hours (–17–22 hour type of culture) were at 0 hour (the time of irradiation) in the G_1 period. If, however, formate C^{14} is added to the culture immediately after irradiation (at 0 hour), then nearly all cells which get labeled between 0 and 5 hours will have been in the *S* period during irradiation. Thus a 0–5 hour type of culture measures the radiosensitivity of the *S* period, a –17–22 hour type of culture the radiosensitivity of the G_1 period. Varying the times from –10–15 to –22–27 hours, "late" or "early" G_1 cells can be investigated, the –17–22 hours referring to "mid-G_1" cells. Effects on the *S* period are best measured by changes in grain counts, since in a 0–5 hour type of culture most of the cells investigated will have been in the *S* period at 0 hour and very little change in the proportion of cells getting labeled can be expected. Effects on the G_1 period, however, should be measured by changes in the proportion of labeled cells (%+ or cpm) as well as grain counts.

Myeloid cells versus nucleated red cells. The results presented in the tables are counts over myeloid cells, except where otherwise stated. In most normal marrows there is only a relatively small proportion of nucleated red cells capable of DNA synthesis. During a culture period of 20 hours many of these will mature into later forms without intervening division. In very active marrows, however (such as post-hemorrhagic marrows, pernicious anemia marrows), there is a sufficient pro-

[2] Evidence for the very limited ability of bone marrow cells *in vitro* to synthesize purines *de novo* and for the limited pool of purine precursors will be published elsewhere (*6*).

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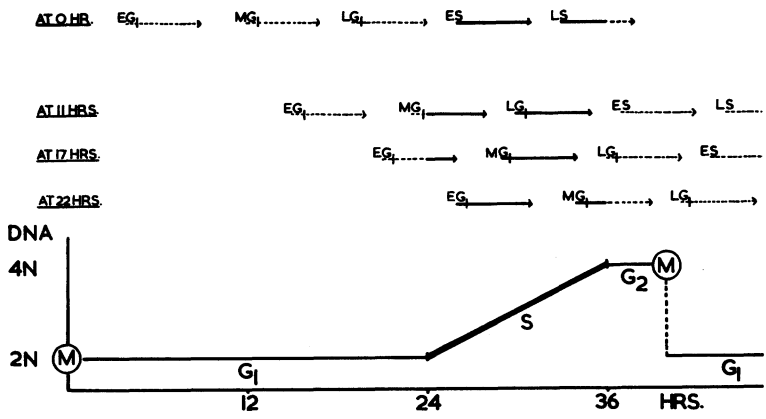

FIG. 2. Scheme of the cell cycle for the average myelocyte. Labeling occurs only in the *S* period. In a nonsynchronized cell population, as the bone marrow, at any given time cells are randomly distributed throughout the cell cycle. Thus at 0 hour (the time of irradiation) there are cells in early, mid-, and late G_1, and early and late *S* periods. Therefore, if label is added to the medium at 0 hour and the cells are incubated for 5 hours (0–5 hour type of culture) the early, mid-, and late G_1 cells will proceed 5 hours but will not have entered the *S* period and will not be labeled. The early *S* and most of the late *S* cells, however, will be labeled. If formate C^{14} is added to the cultures only after 17 hours of incubation, for a 5-hour period (–17–22 hour type of culture), then during the 17 hours preceding the addition of label to the medium all those cells which at 0 hour were in their *S* period will have completed it before the addition of label and will not get labeled. Those cells, however, which at 0 hour were in the mid-G_1 period will have arrived to the *S* period by 17 hours and will be labeled during the 5-hours incubation with formate C^{14}.

2 *N* = diploid amount of DNA; 4 *N* = tetraploid amount of DNA; *S* = DNA synthetic period; G_1 = "long" resting stage; G_2 = "short" resting stage; *M* = mitosis; ⇢ = 5-hour progress of cells during which no labeling occurs; → = 5-hour progress of cells during which they get labeled.

portion of early nucleated red cells for grain counts or %+ counts. Although the nucleated red cells have a shorter cycle time than the myeloid cells (the *S* period is about the same, but the G_1 period is considerably shorter in normoblasts and megaloblasts than in myelocytes), there was no indication of a qualitative difference in the effect of radiation, but usually a greater degree of depression was found in the nucleated red cells.

RESULTS

BASIC OBSERVATIONS

Effect of 5000 to 1000 rads. In the previous work with 5000 rads, adenine C^{14} and P^{32} were used for DNA labeling (*1*). Similar experiments performed with formate C^{14} gave similar results: both the *S* period and the G_1 period were affected by such doses of radiation (Table I).

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TABLE I

EFFECT OF 5000 TO 1000 RADS *IN VITRO*
FORMATE C^{14}

Number and type of experiment		% +	*Grains*	*Remarks*
200	Control	74%+	80	Both G_1 and S affected
3–18 hours	5000 rads	39%+	30	
207	Control	62%+	45	Both G_1 and S affected
1½–18 hours	1600 rads	30%+	10	
210	Control	55%+	40	S slightly affected, G_1 affected
2–20 hours	1000 rads	34%+	30	
212	Control	73%+	50	Both G_1 and S affected
3–22 hours	1000 rads	19%+	30	
217	Control	65%+	50	Both G_1 and S affected
1½–20 hours	1000 rads	40%+	30	
218	Control	53%+	45	Both G_1 and S affected
3–22 hours	1000 rads	28%+	30	
224	Control	74%+	50	S slightly affected, G_1 affected
3–24 hours	1000 rads	46%+	40	
255	Control	45%+	16	S not investigated, G_1 markedly affected
17–24 hours	1100 rads	22%+	9	

Effect of 1000 to 150 rads on the S period. In short-term experiments (0 to 5, 0 to 14 hours) the effect of various doses of X-rays on the S period directly was investigated. The grain counts presented in Table II indicate that, generally, doses above 500 rads directly affect cells which are in the S period and partially inhibit DNA synthesis. Between 300 and 500 rads, the effect becomes uncertain, and below 300 rads no significant effect on the S period directly can be detected.

Effect of 1000 to 100 rads on the G_1 period. In the –17–22 hour type of experiment the effect on cells in the G_1 period was investigated. The results in Table III indicate that, irrespective of the dose within the range of 1000 to 200 rads, the proportion of cells becoming labeled 17 hours after irradiation was depressed by about 40% as compared with the unirradiated controls.

In the dose range of 100 to 250 rads the response appears to follow a sigmoid-shaped curve (Table IV).[3] Maximum response is reached usually above 250 rads, but in radiosensitive marrows it may be reached by 175 rads. The maximum response does not exceed a 50% depression in the proportion of labeled cells (Table III).

Conclusions from the basic observations. These observations indicate that once cells begin DNA synthesis (i.e., enter into the S period) 200 to 300 rads will not inhibit DNA synthesis; however, in cells which have not begun DNA synthesis (i.e., are in the G_1 period) the same dose of radiation will cause about one-half of the cells ap-

[3] Suggesting a multihit process.

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TABLE II

EFFECT OF IRRADIATION ON THE *S* PERIOD DIRECTLY
(Cells in *S* period during irradiation)
FORMATE C^{14}

Number and type of experiment		*Grains/ cell*	*Depression by*	*Number and type of experiment*		*Grains/ cell*	*Depression by*
514	Control	27	50%	513	Control	19	No depression
0–14 hours	1500 rads	13		0–6 hours	300 rads	18	
287	Control	26	15%	513	Control	39	20%
1–6 hours	1000 rads	22		0–14 hours	300 rads	31	
513	Control	19	26%	514	Control	27	22%
0–6 hours	750 rads	14		0–14 hours	300 rads	21	
513	Control	39	36%	287	Control	26	12%
0–14 hours	750 rads	25		1–6 hours	300 rads	23	
287	Control	26	12%	289	Control	37	13%
1–6 hours	750 rads	23		1–6 hours	300 rads	32	
289	Control	37	22%	287	Control	26	No depression
1–6 hours	750 rads	29		1–6 hours	200 rads	26	
254	Control	16	25%	254	Control	16	No depression
0–5 hours	585 rads	12		0–5 hours	195 rads	15	
287	Control	26	8%	259	Control	15	No depression
1–6 hours	500 rads	24		0–5 hours	160 rads	15	
289	Control	37	16%	513	Control	19	No depression
1–6 hours	500 rads	31		0–6 hours	150 rads	21	
289	Control	37	8%	513	Control	39	8%
1–6 hours	400 rads	34		0–14 hours	150 rads	36	
254	Control	16	25%				
0–5 hours	390 rads	12					

Note: Differences below 20% are not considered significant; between 20 and 25% they are questionable; above 25% they are considered significant. Note also the difference in radiosensitivity between individual marrows (e.g., 287 and 254).

parently not to enter DNA synthesis during the observational period. This suggests the presence in the cells of a system connected with but not identical with DNA synthesis (? trigger mechanism) which is more radiosensitive than the process of DNA synthesis itself. Experiments were designed to investigate why only about one-half of the G_1 population appears to be affected (this effect will be referred to as "G_1 depression").

INVESTIGATIONS OF THE MECHANISM OF THE "G_1 DEPRESSION"

Completion of the subsequent S period. The lack of direct effect of small doses of radiation on cells in the *S* period does not exclude the possibility that similar doses delivered in the G_1 period may inhibit the completion of the subsequent *S* period. If damage to the G_1 period would result in allowing the cells to complete only the first 5 to 6 hours of their subsequent *S* period, then in a ~17–22 hour type of culture,

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TABLE III

EFFECT OF IRRADIATION ON THE G_1 PERIOD
FORMATE C^{14}
(1100 to 400 rads)

Number and type of experiment		*%+*	*Cpm*	*Depression by*
255	Control	42%		43%
17–22 hours	1100 rads	24%		
255	Control	48%		58% (nucleated red
17–22 hours	1100 rads	20%		cells)
286	Control	36%	66	49%
17–22 hours	1000 rads	17%	34	
281	Control	33%	32	42%
17–22 hours	800 rads	19%	<20	
286	Control	35%	66	45%
17–22 hours	500 rads	20%	36	
282	Control	35%	67	50%
12–24 hours	400 rads	31%	34	
288	Control		210	38%
17–22 hours	400 rads		131	
294	Control		190	53%
17–22 hours	400 rads		90	
				Average 47%
		(300 rads)		
256	Control	50%		52%
17–22 hours	Irradiated	24%		
260[a]	Control	36%		25%
17–22 hours	Irradiated	27%		
266	Control	60%		37%
12–24 hours	Irradiated	38%		
267	Control	52%		42%
12–24 hours	Irradiated	30%		
268a, b	Control	50%, 67%		40%
12–24 hours	Irradiated	35%, 36%		
279[a]	Control	49%		No depression
10–21 hours	Irradiated	43%		
283a, b, c	Control	31%, 31%, 31%	313, 313, 313	30%
11–21 hours	Irradiated	32%, 28%, 18%	239, 204, 215	
284[a]	Control		164	No depression
17–22 hours	Irradiated		159	
286	Control	35%	66	33%
17–22 hours	Irradiated	20%	52	
288	Control		210, 210	43%
17–22 hours	Irradiated		116, 124	
296	Control		136	32%
17–22 hours	Irradiated		93	
296	Control		247	38%
17–27 hours	Irradiated		151	
				Average 37%

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TABLE III—*Continued*

Number and type of experiment		*%+*	*Cpm*	*Depression by*
		(200 rads)		
258a, b	Control	46%, 52%		52%, 46%
17–22 hours	Irradiated	24%, 28%		
264[a]	Control		164	No depression
17–22 hours	Irradiated		179	
290	Control		212, 212	36%
17–22 hours	Irradiated		149, 122	
291	Control		101, 101	35%
17–22 hours	Irradiated		72, 60	
292	Control		132	35%
17–22 hours	Irradiated		88	
294	Control		190	42%
17–22 hours	Irradiated		110	
				Average 41%

[a] High cell count (20,000/mm^3) or not properly sedimented cultures.

Note: Placing an aliquot of cells in a planchette and counting the activity in a proportional counter was found to be a more accurate measurement than the differential counts expressing the proportion of labeled cells (%+).

TABLE IV

DOSE-RESPONSE EFFECT IN −17–22 HOUR CULTURES FORMATE C^{14}

X-ray dose (rads)	*Degree of G_1 depression*						
	Expt. 292	*Expt. 303*	*Expt. 304*	*Expt. 294*	*Expt. 300*	*Expt. 288*	*Expt. 286*
85	8%						
100		10%	20%				
125	27%						
160				42%			
175	35%	28%	48%				
200					38%		
250		45%	41%				
300				53%		41%	33%
400						38%	
500					51%		45%
1000					51%		49%

Note: Over 250 rads usually maximum effect. Compare experiments 303 and 304 for difference in sensitivity to 175 rads.

where only 5 hours uptake in S is measured (Fig. 2), "5 hours worth" of grain counts would be observed in both control and irradiated samples, but the elimination of the second half of the S period in the irradiated samples would result in an approximately 40 to 50% depression in the proportion of labeled cells. The full completion

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TABLE V

EFFECT OF IRRADIATION ON THE SUBSEQUENT *S* PERIOD
(Cells in G_1 period during irradiation)
FORMATE C^{14}

Number and type of experiment		*Average grains/ cell*	*Depression by*	*Number and type of experiment*		*Average grains/ cell*	*Depression by*
255	Control	22	45%	288	Control	46	22%
17–22 hours	1100 rads	12		17–22 hours	300 rads	36	
255	Control	12	50%	286	Control	26	27%
17–22 hours	1100 rads	6	(nucleated red cells)	17–22 hours	300 rads	19	
				296	Control	26	No depression
286	Control	26	27%	17–22 hours	300 rads	25	
17–22 hours	1000 rads	19		296	Control	29	10%
281	Control	18	11%	17–27 hours	300 rads	26	
17–22 hours	800 rads	16		266	Control	39	15%
286	Control	26	27%	12–24 hours	300 rads	33	
17–22 hours	500 rads	19		267	Control	39	No depression
288	Control	46	22%	12–24 hours	300 rads	39	
17–22 hours	400 rads	36		268a, b	Control	34, 50	No depression
282	Control	22	No depression	12–24 hours	300 rads	34, 49	
12–24 hours	400 rads	23		283a, b, c	Control	22	No depression
294	Control	32	No depression	11–21 hours	300 rads	22, 24	
17–22 hours	320 rads	29				27	
				290	Control	44	14%
				17–22 hours	200 rads	38	
				258a, b	Control	28, 30	No depression
				17–22 hours	200 rads	24, 28	
				291	Control	30	No depression
				17–22 hours	175 rads	33	
				292	Control	28	No depression
				17–22 hours	170 rads	26	
				294	Control	32	No depression
				17–22 hours	160 rads	30	

Note: Differences below 20% are not considered significant.

of the *S* period would be measured by 12 hours of incubation with the labeled compound when maximum grain counts would be, and in fact are obtained. The experiments presented in Table V show, however, that neither in the 5-hour nor in the 12-hour incubation period did doses below 300 rads affect the process of DNA synthesis in the subsequent *S* period. Doses above 500 rads affected the subsequent *S* period similarly to their effect on the *S* period directly.

Total cell counts. Loss of cells in irradiated cultures, although leaving the grain counts over the labeled cells unaffected, would decrease the number of labeled cells, thus producing a decrease in the planchette counts. If this loss is only of cells capa-

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TABLE VI

EFFECT OF IRRADIATION ON TOTAL CELL COUNTS IN 22-HOUR CULTURES

Experiment	*Control*	*Irradiated*	*Remarks*
286b	3,800	3,600	1000 rads
286a	3,800	3,500	500 rads
288b	9,250	8,950	400 rads
294	4,050	5,235	400 rads
283	10,000	10,200	300 rads
284	21,000	22,000	300 rads
288a	9,250	10,050	300 rads
296a	7,800	8,100	300 rads
296b	7,700	6,300	300 rads
290	7,700	8,100	200 rads
291	7,900	10,700	200 rads
292	3,050	2,350	200 rads

Note: All experiments are duplicate bottles except 283 which is triplicate, and 288a which is quadruplicate.

ble of division, it may even affect the differential counts, producing an apparent decrease in the proportion of labeled cells. Total nucleated cell counts failed, however, to indicate any appreciable decrease in the number of cells up to 22 hours after irradiation (Table VI). Differential counts after 150 to 300 rads also failed to show a shift in the proportion of immature cells during the observational period.

"Early" and "late" G_1 sensitivity. As shown in Fig. 2, the –17–22 hour type of culture measures the sensitivity of "mid-G_1" cells. A 40% depression in the proportion of labeled "mid-G_1" cells may be the result of a near 100% sensitivity of early G_1 cells and near 100% resistance of late G_1 cells (or vice versa), the mid-G_1 sample representing both populations, containing an equal number of both. As can be seen from Table VII, however, the early G_1 cells (–22–27 hours) do not differ significantly from the mid-G_1 cells in sensitivity. The late G_1 cells (–11–16 hours) show an apparently lower sensitivity than the mid-G_1 cells, but this is probably due to cells with an S period longer than 12 hours, which in the –11–16 hour type of culture still would be in their S period at the time of irradiation, and as such would not be affected by it (see Table II).

Effect of split doses. Since neither half of the G_1 period appeared to be significantly different in its radiosensitivity, the possibility of a cyclic metabolic process with one sensitive and one resistant phase was explored. The presence of such a system would render about one-half of a cell population sensitive at any given time. In such a case a suitable fractionation of the radiation dose may affect almost the whole cell population.

In the earlier work with large doses of radiations (5000 rads) the time taken for irradiation was 15 minutes at the rate of 333 rads/min. Since the depression in the

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TABLE VII

RADIOSENSITIVITY OF EARLY AND LATE G_1 PERIOD

(%+ or cpm in -11-16 and -22-27 hour type of culture; cells in early (-22-27) or late (-11-16) G_1 period at time of irradiation)

FORMATE C^{14}

Number and type of experiment		*-11-16 hours*		*-22-27 hours*	
		%+ or cpm	*Depression by*	*%+ or cpm*	*Depression by*
276	Control	38	16%	25	40%
	135 rads	32		15	
270	Control	25	32%	22	No depression
	150 rads	17		21	
269	Control	44	10%	45	29%
	150 rads	40		32	
278	Control	32	No depression	31, 31	42%
	160 rads	35		18, 17	
259	Control			24	29%
	160 rads			17	
276a	Control	38	No depression	25	12%
	165 rads	38		22	
278a	Control	32	No depression	31	26%
	165 rads	31		23	
298	Control	186	20%	105	30%
	250 rads	148		75	

Note: Average depression in "mid-G_1" (-17-22 hours) is about 40%. Any measured depression in a -11-16 hour type of culture is likely to be unduly low because of possible admixture of cells with longer than normal S period. Such cells would be in their S period at the time of irradiation and not affected by these low doses.

proportion of labeled cells was still of the order of 50%, it was concluded that the shift from resistant to sensitive phase in the hypothetical metabolic cycle must take much longer than 15 minutes. Table VIII shows the results with split doses of radiation. Throughout the period between doses the cultures were incubated at 37° C. It is clear that no increase in depression is obtained by splitting the dose and using different time intervals up to 3½ hours. It should be noted that a single dose of 160 rads may be sufficient in a sensitive marrow to produce the usual 40% depression, and indeed in experiment 294 a single dose of 160 rads gave the same effect as 2 × 160 rads separated by 1½ hours.

It was concluded, therefore, that there is no evidence in the cells of a cyclic metabolic process with one radiosensitive phase responsible for entry into the DNA synthetic period.

"Old" versus "new" DNA. If during a mitosis all the DNA synthesized during the S period immediately preceding that mitosis ("new" DNA) would go into one daughter cell, and all the DNA from earlier synthetic periods ("old" DNA) into

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TABLE VIII

THE EFFECT OF SPLIT INSTEAD OF SINGLE DOSE OF RADIATION IN −17–22 HOUR TYPE OF CULTURE

Number and type of experiment		*Cpm*
295	400 rads	16
½ hour	2 × 200 rads	20
294	320 rads	48
1½ hours	2 × 160 rads	57
297	488 rads	27
3½ hours	2 × 244 rads	27

another cell, then at any given time 50% of the cell population would consist of cells containing "new" DNA. If, furthermore, the new DNA were more radiosensitive than the old DNA (or vice versa), then certain low doses would affect only the sensitive half of the cell population.

Although this hypothesis did not seem very promising, it was nevertheless examined by measuring the grain count distributions over 28 anaphase and telophase figures. If all the new DNA had gone into one of the daughter nuclei, then on the autoradiographs one daughter nucleus should have contained all the grains and the other should have been negative. In fact, however, each daughter nucleus showed $50 \pm 14\%$ of the grains.[4] It was clear, therefore, that both old and new DNA are transferred into both daughter cells during mitosis, and the depression of half the G_1 population could not be explained by DNA's of different behavior.

DISCUSSION

1. DNA synthesis is defined in this paper as the process during which formate C^{14} is incorporated into DNA. The timing of the synthetic period with formate C^{14} gave similar results to those obtained previously with adenine C^{14} or P^{32} (*1*), and no evidence was found for an intracellular accumulation of low-molecular-weight precursors preceding DNA synthesis.

2. Equimolar (0.75 m*M*) concentrations of adenine C^{14} and formate C^{14} in the medium resulted in a similar degree of incorporation of C^{14} atoms into DNA. Since both compounds were in excess in the medium, and since formate mainly labels the 5-methyl group of DNA thymine, this indicated that very little conversion of adenine into guanine occurs in this system.

3. The fact that formate C^{14} mainly goes into DNA thymine indicated that very little neosynthesis of DNA purines utilizing formate C^{14} occurs in the cells. The high degree of biological labeling (about 1:10) obtainable with both adenine C^{14} and

[4] Similar findings on *Vicia faba* have been published (*7*) with an excellent discussion on the significance of this observation.

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formate C^{14} indicates either a limited pool of precursors or a preferential utilization of added adenine and formate to pool precursors.

4. Throughout the experiments reported in this paper a remarkable constancy was found in the number of C^{14} atoms incorporated by the individual cells during unit time, and in the proportion of labeled cells in a cell population of the same type. Thus myelocytes and basophilic normoblasts took up similar amounts of formate C^{14}, although usually a higher proportion of basophilic normoblasts became labeled in a 0–5 hour type of culture than myelocytes. This was explained by postulating the existence of a shorter G_1 period in the basophilic normoblasts than in the myelocytes. Pronormoblasts and early megaloblasts frequently showed higher grain counts than myelocytes, probably owing to a higher occurrence of tetraploidy in these cells.

It follows, therefore, that marrow samples with a higher proportion of early cell types will show a higher uptake of formate C^{14} (per 1000 cells) than marrows with a low proportion of early cell types.

5. A marked difference in radiosensitivity in respect of G_1 depression was found between individual marrows. This could not be correlated with any other property of the marrow cells, nor with any particular cellular composition of the marrow. Formate C^{14} and adenine C^{14} uptake were equally affected by radiation (*6*).

Further experiments are planned to investigate whether the *in vitro* radiosensitivity of the cells (in respect of G_1 depression) could be correlated with the *in vivo* radiosensitivity of the marrow or the patient.

Preliminary experiments indicated that 400 rads of local irradiation of the sternum *in vivo* affected the subsequent *in vitro* formate C^{14} uptake similarly to radiation delivered *in vitro*.

6. Many authors have pointed out the "50%" depressive effect of irradiations on DNA synthesis since the observation of von Euler and Hevesy (*8*). Most of the published work, however, does not differentiate between effects on the synthetic process itself (S period) and the pre- or postsynthetic periods (G_1 and G_2).

Recently Howard and Pelc (*9*, *10*), who originally described the cell cycle in the bean root cell, reported that the process of DNA synthesis (S period) is not affected by small doses of radiations. The depression which they obtained with small doses they attributed to sensitivity of the G_1 cell population.

Kelly *et al.* (*11*, *12*) and Holmes *et al.* (*13*, *14*), working with regenerating rat liver (a synchronized cell population for about 30 to 40 hours), found that, once DNA synthesis starts, large doses of radiation are necessary to produce a partial inhibition; small doses delivered before commencement of DNA synthesis produce a delay of the onset of DNA synthesis, although not necessarily affecting its rate.

These results are in agreement with ours: Once cells start the process of DNA synthesis, small doses of radiation (<300 rads) do not inhibit it. Large doses may have a direct effect on the S period, and this effect may be dose-dependent. With

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large doses, however, extensive cell destruction may take place with a consequent release of DNA precursors, and studies of the rate of DNA synthesis should take the possibility of increased amounts of unlabeled and/or preferentially utilized pool substances into consideration.

As to the radiosensitivity of G_1 cells in respect to subsequent DNA synthesis, Howard and Pelc find a depression of 50% in the proportion of all labeled cells at 12 hours with recovery after 24 hours (personal communication); Kelly and Holmes find a delay of commencement of DNA synthesis in a synchronized cell population; and we find that apparently about half of the expected number of G_1 cells do not enter synthesis during the observational period (in two experiments no sign of "recovery" was observed up to 43 hours, although mitoses appeared after 15 hours).

7. Having investigated and discarded several explanations of the "G_1 depression," we suggest two possibilities:

a. There are two cell populations in G_1, one sensitive and one resistant to small doses of radiations. The sensitive cells can be prevented from entering the S period; the resistant ones will enter into and proceed in it undisturbed.

b. All G_1 cells are sensitive, and the maximum damage results in slowing down the rate of entry into the S period by about half. This occurs not in a form of accumulation of G_1 cells just before the beginning of the S period, but more likely by slowing down the "progress through the G_1 period."

In both cases the "rate of entry" into the S period would be decreased by half, and since the S cells progress unhindered, eventually a partial depletion of the S population would occur. The time of maximum depletion (which cannot be more than 50%) and of the replenishment of the S period would depend on the length of the G_1 and S periods of the particular cell type studied.

8. It follows from the above that there appears to be in the cells (either in all or in half of the G_1 cells) a system connected with but not identical with DNA synthesis (? trigger mechanism) which is more radiosensitive than the process of DNA synthesis. At this stage it cannot be suggested that this system is a specific trigger mechanism for DNA synthesis, but it is noteworthy that even relatively large doses of radiations will not affect RNA synthesis (*1*), Fe^{59} uptake (*15*), S^{35} sulfate (*16*), or amino acid uptake (*17*). It is hoped that further experiments will elucidate the nature of this system.

9. Since the sensitivities of the S period and the G_1 period are different in respect of radiation effect on DNA synthesis (probably owing to different mechanisms), future work should take this difference into consideration. An over-all rate of DNA synthesis in an organ may be the result of a small proportion of cells synthesizing DNA very fast, or of a large proportion of cells synthesizing very slowly. Therefore for such studies either a synchronous cell population or, better, investigation at the cellular level is necessary.

10. Finally, it should be noted that the effect of irradiation on DNA synthesis

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cannot be simply related to chromosome damage or loss of cell viability. Although the G_1 damage is "saturated" by about 200 to 300 rads, damage to chromosomes and to cell viability are further dose-dependent; and it is not known whether damage to the S period and to chromosomes are related or not.

TABLE IX

RADIOSENSITIVITY OF DNA SYNTHESIS (SUMMARY)
(Human bone marrow cells *in vitro*)
FORMATE C^{14}

X-ray dose (rads)	*Degree of depression*					
	S period directly	*Entry into S period*	*Completion of subsequent S period*	*Cell counts*	*"Early" and "late" G_1 effect*	*Split versus single dose*
750	>30%	40–50%	30%			
>300	20%	40–50%	20%	No depression	Similar depression	Similar depression
300	Dubious	35–45%	Dubious			
200	No depression	35–45%	No depression			

SUMMARY

1. Large doses (>500 rads) of X-radiation directly inhibit the process of DNA synthesis in human bone marrow cells *in vitro*.

2. Small doses (<300 rads) do not inhibit DNA synthesis in cells which already have started DNA synthesis.

3. In cells, however, which are in the presynthetic period at the time of radiation, such small doses will produce a 40 to 50% depression of the number of cells entering the subsequent synthetic period in a given time, without affecting the rate of subsequent DNA synthesis.

4. It is suggested that unless two populations are involved the damage suffered by the presynthetic cells results in a half-speed progress through the presynthetic period.

5. It appears that in the cells there is a system connected with but not identical with DNA synthesis (? trigger mechanism, ? specific) which is more radiosensitive than the process of DNA synthesis.

6. In studies of radiation effects on DNA synthesis a clear differentiation has to be made between effects on the process of DNA synthesis (synthetic period) and those of the presynthetic period, since different mechanisms may be affected.

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