RELATIONSHIP BETWEEN S AND G_2 PERIODS AND TOTAL SEGMENT OF INTERPHASE RELATIVELY INSENSITIVE TO ACTINOMYCIN IN REGENERATING MOUSE LIVER CELLS

I. D. Belyaeva

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In regenerating mouse liver cells the S and G_2 periods of interphase are relatively insensitive to the action of actinomycin (0.25 μg/g). Shortening of the insensitive section of interphase when associated with a decrease in its total duration is evidently connected with a shortening of the S period.

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Previous investigations showed that disturbance of RNA synthesis in regenerating mouse liver cells by actinomycin during a considerable part of interphase causes almost total suppression of mitoses; during the last 12 h before mitosis, however, regenerating liver cells are relatively insensitive to actinomycin [1, 2].

The object of the present investigation was to discover whether the duration of the actinomycin-insensitive segment of interphase changes when the total duration of interphase is shortened. It was also important to determine which periods of interphase correspond to the segments sensitive and insensitive to the action of actinomycin. For this purpose an autoradiographic method was used to determine the duration of the S and G_2 periods of the mitotic cycle of regenerating mouse liver cells (corresponding data could be found in the literature only for rats [3]).

EXPERIMENTAL METHOD

Male mice (CBE/C57 hybrids) weighing 20-22 g were used. Partial hepatectomy was performed by the usual method. In the experiments of series I the operation was performed on the animals in the morning (9-10 A.M.) and the animals were sacrificed on the 2nd day at 8 A.M. In this case the mitotic index in the regenerating liver was determined 46-47 h after operation; interphase of cells undergoing division at this time was conventionally described as "46-h" because of its duration. In the experiments of series II operations were carried out on the animals in the evening (7-8 P.M.) and they were sacrificed on the 2nd day also at 8 A.M. In this case the mitotic index in the regenerating liver was determined 36-37 h after the operation, and interphase of cells dividing at this time was conventionally described as "36-h." These definitions of the duration of interphase are conventional, because it was not known at what time after the operation the cells entered the G_1 period—the first period of interphase.

In both series of experiments the animals were given actinomycin C in a dose of 0.25 μg/g body weight at different times after the operation. The number of mitoses (per 5000 cells) was determined in histological sections and expressed in promille.

In the experiments of series III and IV the duration of the S and G_2 periods was determined in regenerating mouse liver cells for "46-h" and "36-h" interphases. To do this, the animals were injected with thymidine-H^3 (dose 0.3 μCi/g, specific activity about 800 mCi/mmole) at different times before sacrifice (between 15 and 1.5 h). The percentage of labeled mitoses was determined in sections coated with emulsion (after exposure at 4° for 2 h) by counting 100 mitoses for each animal. The mitoses were understood to be labeled if the cells were synthesizing DNA at the time of injection of thymidine-H^3. Unlabeled mitoses in-

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TABLE 1. Mitotic Index (in %) in Regenerating Mouse Liver Following Administration of Actinomycin (0.25 μg/g) at Different Times before Sacrifice

Interphase	Control	Time of injection of actinomycin (hours before sacrifice)				
		Immediately after oper.	18	12	9	6
46-h	36,0	0,3	1,0	11,3	53,0	18,4
	0,0	0,0	0,0	5,2	35,0	28,7
	73,0	0,0	0,0	14,4	13,4	19,4
	13,0	0,0	2,0	48,0	19,2	16,6
	62,0	4,3	0,2	18,0	6,2	26,8
	41,0	0,0	0,5	6,0	2,6	1,3
	0,6		0,36	18,4	23,5	33,6
	43,0		1,8	23,0	1,2	
	17,0		4,9	13,0	22,6	
	16,0		1,7	21,5	24,8	
			0,0	20,5	46,0	
				16,8		
Mean	30,1	0,76	0,13	18,1	22,5	20,7
36-h	1,8		0,0	4,0	30,0	0,2
	39,0		0,0	0,7	11,3	18,0
	29,0		0,0	0,5	5,7	26,0
	40,0		0,2	0,0	18,6	30,0
	19,0		0,0	0,2	23,3	28,0
				4,0	18,0	16,0
Mean	25,8		0,04	1,6	17,8	19,7

TABLE 2. Percentage of Labeled Mitoses in Regenerating Mouse Liver Following Administration of Thymidine-H^3 at Various Times before Sacrifice

Interphase	Time of injection of thymidine-H^3 (hours before sacrifice)									
	15	13½	12	10½	9	7½	6	4½	3	1½
46-h	3	4	83	92	99	90	54	36	5	0
	4	34	88	78	95	95	99	72	30	0
	2	2	82	77				52		0
		73	80							
			69							
Mean	3	28	80	82	97	93	77	53	17	0
36-h	2	39	12	81	97	86	99	9	1	0
	4	28	31	91	100	96	98	44	9	0
		29	70		100		100		0	0
			30							
			4							
			24							
			75							
Mean	3	32	35	86	99	91	99	26	3	0

dicated that at the time of injection of thymidine-H^3 the cell was not synthesizing DNA and, consequently, was in the presynthetic (G_1) or postsynthetic (G_2) period.

EXPERIMENTAL RESULTS AND DISCUSSION

The results of the experiments of series I and II are given in Table 1. In the case of "46-h" interphase, when actinomycin was given immediately after operation and 18 h before sacrifice, mitotic divisions in the regenerating mouse liver were very strongly suppressed, but if actinomycin was injected 12, 9, and 6 h before sacrifice, inhibition was much weaker (by about 30% of the control level). Mitotic cell divisions in the case of "36-h" interphase were suppressed strongly when actinomycin was injected 18 or 12 h before sacrifice. Only if injected 9 and 6 h before sacrifice were mitoses inhibited to a considerably lesser degree (the number of mitoses was reduced by about 30% compared with the control).

Consequently, in the case of "36-h" interphase the segment of interphase insensitive to actinomycin was somewhat shorter than in 46-h interphase.

The results of the experiments of series III and IV are shown in Table 2. If thymidine-H^3 was injected 15 h before sacrifice, the overwhelming majority of mitoses were unlabeled in the case of both "46-h" and "36-h" interphase. Consequently, 15 h before mitosis the cells had not yet started DNA synthesis. If thymidine-H^3 was injected 13.5 h before sacrifice, in both cases labeled mitoses were seen (28 and 32%), although most cells (72 and 68%) remained unlabeled. If thymidine-H^3 was injected 12 h before sacrifice, then in the case of "46-h" interphase most mitoses in all animals were labeled and, consequently, most cells at this time had begun to synthesize DNA (in the S period). In the case of the "36-h" interphase 12 h before sacrifice, most cells in most animals (5 of 7) had not yet started synthesizing DNA. In this series of experiments most mitoses were labeled only if thymidine-H^3 was injected 10.5 h before sacrifice. Consequently, in the case of the "36-h" interphase, cells begin to synthesize DNA (i.e., enter the S period) somewhat later than in the case of "46-h" interphase. If thymidine-H^3 was injected 9, 7.5, and 6 h before sacrifice, irrespective of the duration of interphase the great majority of mitoses were labeled, indicating DNA synthesis was taking place at this time, i.e., that the cells were in the S period. DNA synthesis ends 4.5-3h before mitosis. If thymidine-H^3 was injected at these times most mitoses were unlabeled, indicating that the cells had moved into the G_2 period. All 100% of cells were in the G_2 period 1.5 h before mitosis.

Hence, in the case of "46-h" interphase, DNA synthesis or the S period in most regenerating mouse liver cells began roughly 12 h before mitosis. Since inhibition of mitotic division was only slight when actinomycin was injected 12 h before sacrifice, it can be postulated that with passage of the cells into the S period they become insensitive to disturbance of RNA synthesis by actinomycin. In "36-h" interphase most cells move into the S period somewhat later and the segment of interphase insensitive to actinomycin becomes shorter. Consequently, in this case also, the segment of interphase insensitive to actinomycin is evidently associated with passage of the cells into the S period.

Since in the case of "36-h" interphase the cells begin to synthesize DNA, i.e., move into the S period, rather later than in the case of "46-h" interphase, and they move into the G_2 period in both cases at about the same time, it can be concluded that in the "36-h" interphase some shortening of the S period takes place. The considerable variability of the time when the cells enter the S period and leave it for the G_2 period in individual animals indicates differences in the duration of these periods in cells entering on mitosis simultaneously.

LITERATURE CITED

1. I. D. Belyaeva, Dokl. Akad. Nauk SSSR, 161, No. 5, 1205 (1965).
2. I. D. Belyaeva, in: Nucleic Acids [in Russian], Moscow (1966), p. 338).
3. E. Stöcker and U. Pfeifer, Naturwissenschaften, 52, 663 (1965).