*THE RATE OF DNA REPLICATION AT THE MOLECULAR, CHROMOSOMAL, CELLULAR, AND INTERCELLULAR LEVELS IN REGENERATING RAT LIVER**

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Previous autoradiographic and cytochemical studies on DNA synthesis in individual cells of regenerating rat liver indicate that the rate of DNA synthesis changes during the eight-hour period of DNA replication.[1] Coordinated studies of DNA replication at the molecular, chromosomal, cellular, and intercellular levels should provide additional insight into the mechanisms of the replication of DNA, as well as the interrelationships of these mechanisms at the different organizational levels.

The present investigation has been confined to the study of the replication of the first cycle of hepatocytes synthesizing DNA following partial hepatectomy. The changing rates of DNA synthesis in individual cells and individual chromosomes were measured by quantitative autoradiography. The changing rates of DNA synthesis in the total cell population were measured by biochemical methods. Calculations for the rates of DNA synthesis at the molecular level have been made from these experimental results.

Materials and Methods.—The biochemical and autoradiographic methods are described in previous reports.[2, 3] Studies of synthesis in the total population of cells and in individual cells during the eight-hour period of DNA replication were made by intravenous administration of 50 μc tritiated thymidine (5-methyl-TdR-H^3) with a specific activity of 3 c/mmole in 1 ml of normal saline solution, at 13, 15, 17, 21, 23, and 25 hours after partial hepatectomy. The rats were sacrificed one hour after thymidine administration. Six animals were used routinely to define each point.

DNA replication in individual chromosomes was investigated by the administration of 50 μc tritiated thymidine at 13, 15, 17, 19, 21, and 23 hours after partial hepatectomy. All of the animals were given 1 cc of a 1 per cent colchicine solution at 26 hours, and were sacrificed at 28 hours after partial hepatectomy.

Specimens for the chromosome study were prepared by a slightly modified method of Tjio.[4] Following fixation, they were stained by the Feulgen technique prior to the preparation of the autoradiographs. Corrections for background were made by counting the number of grains in areas adjacent and equivalent to those covered by the metaphase chromosomes. This background correction was made for chromosomes having one grain as well as for those having more than one. The net labeled chromosomes and grain counts per chromosome are presented in this report. Determinations were made on seven to ten cells in metaphase from three randomly selected slides for each of the six rats. The number of labeled cells in different stages of the mitotic cycle was determined for each animal by counting approximately 250 cells on each of three slides.

Results.—The change in the rate of DNA synthesis in the total population of cells,

TABLE 1

DNA SYNTHESIS AT DIFFERENT TIMES DURING "S" PERIOD (AUTORADIOGRAPHIC AND BIOCHEMICAL DATA)

	Time after hepatectomy (hr)	No. of animals	Grain counts per nucleus (mean ± S.E.*)	Per cent labeled (mean ± S.E.)	Specific activity,† cpm/mg DNA‡ (mean ± S.E.)	Acid-soluble fraction, cpm/gfw§
Controls	13	6	14.7 ± 3.6	0.7 ± 0.1	4,257 ± 245	254,072 ± 15,163
	15	6	32.2 ± 5.9	3.7 ± 1.5	5,178 ± 2,260	132,398 ± 8,256
	17	5	50.5 ± 8.6	9.2 ± 2.4	18,360 ± 5,437	152,415 ± 22,840
	21	6	59.6 ± 7.1	19.1 ± 2.8	56,413 ± 11,535	160,139 ± 38,269
	23	7	52.7 ± 5.6	21.1 ± 3.7	51,304 ± 7,078	138,173 ± 8,695
	25	7	49.8 ± 8.1	17.6 ± 3.0	42,822 ± 10,441	137,813 ± 41,881

* Standard error of the mean.
† All values have been corrected for quenching.
‡ Counts per minute per milligram of DNA.
§ Counts per minutes per gram fresh weight.

determined biochemically, is given in Table 1. There is a relatively low rate at 13, 15, and 17 hours, with a rapid increase to a maximum at 21 hours after partial hepatectomy (Fig. 1). The change in the relative rate of DNA synthesis in individual cells and in new cells beginning DNA synthesis, determined autoradiographically, is given in Table 1 and Figure 2. The maximum rate of DNA synthesis was reached at 21 hours, with a decline at 23 and 25 hours, in both the autoradiographic and biochemical results. The maximum number of cells synthesizing DNA was reached at 23 hours, with a decline at 25 hours after partial hepatectomy.

The change in per cent labeled chromosomes is shown in Figure 2. The total number of chromosomes counted for each point is given in Table 2. No attempt was made to distinguish between the different chromosomes, since the primary objective of the study was to obtain a composite picture for the changing number of labeled chromosomes during the eight-hour period of DNA replication. This is shown in the variation of the total chromosomes in column 5 of the table. The

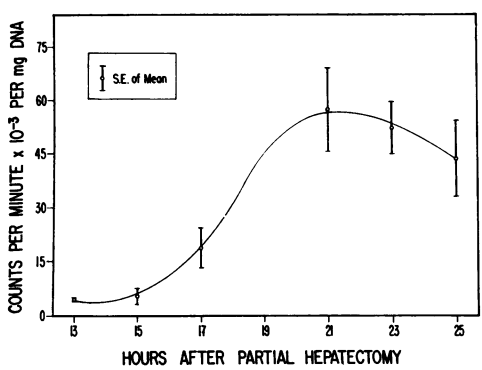

FIG. 1.—The specific activity at different times after partial hepatectomy is a reflection of the changes in both the rate of synthesis in individual cells and in the number of cells synthesizing DNA during the replication period.

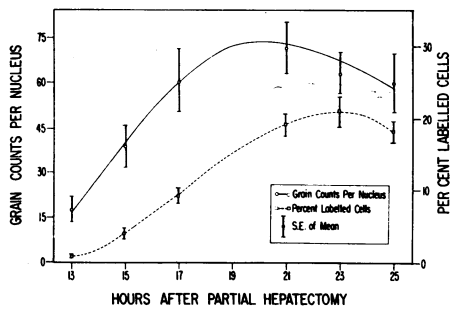

FIG. 2.—The solid curve represents the grain counts per nucleus, which is a reflection in the change in rate of DNA synthesis in individual cells at different times during the DNA synthetic period. The broken curve represents the number of hepatocytes which are labeled and are in the process of DNA synthesis at the time of thymidine administration. The increase in per cent labeled cells is a measure of the rate of initiation of DNA synthesis, and the decrease should reflect the completion of synthesis in this first cycle of hepatocytes following partial hepatectomy.

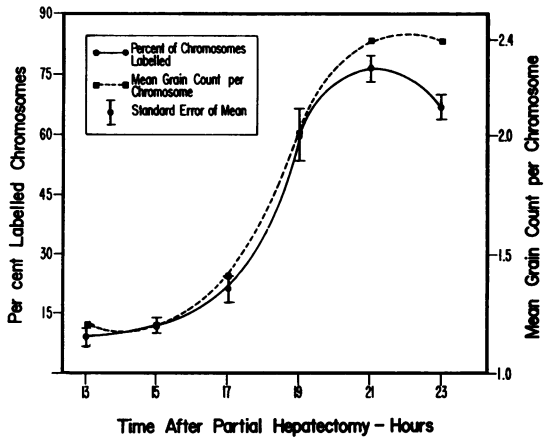

FIG. 3.—The solid curve is the per cent of chromosomes labeled at different times during the DNA synthetic period. Only a small fraction of the total number of chromosomes is labeled during the first part of the replication period. There is a marked increase in the latter part of the period, reaching a maximum of approximately 80% of the total just before completion of the DNA synthetic period.

The broken curve is the mean grain count per chromosome. The increase in grains per chromosome follows closely the increase in per cent labeled chromosomes. The maximum rate of synthesis in individual chromosomes also is reached just prior to completion of the DNA synthetic period.

change in the per cent of labeled chromosomes, in column 6, demonstrates the changing number of chromosomes synthesizing DNA within the period of replication. The per cent labeled chromosomes at 13, 15, and 17 hours after partial hepatectomy is 9.3, 14.5, and 21.1, respectively. This is followed by a marked rise to 60.3 per cent at 19 hours, with a maximum of 77.3 per cent at 21 hours after partial hepatectomy. The time of the maximum number of chromosomes synthesizing DNA is also the time of the maximum rate of DNA synthesis in the total population of cells, determined biochemically, as well as the time of the maximum rate of DNA synthesis in individual cells, determined autoradiographically. There is a decline in the number of labeled chromosomes at 23 hours, as well as a decline in the rate of DNA synthesis, measured biochemically and autoradiographically.

There is an increase in both the number of labeled chromosomes and rate of DNA synthesis in individual chromosomes up to 21 hours after partial hepatectomy (Table 3 and Fig. 3). A gradual increase in both the number of labeled chromosomes and the number of grains per chromosome occurred at 13, 15, and 17 hours. There was a large increase at 19 hours, reaching a maximum at 21 hours, as shown in the previous results at the cellular and intercellular levels. The mean grain count of 2.4 per chromosome was the same at 23 hours as at 21 hours; however, there was a reduction in number of chromosomes with lower grain counts. The mean

TABLE 2

CHROMOSOMES SYNTHESIZING DNA AT DIFFERENT TIMES DURING THE "S" PERIOD

Hours (after partial hept.)	No. of animals		Unlabeled chromosomes (mean)	Labeled chromosomes (mean)	Total chromosomes (mean)	Labeled chromosomes (% of total)	Chromosome number (mean)
13	5	Mean	393.3	40.5	433.7	9.3	43
		S.E.	18.3	7.1	54.2	2.5	
15	6	Mean	347.8	60.2	413.4	14.5	41
		S.E.	50.4	6.4	57.1	1.9	
17	6	Mean	298.9	80.2	379.2	21.1	38
		S.E.	25.4	14.1	27.4	3.4	
19	6	Mean	115.0	174.9	289.9	60.3	39
		S.E.	29.5	24.3	34.5	7.7	
21	6	Mean	72.2	246.3	318.6	77.3	42
		S.E.	10.6	16.6	21.9	2.5	
23	6	Mean	94.0	186.9	280.8	66.6	46
		S.E.	13.4	10.3	18.7	3.0	

TABLE 3

DISTRIBUTION OF GRAINS OVER CHROMOSOMES

Hours (after partial hept.)	1 Gr	2 Gr	3 Gr	4 Gr	5 Gr	6 Gr	7 Gr	8 Gr	+	Av. grains per chrom.
13	34.8	7.1	0.9	0	0	0	0	0	0	1.2
15	54.6	9.6	1.4	0.1	0	0	0	0	0	1.2
17	56.0	18.7	4.2	1.1	0.2	0	0	0	0	1.4
19	71.0	53.1	29.7	13.2	4.5	1.4	0	0	0	2.0
21	72.4	74.1	54.5	27.3	12.1	2.9	0.7	0.7	0	2.4
23	53.6	57.6	38.7	15.9	9.7	6.0	1.1	0.1	0.1	2.4

value of 2.4 at both 21 and 23 hours is the result of a few chromosomes with relatively high rates of synthesis (six, seven, and eight grains per chromosome). Thus, the chromosomes appear to reach a maximum rate of synthesis shortly before the completion of replication of DNA in the chromosome.

The suitability of the technique was tested by determining the closeness of the approximation of the frequency distribution of grains per chromosome in the same sample population with a Poisson series (Fig. 4). A logarithmic plot should give a straight line if the frequency distribution of the samples approximates a Poisson series. The closeness of the observed frequency to a straight line is shown by curve *B*. This suggests that the techniques were satisfactory for obtaining adequate samples for assessing the change in the rate of DNA synthesis in individual chromosomes.

Discussion.—Liver regeneration occurs after removal of a liver segment. The original liver weight and cell number are restored within three weeks after the removal of two thirds of the organ.[5] Exponential equations first utilized by Sir D'Arcy Thompson for the description of growth in general were similar to the equations utilized to describe the change in the rate of DNA replication at different

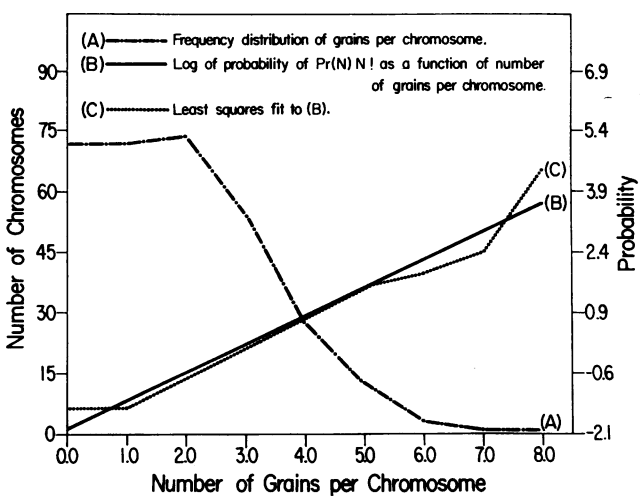

FIG. 4.—The frequency distribution of the number of grains per chromosome at 21 hr after partial hepatectomy is shown in curve (*A*). The logarithm of the Poisson equation for the frequency distribution of grain counts is shown in curve (*B*). The least-squares fit is shown in curve (*C*).

organizational levels in this and previous studies.[1, 6] It is demonstrated in the current study that the time and magnitude of the changing rates of DNA synthesis at the four organizational levels are similar. Although the nature of the stimulus for initiation of DNA synthesis in liver cells following partial hepatectomy is unknown, the number of cells entering DNA synthesis increases in an exponential manner and then decreases. The equation is as follows:

$$y = b_0 + b_1{}^t + b_2 e^{-\left(\frac{t-22}{4}\right)^2},$$

where y = cells in synthesis at any time "t"; b_0, b_1, and b_2 are constants equal to -8.9, 0.81, and 11.8, respectively; t = time in hours, and e = base of natural logarithm. The rate of DNA synthesis in individual cells, determined autoradiographically during their eight-hour period of replication, can also be expressed as an exponential equation. This is, in all probability, a reflection of an exponential increase in both the number of chromosomes synthesizing DNA and the rate of synthesis in individual chromosomes.

Shea[7] also found that exponential growth could be deduced from the data of Grisham[8] on DNA replication in regenerating liver during the first 20 hours after partial hepatectomy. Shea also found from his results that the growth rate decays exponentially between 27.5 and 79.5 hours postoperatively.

The information obtained on the replication of DNA in the rat chromosome has been used in an attempt to determine the correlation, if any, between DNA replication in higher and lower organisms. Cairns[9] has suggested that a universal replication rate for DNA may exist. If we assume that the rate of DNA replication at the molecular level is invariant for all organisms, it does seem possible to reconcile the times of replication of DNA in viruses, bacteria, and mammals, in spite of the large differences in DNA contents. The 2×10^{-16} gm of DNA in bacteriophage is replicated in one to two minutes. The DNA content of diploid liver cells of the rat (6.5×10^{-12} gm) is on the order of 650 times the DNA content of a bacterium (*E. coli*, 1×10^{-14} gm).[10] The time for replication of the DNA content of the rat is eight hours, or on the order of 12 times the period of DNA, replication in bacteria (40 min).[11] It becomes apparent that the DNA content of the 42 rat chromosomes can replicate at the same rate at the molecular level as *E. coli* and bacteriophage, if multiple sites in each chromosome are replicating DNA.

In spite of the changes in length from early to late metaphase, the largest rat chromosome was between 6.5–6.8 times greater in length than the smallest chromosome. Since correlation has also been shown between total DNA content and chromosome length in mammalian chromosomes,[12, 13] the DNA content of the largest rat chromosome was calculated to be 39×10^{-14} gm, and the DNA content of the smallest chromosome was calculated to be 6×10^{-14} gm. If all of the DNA in the chromosome were one DNA molecule, the smallest chromosome would have a molecular weight of 3.6×10^{10}. This is an order of magnitude greater than the molecular weight of 6×10^9 for *E. coli*, and 2 orders of magnitude greater than the molecular weight of 1.2×10^8 for T2 phage.

If we assume that the smallest rat chromosome is one DNA molecule, then it would take 600 minutes to synthesize the DNA at a rate equal to 1×10^{-16} gm of DNA per minute. This study has shown that all chromosomes do not replicate

throughout the eight-hour replication period. The increase by a factor of 2 in the mean rate of DNA synthesis in individual chromosomes during the time of replication would suggest either an increase in DNA synthetic rate at the molecular level or an increase in the number of sites of synthesis during the replication of the DNA.[14] If we assume that each chromosome has two molecules of DNA of 3×10^{-14} gm, then the time for replication would be 300 minutes provided that both molecules synthesized DNA simultaneously at a rate of 1×10^{-16} gm per minute. The experimental evidence of the exponential rate of increase from this study suggests that an intermediate pattern between simultaneous and sequential replication of the two molecules would occur. This would give a replication time intermediate between 300–600 minutes, which is within the experimentally determined limits for the replication time of the total DNA content of the cell of 480 minutes.

Estimates of the replication time for the largest chromosome with 39×10^{-14} gm of DNA would be 3900 minutes, based on the assumption of one large molecule synthesizing at a rate of 1×10^{-16} gm per minute. This is evidently not possible, since the total replication time for the DNA of the cell is 480 minutes. However, if we assume that the largest chromosome has 13 molecules, the same molecular weight as assumed for the smallest chromosome (3×10^{-14} gm), then the replication time would be within the experimentally determined time for the total DNA content of the cell.

It is assumed that if 3×10^{-14} gm of DNA is a basic unit of DNA in rat liver, a maximum of 200 sites of this magnitude would be available in the whole nucleus, with 6.5×10^{-12} gm of DNA. There would be approximately 20 of the possible 200 sites actively synthesizing DNA at 13 hours after partial hepatectomy, based on the per cent labeled chromosome results. The number would increase to 40 at 17 hours, with a maximum of 150 of the possible 200 sites reached at 21 hours after partial hepatectomy.

Summary and Conclusions.—The rate of increase in DNA synthesis in the total liver could be described by an exponential equation between the onset of synthesis and the maximum rate of synthesis at 21 hours after partial hepatectomy. Changes in rate of DNA synthesis in individual cells and individual chromosomes could also be described by an exponential equation during the same time period. These exponential changes in rate at the higher organizational levels can be explained by increasing numbers of sites of DNA replication in the different biological units. These findings would be consistent with the assumption that DNA synthesis at the molecular level could proceed at a linear rate on the order of 1×10^{-16} gm of DNA per minute.

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