THE REPLICATION TIME AND PATTERN OF CARCINOGEN—INDUCED HEPATOMA CELLS

JOSEPH POST, M.D., and JOSEPH HOFFMAN, M.D.
With the technical assistance of TANYA MIHEYEV and ARLINE SASLOW

From the New York University Research Service, Goldwater Memorial Hospital, New York

ABSTRACT

The replication time and pattern have been investigated in hepatoma cells induced by feeding 3'Me-DAB to male rats for 5 months. With the use of tritiated thymidine as a DNA label along with autoradiography, mitotic nuclear labeling has been studied 0.5 to 72 hours after the administration of the label. The following time intervals have been estimated: replication time, 31 hours; DNA synthesis, 17 hours; G_2 plus Mitosis, 2 hours; G_1, 12 hours. Only about 8 per cent of the tumor cell (interphase) population is "flash" labeled, following a single dose of 50 μc of H^3TDR. This group of cells has been followed through three cycles of division. The repeated rhythmic passage of tumor cells through cell division is similar to that previously reported for normal liver cells in the growing rat. However, tumor cells have longer replication and DNA synthesis times. In addition, the several time intervals studied vary more in the tumor cell population than they do in the growing normal cell population.

INTRODUCTION

Inasmuch as the uncontrolled proliferation of tumor cells is their most important characteristic, it is of interest to investigate their patterns of multiplication. The present report is concerned with the replication time and pattern of carcinogen-induced hepatoma cells in the liver of the 6-month-old male rat. This autogenous tumor was selected because it simulates the growth *in vivo* of a human cancer. The replication time, its component intervals, and the rhythm of cell division have been studied in these tumor cells. The data have been compared with those previously reported, from a rapidly growing population of normal liver cells (24). The results show that hepatoma cell multiplication follows a relatively orderly rhythm, similar to that of normal cells in the growing liver. On the other hand, striking differences have been found between these cell populations, with regard to the replication times and their respective component intervals.

METHODS

Three-week-old male rats of the Wistar strain were fed *ad libitum* a diet of Purina checkers containing 0.08 per cent 3'-methyl-4-dimethylaminoazobenzene (3'Me-DAB) dissolved in corn oil. After animals have been fed this diet for about 5 months their livers show extensive infiltration by hepatoma and cholangiocarcinoma. In addition, metastases to omentum, diaphragm, and lung are observed (5). Most of the animals die during the 5th and 6th months with cachexia and almost complete replacement of liver tissue by tumor and cysts. Livers may enlarge to 60

per cent of the total body weight. Accordingly, after 5 months of 3'Me-DAB feeding, rats weighing 245 to 313 grams (average 282) were injected intraperitoneally with 50 μc of tritiated thymidine (H^3TDR), specific activity 0.36 c per mmole, contained in 1 ml of distilled water. At intervals of 0.5 to 72 hours later, pairs of animals were killed. At least 4 different hepatoma-bearing regions of each liver were sampled. The tissues were fixed in Carnoy's fluid and 5-μ sections were stained by the Feulgen technique. Strips of Kodak AR 10 film were applied in the dark and the slides were stored for 30 days in the cold, after which time they were developed.

The sections, which measured about 20 to 30 mm^2, were examined under oil immersion to determine the percentage of the four mitotic stages. At least 100 tumor cell mitoses were scored per animal. These were counted in tissues which showed well labeled tumor interphase nuclei and which were free of necrosis. In those animals sacrificed at 0.5, 2, 4, and 6 hours after H^3TDR administration the percentages of interphase nuclear labeling and of mitoses were determined from the random scoring of over 5,000 hepatoma cells per animal.

The estimation of the replication time and of its component intervals may be made from the time-phase waxing and waning of mitotic nuclear labeling of 2 successive cycles of cell division (24, 26). The cells are "flash" labeled during interphase, following the injection of H^3TDR. Each labeled mitosis is derived from a labeled interphase nucleus. If the curves of mitotic labeling for two successive cycles are similar and symmetrical, or nearly so, the replication time may be estimated from the difference between 2 points comparably placed on each of the cycle curves. These may be chosen at the initiation of labeling, at the respective times of 50 per cent of population labeling, and at the peaks of labeling. This method of analysis may be employed if the initially labeled interphase nuclei divide and remain in phase in each successive division cycle, so that the labeled cells in each cycle are derived from the labeled cells of the preceding cycle. In the present studies the replication time was estimated from the interval between the initiations of labeling in the 2 cycles of prophase nuclear labeling (24). This mitotic stage was chosen because the numbers of grains over these nuclei were usually greater than those found over the nuclei of other mitotic stages. In addition, the labeling curve for the prophase mitoses was smoother than those for the other phases of mitosis.

The average time for deoxyribonucleic acid (DNA)

TABLE I

Summary of Nuclear Labeling of Hepatoma Cells

Hours after H^3TDR	No. nuclei scored	Lab Int / 100 Int	Per cent Mit	Lab Mit / 100 Mit	Pro / 100 Mit	Lab Pro / 100 Pro	Met / 100 Mit	Lab Met / 100 Met	Ana / 100 Mit	Lab Ana / 100 Ana	Tel / 100 Mit	Lab Tel / 100 Tel
0.5	287	8.1	2.8	3.3	54.0	4.9	33.6	2.0	8.6	0	4.0	0
1	305	5.8	2.0	8.7	35.1	6.6	50.6	11.0	10.7	8.8	4.4	0
2	337	8.3	2.8	54.0	27.3	45.7	61.7	60.1	8.6	51.7	2.4	0
4	540	9.4	3.2	86.4	16.0	83.3	45.2	93.4	25.3	80.3	13.6	78.1
6	621	7.7	2.6	87.2	34.2	80.0	59.2	92.5	3.9	84.6	2.7	66.7
9	285	—	—	73.3	23.5	77.6	61.4	76.0	9.8	57.1	5.3	53.3
12	229	—	—	55.0	17.0	56.4	71.6	56.1	8.7	50.0	2.6	33.3
14	293	—	—	54.6	20.5	58.3	58.0	56.5	16.4	47.9	5.1	40.0
20	318	—	—	53.1	24.2	44.2	59.1	58.5	14.5	50.0	2.2	28.6
24	274	—	—	41.6	24.4	30.3	66.1	45.3	6.6	44.4	3.3	44.4
28	223	—	—	14.4	39.9	14.6	50.7	15.9	7.2	6.3	7.2	0
32	344	—	—	21.9	31.6	20.4	56.1	24.1	9.7	20.0	2.6	0
36	291	—	—	50.6	39.9	46.5	52.8	55.3	3.4	33.3	3.4	50.0
40	205	—	—	50.9	39.0	50.0	42.4	54.0	10.2	58.3	8.5	30.0
44	381	—	—	53.0	29.3	54.7	58.0	53.3	6.1	45.5	6.6	50.0
48	212	—	—	43.9	26.9	35.1	58.5	47.6	11.8	52.0	2.8	20.0
72	192	—	—	—	30.7	—	58.3	—	7.8	—	3.1	—
Mean		7.5	2.7	—	31.7	—	52.0	—	11.2	—	5.4	—

Lab, labeled; Int, interphase; Mit, mitoses; Pro, prophase; Met, metaphase; Ana, anaphase; Tel, telophase.

synthesis was estimated from the interval between the times of one-half of population labeling on the ascending and descending limbs of the prophase labeling curve of the first cycle. The average time for post-DNA synthesis (G_2) plus mitosis may be approximated from the time required for one-half of the total mitotic nuclear population to become labeled. The estimations of the intervals for G_2 and for each stage of mitosis were not made from these data because of the marked variability of the time sequences of labeling of the different stages of mitosis. The time for the postmitotic gap (G_1) was calculated by subtracting, from the total replication time, the sum of the times for G_2 + mitosis and for DNA synthesis. Grain counts have been made of randomly selected interphase nuclei at 4 and 6 hours, 36 and 40 hours, and at 72 hours after injection of H^3TDR. The number of grains over each labeled nucleus was always many times that over background, in an area equal to that of the nucleus being scored. For each animal studied, the grain counts for 100 or more labeled hepatoma interphase nuclei have been made. Interphase grain counts were preferred to mitotic nuclear grain counts to determine cell line continuity, because the former numbers were larger.

RESULTS

Within 0.5 hour, 8.1 per cent of the tumor interphase nuclei are labeled and this percentage level is maintained during the first 6 hours. By 1 hour, significant labeling of prophase, metaphase, and anaphase nuclei occurs (Table I, Fig. 1 *a* and *b*). At 4 hours the respective labeling percentages reach their peaks and by 12 hours have begun to decline (Fig. 2). They reach their lowest points

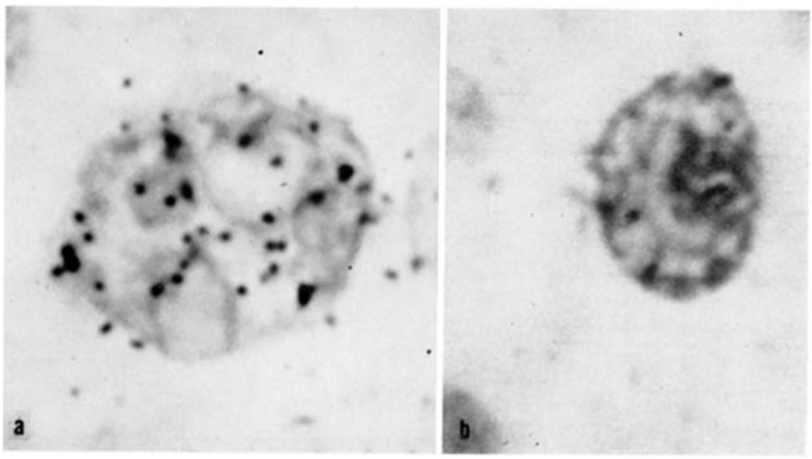

FIGURE 1 *a* Labeled hepatoma interphase nucleus. × 1125.

FIGURE 1 *b* Labeled hepatoma prophase nucleus. × 1125.

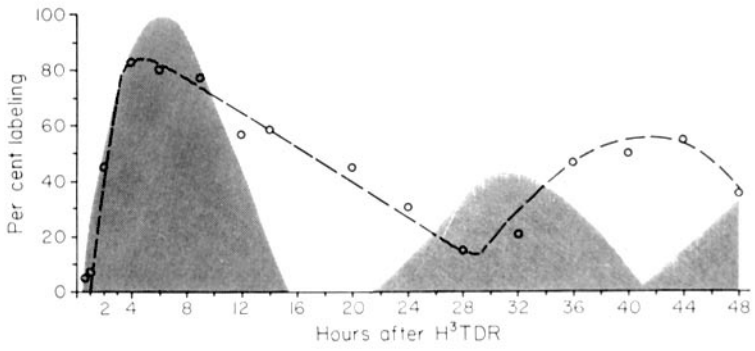

FIGURE 2 Labeling of prophase mitoses of hepatoma cells and of normal liver cells (shaded areas) of growing rat (24), during 48 hours after H^3TDR administration.

TABLE II

Grain Counts of Interphase Hepatoma Cells

Hours after H^3TDR	No. rats	No. nuclei examined	Mean grain count*	Prob. error of mean	Background grain count
4 and 6	4	500	12.5	0.1	<1
36 and 40	4	410	6.9	0.1	0
72	2	200	4.7	0.2	0

* The mean grain counts of all groups differ significantly from each other.

at 28 hours, and begin to rise again at 32 hours and the pattern is repeated. By 48 hours, the per cent of labeling in the second cycle is in its descent. At 72 hours, mitotic labeling is too light for accurate estimates to be made.

The curve for prophase labeling (Fig.2) has been used for the estimation of the replication time. Ideally, the curve for each cycle should be symmetrical and identical. Distortion of symmetry might be expected if there were variations in the time intervals among the cells. There is skewing in the descending limb of the first cycle, and the second cycle is markedly widened. The highest level of the first cycle portion of the curve is between 80 and 90 per cent labeling and that of the second part is at about 50 per cent labeling. It is likely that these values would reach 100 per cent were it not for the "loss" of the lightly labeled cells in the autoradiograph. This loss would be expected to occur to a greater degree in the second cycle, when labeled DNA has been diluted with unlabeled DNA.

The interval between the beginning of labeling in the first cycle (1 hour) and that in the second cycle (32 hours) is 31 hours. The time period between one-half labeling of the ascending limb of the first cycle (42 per cent, at about 2 hours) and that of the second cycle (25 per cent, at about 32 hours) is about 30 hours. Since the peaks of the curve are not sharply defined, the time interval between them is not accurately estimated. The replication time of 31 hours, calculated from the interval between the initiations of prophase labeling in the two cycles, has been used in this paper.

The proof of cell continuity from one cycle to the next cannot be established in this type of *in vivo* experimental design. Cinematographic ob-

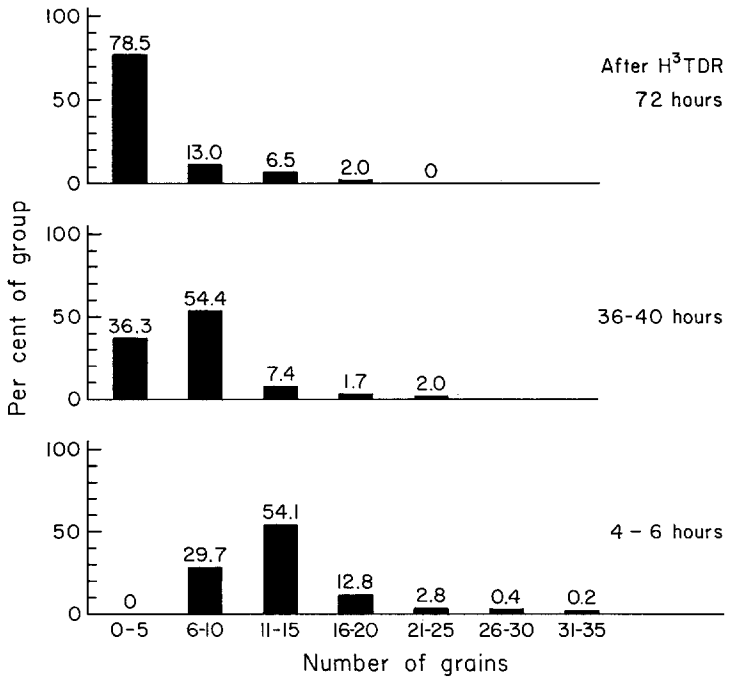

FIGURE 3 Frequency distribution of grain counts of hepatoma cells (interphase) at several intervals after H^3TDR administration.

servation is the best technique for obtaining such proof and obviously this is not adaptable to the present experiment. An alternate method, although not definitive, is to study the grain counts of the cell populations of successive cycles. Theoretically, when a labeled interphase nucleus divides, its daughter nuclei should each have one-half of the original grains. The observed grain counts (Table II, Fig. 3) in each group are probably weighted on the high side, because many of the lightly labeled nuclei are lost in the autoradiograph. The data show a significant reduction in the interphase nuclear grain counts between 4 and 6 hours (first cycle) and 36 and 40 hours (second cycle). These findings offer supporting evidence that the labeled cells in the second cycle are derived from those in the first cycle. The further significant decrease in grain count at 72 hours, as compared with the 36 to 40 hour level, suggests that a third cycle has occurred. Beyond 72 hours the grain counts are too light to be employed. The rising and falling values of mitotic labeling indicate that this group of labeled cells is passing through cell division cycles, alternating with non-labeled tumor cells. The occurrence of the low points of labeling for each stage of mitosis at the same time, 28 hours, indicates that the labeled tumor cell population is dividing in phase.

The average time for DNA synthesis calculated from the 50 per cent labeling time on the ascending and descending limbs of the first cycle is about 17 hours. There is a wide range of time for this interval as evidenced by the skewed curve. The time for G_2 + mitosis, estimated from the time of 50 per cent labeling of the total mitotic population, is about 2 hours (Fig. 4). Of interest is the absence of the time lag between the labelings of the successive mitotic stages. Metaphase labeling appears to proceed more rapidly than does prophase nuclear labeling (Table I). Anaphase nuclear labeling seems to progress as rapidly as does prophase labeling. These paradoxical findings may be explained if there were marked variations among the tumor cells with regard to these time intervals. The estimation of G_1 is 12 hours. All of these intervals are approximations.

It is noteworthy that where hepatoma cell metastases are studied, at 4, 9, and 20 hours after H^3TDR, the percentages of labeling of prophase and metaphase mitoses are similar to those found

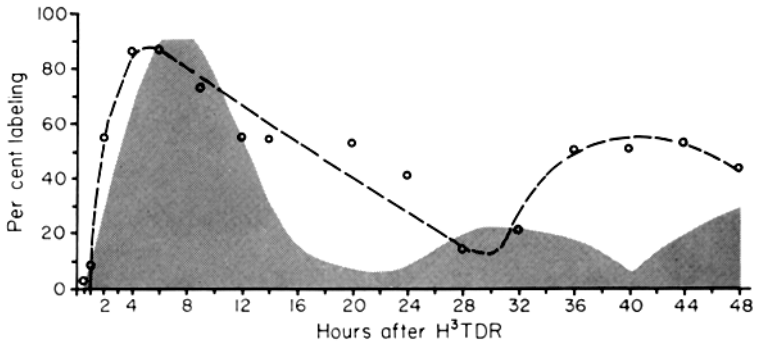

FIGURE 4 Total mitotic labeling of hepatoma cells and of normal liver cells (shaded areas) of growing rat (24), during 48 hours after H^3TDR administration.

TABLE III

Labeling of Intrahepatic and Peritoneal Metastatic Hepatoma Cells

Hours after H^3TDR	Intrahepatic				Metastatic			
	Lab Pro / 100 Pro	Lab Met / 100 Met	Lab Ana / 100 Ana	Lab Tel / 100 Tel	Lab Pro / 100 Pro	Lab Met / 100 Met	Lab Ana / 100 Ana	Lab Tel / 100 Tel
4	83.3	93.4	80.3	78.1	100.0	94.3	100.0	100.0
9	77.6	76.0	57.1	53.3	75.0	90.0	100.0	100.0
20	44.2	58.5	50.0	28.6	69.2	71.1	87.5	100.0

TABLE IV

Replication Times of Mammalian Normal and Tumor Cells

Cell Type		Replication	DNA synthesis	G_2	Mitosis	G_1	Reference
		(*hours*)					
Normal cells							
Human normoblast	*in vivo*	15–18.0	9.0	3.0	—	3.0–6.0	2
" neutrophile precursor	" "	48.0	—	—	—	—	9
" colon mucosa	" "	24.0	11.0–15.0	G_2 + Mitosis = 1.0–3.0	—	10.0+	15
Rat (growing) liver cell*	" "	21.5	9.0	G_2 + Mitosis = about 3.5*		9.0	24
" (adult) " " (posthepatectomy regeneration)	" "	—	8.0	—	—	—	16
" tibial metaphysis	" "	36.0	8.0	1.0–1.5	—	—	32
" " endosteum	" "	57.0	8.0	1.0–1.5	—	—	32
" " periosteum	" "	114.0	8.0	1.5–2.0	—	—	32
" primary spermatocyte	" "	—	14.0	—	—	—	20
Mouse ear epidermis	" "	24.4 days	30.0	4.6	3.8	>22 days	28
" duodenal mucosa	" "	11.5	—	—	1.38	—	4
" jejunal "	" "	18.75	7.5	G_2 + Mitosis = 1.5		9.5	26
" colon "	" "	16.0	6.5	G_2 + Mitosis = 1.5		8.0	14
" hair follicle	" "	13.0	7.5	—	—	—	17
Dog myelocyte	" "	16.0–18.0	—	—	—	—	22
" promyelocyte	" "	9.0	—	—	—	—	22
" myeloblast	" "	9.0	—	—	—	—	22
Hamster pouch lining cell	" "	—	8.0–12.0	2.0–3.0	—	—	27

Fetal calf liver cell	*in vitro*	31.0	8.0	6.0	1.0	16.0	13
Hamster fibroblast	" "	11.0	5.8	G_2 + Mitosis = 2.5		2.7	8
" connective tissue cell	" "	14.0	6.0	2.5	0.5	5.0	30
Kitten lung cell	" "	18.3	DNA_s + G_2 + Prophase + Metaphase = 12.0			Anaphase + Telophase + G_1 = 6.3	29
		23.6	" + " + " + "		= 11.7	" " " = 11.9	29
Human amniotic cell	" "	18.2	" + " + " + "		= 10.9	" " " = 7.3	29
		21.4	" + " + " + "		= 11.1	" " " = 10.3	29
Tumor cells							
Human leukemic myeloblast	*in vivo*	50.0–60.0	—	—	—	—	9
Human multiple myeloma	" "	2.0–6.0 days	—	—	—	—	10
Rat hepatoma cell	" "	31.0	17.0	G_2 + Mitosis = 2.0		12.0	this paper
Mouse Ehrlich ascites cell	" "	20.0	8.0	—	—	—	11
" " " "	" "	18.0	8.5	1.5	5.1	3.0	3
" " " "	" "	18.0	11.0	2.0	Prophase = 5.0	0	1
					Metaphase = 1.0		1
" " " "	" "	18.0±	12.0	6.0	0.5	0	6
" mammary carcinoma transplanted	" "	1.0–3.5 days	9.0–13.0	1.0–4.0	—	—	18
Hamster pouch carcinoma	" "	—	6.0	2.0	—	—	27
Hela cell	*in vitro*	30.5	—	—	—	—	7
" "	" "	28.0	8.5	3.0–10.0	—	—	23

* Estimated from the curve of total mitotic labeling (24).

in hepatoma cells within the liver substance, at the same respective times after H^3TDR (Table III). The marked discrepancy in telophase labeling is unexplained, but might reflect variations among the tumor cell population. Relatively few telophase mitoses were scored.

DISCUSSION

The data show that the hepatoma cells multiply in a relatively orderly fashion. A group of cells has been followed through two division cycles. If the replication time of about 31 hours is projected, the decrease in the grain count of the interphase nuclei at 72 hours indicates that a third cycle of division is occurring in this group of labeled cells.

It is of interest to compare the data from this proliferating tumor cell population with those from a growing normal cell population (24) (Table IV, Figs. 2 and 4). The normal cells have a shorter replication time, 21.5 hours. Indeed, in normal cells 2 cycles and the beginning of a third are seen during the time when 2 cycles occur in the tumor cells. Most of the difference is due to the shorter DNA synthesis time of the normal cells, 9 hours, compared with that for hepatoma cells, 17 hours. Since hepatoma cells are relatively large, aneuploid, and contain greater amounts of DNA than do normal cells (5, 12), it may be that more time is required to synthesize more material. Finally, there is relative uniformity of the time intervals in the normal cell population, as evidenced by the fairly symmetrical curve of prophase labeling and the orderly time lag of mitotic labeling from prophase to telophase. This is in sharp contrast with the variations of these times among the hepatoma cells, manifested in the marked skewing of the prophase labeling curve, and the absence of the orderly labeling of the several mitotic stages.

In both groups, at any one time, a relatively small population is synthesizing DNA in preparation for cell division, about 4 per cent of the normal cells (24) and 8 per cent of the tumor cells. The percentages of mitoses are 1.0 and 2.7, respectively, for normal and tumor cells. In each case the estimated proliferating pool is small (26, 11) where:

$$\text{Proliferating pool (per cent of population)} = \frac{\text{(per cent of interphase labeling)} \times \text{(replication time)}}{\text{DNA synthesis time}}$$

These are 10 per cent for the normal cells and 15 per cent for the hepatoma cells.

It is interesting to examine some of the published data relating to the replication times of tumor cells and of normal cells (Table IV). The replication times of the latter vary from 9 hours for the dog myeloblast and promyelocyte (22) to 24.4 days for the mouse epidermis cell (28). Tumor cell replication times have been reported as widely different as 18 to 20 hours for the transplanted Ehrlich ascites tumor cells (1, 3, 6, 11) on the one hand, and 1 to 3.5 days for the transplanted mouse mammary cancer cells (18), on the other. It is obvious from these data that the few tumors studied have not had unusually short replication times, when compared with normal cell lines such as those from bone marrow and intestinal epithelium.

How does one account for the overgrowth of the tumor? In the present studies this may be explained, in part, in terms of the relative percentages of replicating cells in the tumor and in the organ where the tumor is growing. The host animal is the 6-month-old rat, whose liver shows little cell division. It has been found that the per cent of cells synthesizing DNA, (labeled interphase) 2 hours after administration of 50 μc H^3TDR, is less than 0.2 (25). Thus, greater numbers of hepatoma cells are multiplying than of the liver cells of the 6-month-old animal. In the cases of other tumor cells which may have replication times longer than the normal tissues which they invade, other factors probably play significant roles. Thus, tumor cells have the unique property of overgrowing and of destroying normal tissue (31). In addition, there is the tendency for some organs to be the sites of metastases whereas other organs are rarely invaded. Local tissue factors are involved which are not well understood at this time.

The traditional picture of tumor cells growing very rapidly has been questioned, and it has been noted that tumors do not grow so quickly as do fetal tissues or regenerating tissues following injury (21). Many mammary cancer cells are not involved in cell division (19). The present studies show that hepatoma cell multiplication has a fairly regular rhythm, with a relatively small calculated proliferating pool. Furthermore, a particular cohort of cells replicates time after time, without pause. These findings indicate that the multiplication of tumor cells is under control, and is not chaotic. It cannot be determined, from the data, how many times a particular tumor cell line passes

through cell division. Furthermore, it is not known whether the replication time, its component intervals, and the proliferating pool vary during the life of the tumor.

At this time there are insufficient published data relating to autogenous tumors, studied *in vivo*, to know whether the characteristics of multiplication described for the hepatoma cells are shared by other tumors. Such information about tumors is of significance in the problem of their control. Thus, if most of the cells of a given tumor population have longer replication times than do intestinal epithelial and bone marrow cells, the special toxicity of antitumor compounds for these latter normal tissues may be due to the competitive incorporation of the cytotoxins in greater amounts by the more active normal cells. Herein may lie an explanation for the ultimate limitations of the many cytotoxic antimetabolic and alkylating agents in the control of tumor growth *in vivo*. Among the tumor cell population there may be cells with shorter replication times, in relation to bone marrow and gut cells. These tumor cells may be destroyed by the cytotoxins in specific situations of "responsive" tumors.

This work was supported in part by the United States Public Health Service under grants CA 03917-06 and HD 00672-08, in part by the Atomic Energy Commission under grant AT(30-1)-2778, and in part by the Health Research Council of the City of New York under contract U-1089.

Received for publication, October 3, 1963.

BIBLIOGRAPHY

1. Baserga, R., Mitotic cycle of ascites tumor cells, *Arch. Path.*, 1963, **75,** 156.
2. Bond, V. P., Fliedner, T. M., Cronkite, E. P., Rubini, J. R. and Robertson, J. S., Cell turnover in blood and blood-forming tissues studied with tritiated thymidine, *in* The Kinetics of Cellular Proliferation, (F. Stohlman, Jr., editor), New York, Grune and Stratton, 1959, 188.
3. Edwards, J. L., Koch, A. L., Yoncis, P., Freese, H. L., Laite, M. B., and Donalson, J. T. Some characteristics of DNA synthesis and the mitotic cycle in Ehrlich ascites tumor cells, *J. Biophysic. and Biochem. Cytol.*, 1960, **7,** 273.
4. Fry, R. J. M., Lesher, S., and Kohn, H. L., Estimation of time of generation of living cells, *Nature*, 1961, **191,** 290.
5. Hoffman, J., Klein, A., Miheyev, T., and Post, J., Responses of the liver to injury: nuclear DNA changes during the evolution of 3'-methyl-4-dimethylaminoazobenzene hepatomas, *Arch. Path.*, 1961, **71,** 202.
6. Hornsey, S., and Howard, A., Autoradiographic studies with mouse Ehrlich ascites tumor, *Ann. New York Acad. Sc.*, 1956, **63,** 915.
7. Hsu, T. C, Generation time of Hela cells determined from cine records, *Texas Rept. Biol. and Med.*, 1960, **18,** 31.
8. Hsu, T. C., Dewey, W. C., and Humphrey, R. M., Radiosensitivity of cells of chinese hamster *in vitro* in relation to the cell cycle, *Exp. Cell Research*, 1962, **27,** 441.
9. Killmann, S. A., Cronkite, E. P., Robertson, J. S., Fliedner, T. M., and Bond, V. P., Estimation of phases of the life cycle of leukemic cells from *in vivo* labeling in human beings with tritiated thymidine, *Brookhaven Nat. Lab.* 6602, 1961.
10. Killmann, S. A., Cronkite, E. P., Fliedner, T. M., and Bond, V. P., Cell proliferation in multiple myeloma studied with tritiated thymidine *in vivo*, *Lab. Inv.*, 1962, **11,** 845.
11. Kisieleski, W. E., Baserga, R., and Lisco, H., Tritiated thymidine and the study of tumors, *Atompraxis*, 1961, **7,** 81.
12. Koulish, S., and Lessler, M. A., Cytologic aspects of normal and tumorous liver, *Cancer Research*, 1962, **22,** 1188.
13. Kuyper, C. M. A., Smets, L. A., and Pieck, A. C. M., The life cycle of a strain of liver cells cultivated *in vitro*, *Exp. Cell Research*, 1962, **26,** 217.
14. Lipkin, M., and Quastler, H., Cell population kinetics in the colon of the mouse, *J. Clin. Inv.*, 1962, **41,** 141.
15. Lipkin, M., Sherlock, P., and Bell, B. M., Generation time of epithelial cells in the human colon, *Nature*, 1962, **195,** 175.
16. Looney, W. B., The replication of desoxyribonucleic acid in hepatocytes, *Proc. Nat. Acad. Sc.*, 1960, **46,** 690.
17. McCarter, J. A., and Quastler, H., Effect of dimethylbenzanthracene on the cellular proliferation cycle, *Nature*, 1962, **194,** 873.
18. Mendelsohn, M. L., Dohan, F. C., Jr., and Moore, H. A., Jr., Autoradiographic analysis of cell proliferation in spontaneous breast cancer of C_3H Mouse. 1. Typical Cell Cycle and Timing of DNA Synthesis, *J. Nat. Cancer Inst.*, 1960, **25,** 477.
19. Mendelsohn, M. L., Chronic infusion of tritiated

thymidine into mice with tumors, *Science*, 1962, **135,** 213.

20. MONESI, V., Autoradiographic study of DNA synthesis and the cell cycle in spermatozoa and spermatocytes in mouse testis using tritiated thymidine, *J. Cell Biol.* 1962, **14,** 1.
21. OBERLING, C., and BERNHARD, W., The morphology of the cancer cells, *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York, Academic Press, Inc., 1961, **5,** 441.
22. PATT, H. M., and MAHONEY, M. A., Patterns of neutrophilic leukocyte development and distribution, *Ann. New York Acad. Sc.*, 1959, **77,** 766.
23. PAINTER, R. B., and DREW, H. M., Studies on deoxyribonucleic acid metabolism in human cancer cell cultures (Hela) 1. The temporal relationships of deoxyribonucleic acid synthesis to mitosis and turnover time, *Lab. Inv.*, 1959, **8,** 278.
24. POST, J., HUANG, C. Y., and HOFFMAN, J., The Replication time and pattern of the liver cell in the growing rat, *J. Cell Biol.*, 1963, **18,** 1.
25. POST, J., and HOFFMAN, J., Changes in the replication times and patterns of the liver cell during the life of the rat, *Exp. Cell Research*, in press.
26. QUASTLER, H., and SHERMAN, F. G., Cell population kinetics in the intestinal epithelium of the mouse, *Exp. Cell Research*, 1959, **17,** 420.
27. RUSKIN, A. B., and MENDELSOHN, M. L., A comparison of DNA synthesis in induced carcinomata and their normal counterpart, *Proc. Am. Assn. Cancer Research*, 1962, **3,** 353.
28. SHERMAN, F. G., QUASTLER, H., and WIMBER, D. R., Cell population kinetics in the ear epidermis of mice, *Exp. Cell Research*, 1961, **25,** 114.
29. SISKEN, J. E., and KINOSITA, R., Timing of DNA synthesis in the mitotic cycle *in vitro*, *J. Biophysic. and Biochem. Cytol.*, 1961, **9,** 509.
30. TAYLOR, J. H., Nucleic acid synthesis in relation to the cell division cycle, *Ann. New York Acad. Sc.*, 1960, **90,** 409.
31. WOLFF, E., La culture des cellules tumorales sur des explants d'organes *in vitro*, *Experimentia*, 1956, **12,** 321.
32. YOUNG, R. W., Regional differences in cell generation time in growing rat tibia, *Exp. Cell Research*, 1962, **26,** 562.