THE KINETICS OF CELLULAR PROLIFERATION IN REGENERATING LIVER

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ABSTRACT

The study concerns the kinetics of cellular proliferation in the different cell populations of the normal and regenerating rat liver. A detailed analysis is presented, which includes techniques of in vivo labeling of DNA with tritiated thymidine and high-resolution radioautography, of the temporal and spatial patterns of DNA synthesis and cell division in the parenchymal cells, littoral cells, bile duct epithelium, and other cellular components in the liver during the first 64 hr of regeneration after partial hepatectomy. The analysis of cell population kinetics indicates that (*a*) the rate of entry of parenchymal cells into synthesis, after an initial burst of proliferative activity, was an orderly progression at 3–4%/hr; (*b*) most cells divided once and a few twice, a large proportion of the cell deficit being replaced by 72 hr after the onset of proliferation; (*c*) T_s was ~8.0 hr; $T_{g_2+m/2}$, 3.0 hr; and M, ~1.0 hr. Littoral cell proliferation began about 24 hr after the onset of parenchymal cell proliferation; the rate of entry of littoral cells into synthesis was greater than 4%/hr. Interlobular bile duct cell proliferation lagged well behind the parenchymal and littoral cell populations both in time and extent of proliferation.

INTRODUCTION

The mammalian liver is a conditional cell renewal system which ordinarily undergoes little proliferation, but may do so in response to a demand situation to compensate for a loss of cells, for example, after partial hepatectomy. While a considerable amount is known about the biochemical events in the regenerating liver (3, 13, 30, 31), the complex cellular kinetics of the controlled compensatory growth are not, as yet, elucidated. In particular, the rapid changes in the patterns of DNA synthesis and cell division in the different cell populations which are constantly altering the relevant kinetic parameters, such as proliferation rates, transit rates, and compartment sizes, have not been fully investigated. The present research is a radioautographic study of DNA synthesis and the cell cycle in the regenerating rat liver and is concerned with the analysis of cell population kinetics[1] (15, 24) during the period of

[1] The following abbreviations are used in analysis of cell population kinetics (24): C, cells in cell cycle; M, cells in mitosis (M period); S, cells in DNA synthesis (S period); G_1, cells in presynthetic period; G_2, cells in postsynthetic period; G_0, potentially proliferative cells; Tr, transitional and mature cells; N, number of cells in the population; N_t, number of cells in the population at time t; N_m, number of cells in the population in mitosis; N_s, number of cells in the population in DNA synthesis; T_c, duration of cell cycle; T_m, duration of M period; T_s, duration of S period; T_{g_1}, duration of G_1 period; T_{g_2}, duration of G_2 period; $T_{g_2+m/2}$, duration of (G_2 + M/2) complex period; MI, mitotic index (N_m/N), LI, labeling index (N_s/N).

rapid cell proliferation following two-thirds partial hepatectomy. By using techniques of in vivo labeling with tritiated thymidine (TdR-^{3}H), a specific DNA precursor, combined with high-resolution radioautography, we made a detailed investigation of the kinetics of cellular proliferation of the parenchymal cells, littoral cells, bile duct epithelium, and other cell populations of the resting liver and during the first 64 hr after partial hepatectomy of the regenerating liver in order to (*a*) examine their temporal and spatial patterns of proliferation and (*b*) evaluate the dynamic contributions of the cell populations taking part in the regenerative process leading to restoration of the hepatic tissue deficit.

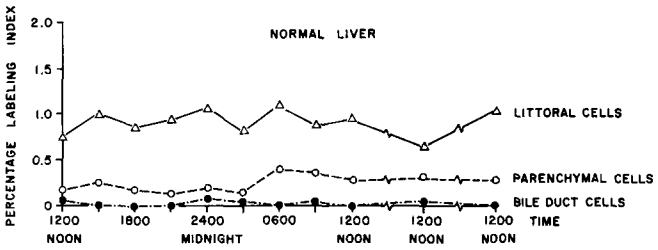

FIGURE 1 DNA synthesis (percentage labeling indices) in the proliferating parenchymal, littoral and bile duct cell populations in the August rat liver at various times during the day. Mean values for four rats per interval.

MATERIALS AND METHODS

Animals

6–8-wk old August strain male rats weighing 90–110 g and bred in the Department of Biophysics, Institute of Cancer Research (Surrey Branch, England) were used in all experiments. They were housed in groups of five and were fed food (Medical Research Council. Diet No. 41 B) and water ad libitum. The number of rats and intervals of sacrifice are described for each study.

Partial Hepatectomy

The technique for partial hepatectomy (14) consisted of the surgical removal of the median and left lateral lobes. Liver-weight determinations were made on 10 rats, and 65.2% of the liver was removed. Light ether anesthesia was used, and all operations were performed between 9:00 a.m. and 12:00 p.m.

Histological Preparations

All animals were sacrificed by heavy ether anesthesia and cervical dislocation. Since the proliferating cell populations in the resting and regenerating liver are distributed in similar patterns throughout the lobes of the liver or liver remnant in each case (9), the right lobe of the liver was considered representative and used for all quantitative histological studies unless stated otherwise. Liver tissue (1–2 g) was fixed for 24 hr (20 parts 70% ethanol, two parts 10% formalin, $CaCO_3$ in excess, and one part glacial acetic acid). Histological sections (4–5 μ thick) were obtained from wax-embedded tissue and stained with hematoxylin and eosin after radioautography.

Radioautography

The liquid emulsion-dipping method for high-resolution radioautography was used. Tissue sections prepared on chemically clean gelatin–alum–coated microscopic slides were dipped in Ilford K-5 Nuclear (Ilford, Ltd., Ilford, Essex, Eng.) emulsion (50% emulsion, 50% distilled water at 50°C), dried, and stored with desiccant in light-tight boxes at 4°C. The exposure time for the identification of heavily labeled cells was 3 wk, and for grain counting and labeled mitoses studies, 2 wk. Slides were developed in Kodak D19b solution (Kodak, Ltd., London, Eng.) fixed for 5 min in Johnson FixSol (Johnson's of Hendon, Ltd., London, Eng.) solution at 16°C, washed, dried, stained, and mounted.

Tritiated Thymidine

TdR-^{3}H, specific activity 10–15 c per mmole (Radiochemical Centre, Amersham, Eng.), in 100 μc/ml solution, was injected intraperitoneally, 0.5 μc/g body weight. This dose produced good radioautographs within the reasonably short exposure time. The animals were sacrificed 1 hr following TdR-^{3}H for the temporal and spatial studies on cell proliferation in normal and regenerating liver, and at designated intervals following pulse labeling for the labeled mitoses data experiment, unless stated otherwise.

Cell Proliferation in the Liver

The lobule was considered the morphological and functional unit of the liver, and all labeled and unlabeled parenchymal and littoral cells were counted in representative cross-sections of intact liver lobules. Labeling and mitotic indices were determined as a

percentage of all nuclei of cells of that population in the lobule. Counts of 5000–7500 parenchymal and littoral cell nuclei and of all interlobular bile duct cells, mesothelial lining cells, vascular endothelium, and other connective tissue cells in a section were recorded. Radioautographs were selected for low background, and in slides with background counts of less than 1–2 grains per nucleus, parenchymal cell nuclei with more than 10 grains, and nonparenchymal cell nuclei which were smaller, with more than 5 grains were scored as labeled. These values were chosen arbitrarily, but the lower limits did not seriously affect interpretation, since most cells scored were heavily labeled.

Studies on Resting Liver

The distribution of the different cell populations in the normal liver was examined in 44 rats, four rats per group, at 3-hr intervals for 24 hr, and at noon on 2 consecutive days. Percentage labeling indices, mitotic indices, and mean grain counts of parenchymal, littoral and bile duct cells, and a miscellaneous group of nonparenchymal cells, were determined.

Studies on Regenerating Liver

The temporal and spatial changes in the patterns of DNA synthesis and mitosis in the parenchymal and littoral cell populations after partial hepatectomy were examined in 92 rats at very frequent intervals during the initial 64 hr of regeneration, i.e., the period of greatest proliferative activity. Animals were partially hepatectomized, and sacrificed in groups of four 1 hr after TdR-^{3}H labeling at 2-hr intervals from 8–40 hr, and then at 4-hr intervals to 64 hr, thereafter. Percentage labeling and mitotic indices in the parenchymal, littoral, and bile duct cell populations were determined.

Studies on the Phases of Proliferating Parenchymal Cells During Regeneration

The percentage labeled mitoses technique involves pulse labeling a population of proliferating cells during DNA synthesis, and following them through the cell cycle. The cell cycle consists of at least four phases: M, G_1, S, and G_2. In the regenerating liver, the system may contain a G_0 population of potentially proliferative cells. 30 rats were partially hepatectomized, injected with TdR-^{3}H 20 hr later, and sacrificed in pairs at frequent intervals from 1 to 44 hr thereafter. A minimum of 100 mitoses were counted for each interval, and the percentage of mitoses labeled (5 grains or more) with time was scored. T_{g_2}, T_m, and T_s were measured according to the method of Quastler and Sherman (25). These data are reported in detail separately (10).

RESULTS

Resting Liver

The distribution of labeled nuclei of the different cell populations of the liver is presented in Table I. Fig. 1 shows the temporal pattern of labeling of parenchymal cells, littoral cells, and bile duct epithelium. All labeled parenchymal and littoral cells were distributed randomly throughout the liver and within the individual lobes and lobules. No appreciable diurnal variation was noted in the labeling patterns of the different cell populations. The mean percentage labeling indices agreed with results of previously reported studies (6, 7, 12, 20, 26). Parenchymal cell percentage labeling indices and mean grain counts were approximately the same during a 24 hr period. Littoral cells were most frequently labeled and synthesized more than half of the DNA in the liver; grain counts varied greatly and were less than over hepatocytes. Bile duct cells were rarely labeled, and often no labeled cells were seen in numerous sections from the same liver.

TABLE I

Distribution of Labeled Nuclei in the Rat Liver

Cell type	Percentage of all cells	Percentage of nuclei labeled (range)	Percentage of all labeled nuclei	Mean grain count (±SE)
Parenchymal	60.2	0.3 (0.2–0.4)	34.1	44.6 ± 14.5
Littoral	35.8	0.9 (0.7–1.2)	60.7	35.3 ± 7.8
Bile ductal	1.1	0.02 (0.0–0.1)	0.2	33.2 ± 6.9
Miscellaneous	2.9	0.8 (0.5–1.0)	5.0	

Regenerating Liver

TEMPORAL PATTERN OF PARENCHYMAL CELL PROLIFERATION: The temporal pattern

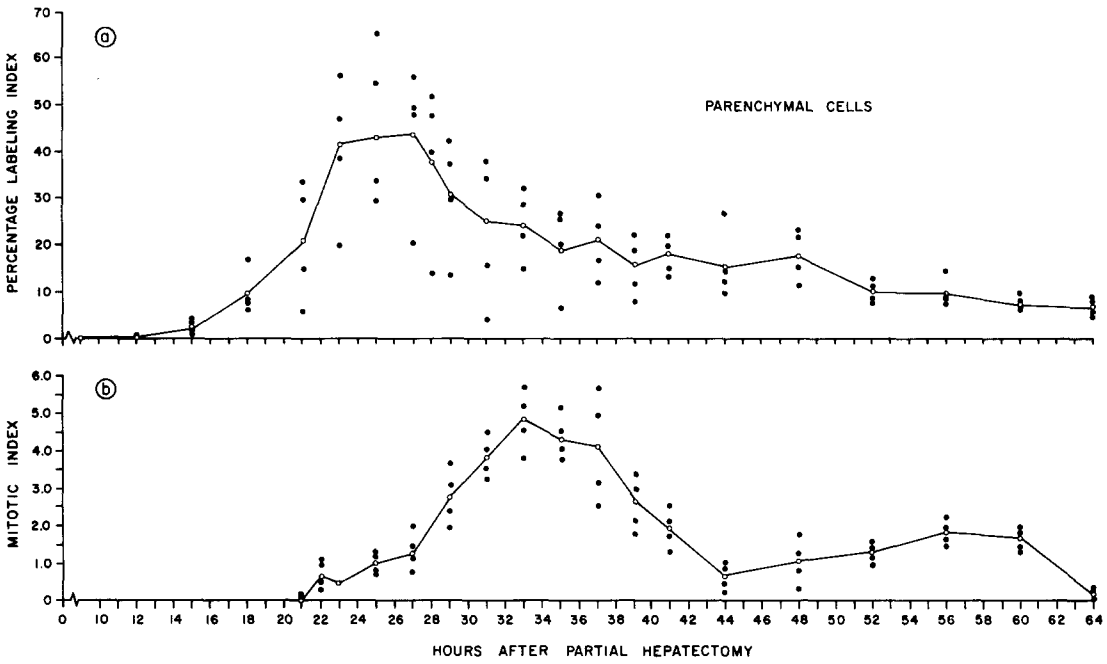

FIGURE 2 *a*, temporal pattern of incorporation of TdR-^{3}H into proliferating parenchymal cells in the rat liver during the 64 hr following partial hepatectomy. Mean values for four rats per interval. *b*, the temporal pattern of parenchymal cell mitoses during the 64 hr following partial hepatectomy. Mean values for four rats per interval.

of labeling of parenchymal cells during the 64 hr following partial hepatectomy is illustrated in Fig. 2 *a*. There was little or no DNA synthesis until 12 hr at which time the first labeled cells were seen; this was followed by a rapid rise in the percentage labeling index to a peak of 44% at 25 hr, and then by a gradual decline to 64 hr. The binucleate cells had higher labeling indices and therefore accounted for a greater proportion of the proliferative activity than their number would suggest. Labeling indices remained elevated for a number of days thereafter, and returned to normal after 2–3 wk when regeneration was complete.

Cells in mitosis first appeared at 21 hr; the mitotic index rose rapidly to 5% at 33 hr; this was followed by a gradual decline to approximately 1% at 44 hr (Fig. 2 *b*). A second and smaller wave occurred between 52 and 60 hr. The initial wave of mitosis followed that of labeling by some 8 hr, and allowing for this interval, there were 5–10 times as many cells labeled as in division. The mitotic index remained elevated for a number of days thereafter, and occasionally cells in division were observed 1 and 2 wk later.

SPATIAL DISTRIBUTION OF PROLIFERATING PARENCHYMAL CELLS: The distance between the portal triad and the central vein in the liver lobule was subdivided into thirds; an outer, middle, and inner zone (Fig. 3) were thereby defined. During the first 64 hr after partial hepatectomy, all lobules in the remnant were similarly labeled. That mean grain counts were of the same order indicates that the TdR-^{3}H was available equally to all cells in synthesis. The earliest evidence of labeling of parenchymal cells occurred in the periportal area; at the peak of synthetic activity, the labeled nuclei were concentrated in the outer zone and, with time, labeling of cells appeared to extend toward the middle and inner zones, and eventually became randomly distributed throughout the lobule. Counts of the percentage of parenchymal cells labeled in each zone with time after partial hepatectomy indicated that during the 64 hr the proliferative activity appeared earliest and was greatest in the outer zone and was least and appeared much later in the inner zone (Fig. 4 *a*). During the peak of DNA synthesis approximately 75% of labeled cells were

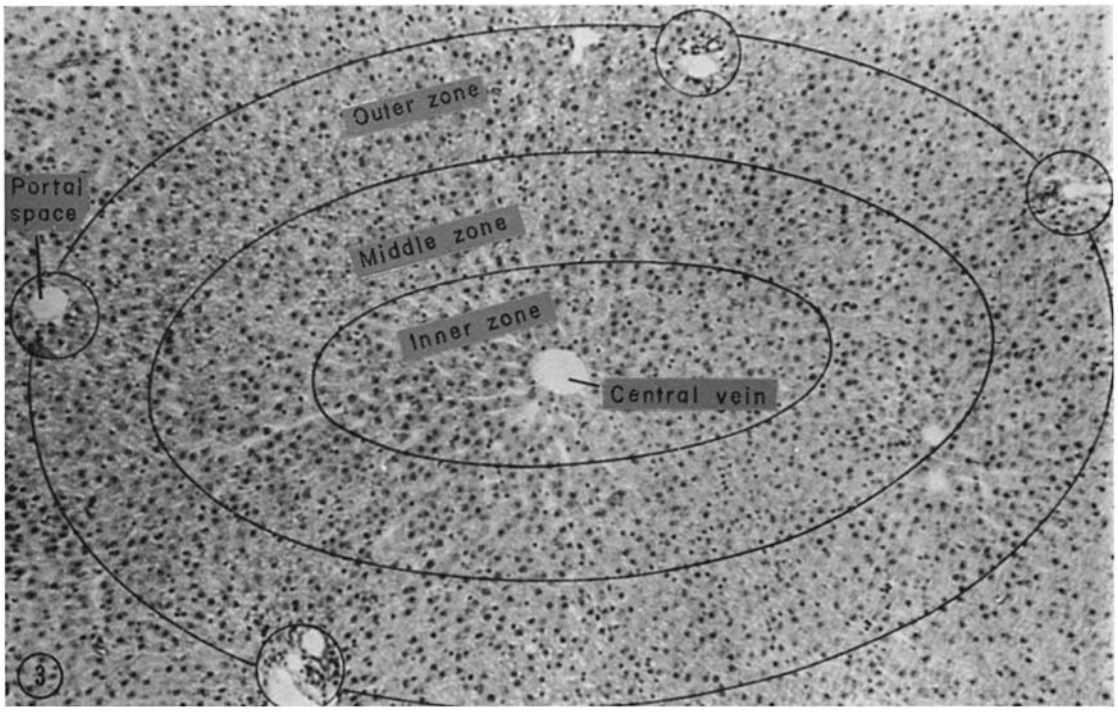

FIGURE 3 Subdivision of the liver lobule into outer, middle, and inner zones, based on the length of the hepatic cords or sinusoidal paths (see text). × 85.

in the outer zone, 20% in the middle zone and only 5% in the inner zone. After 48 hr, a second population of labeled cells occurred primarily in the outer zone and to a lesser extent in the outer half of the middle zone. It appeared that most of the outer zone cells entered synthesis and divided more than once, whereas a number of inner zone hepatocytes did not proliferate at all during regeneration.

The wave of mitotic activity which followed DNA labeling exhibited the same pattern of temporal and spatial distribution within the lobule (Fig. 4 *b*). Initially, almost all mitoses occurred in the outer zone and by 29 hr, the percentage of cells in mitosis in the zone rose to almost 13%. The rapid birth of new cells resulted in the redistribution of zonal boundaries. With subsequent cell division, the mitotic index in the middle zone increased rapidly to 13% at 37 hr. By 33 hr, the outer half of the lobule contributed the greatest number of new cells. The second wave of mitoses was due primarily to cell division in the outer zone.

DURATION OF THE PHASES OF PROLIFERATING PARENCHYMAL CELLS: Following TdR-^{3}H, the relationship between the percentage of mitoses labeled and time and the phases of the cell cycle was examined (Fig. 5). The shortest T_{g_2} was 2.0 hr, and the $T_{g_{2+m/2}}$ complex period was 3.0 hr. The mean T_s, measured at the 50% levels of the ascending and descending limbs of the wave of labeled mitoses, was ~8.0 hr. A second wave was absent, and since analysis by this method requires at least two divisions for the accurate determination of the cell cycle time (and thus, T_{g_1} as well), only T_{g_2}, T_m, and T_s could be measured with precision. Labeled mitotic figures with very low grain counts were seen 44 hr after TdR-^{3}H; this indicates that a few parenchymal cells probably divided two or more times during the first 64 hr of regeneration (10).

PROLIFERATION OF LITTORAL CELLS: The earliest appearance of labeled littoral cells occurred at 18 hr following partial hepatectomy (Fig. 6 *a*). There was a gradual increase in the percentage labeling index to approximately 30%

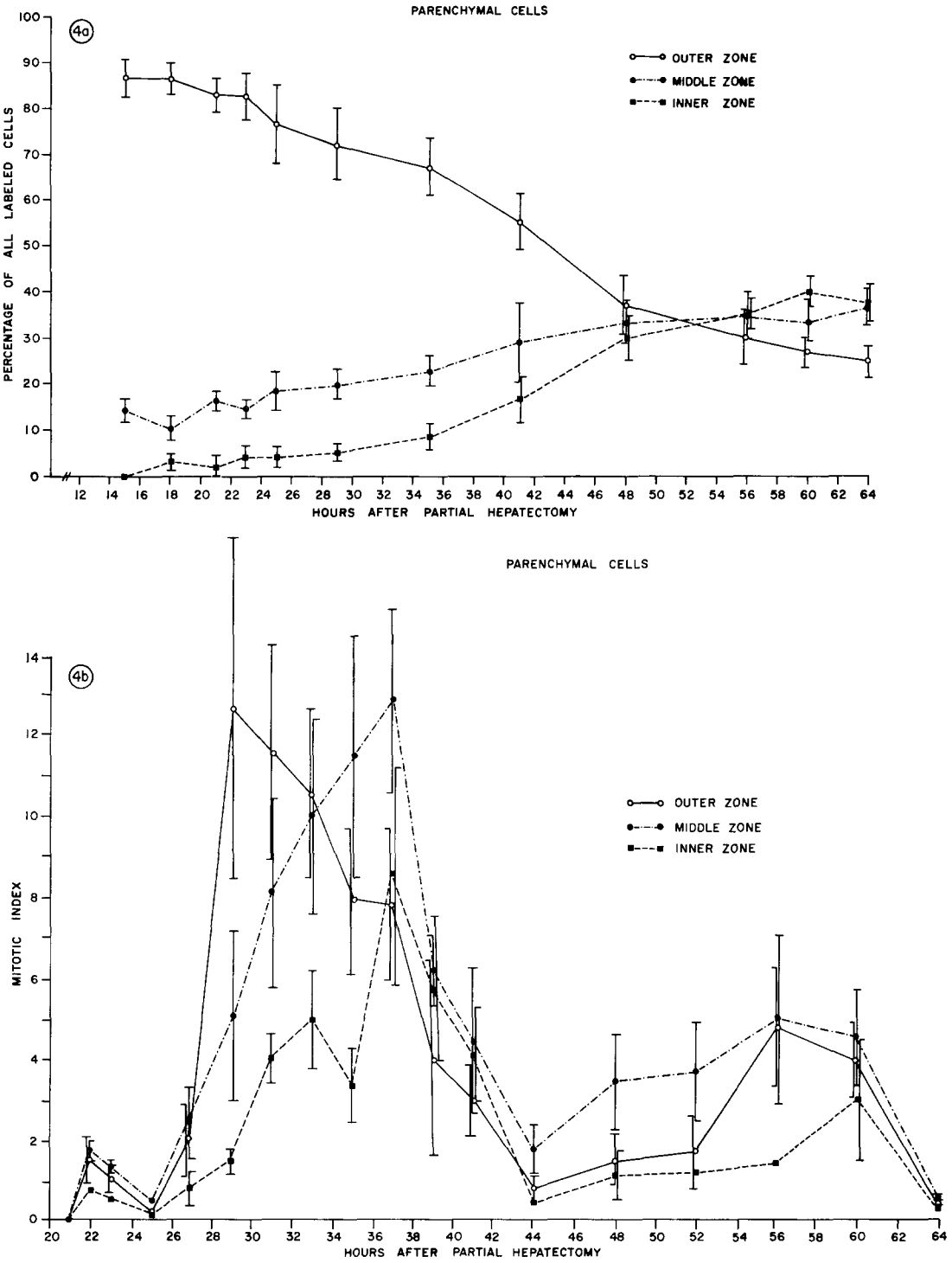

FIGURE 4 Cell proliferation in the regenerating liver; zonal distribution of labeled cells. *a*, temporal pattern of parenchymal cell labeling indices in each zone in the liver lobule during the 64 hr after partial hepatectomy. Mean values and range for four rats per interval. *b*, temporal pattern of parenchymal cell mitotic indices in each zone in the liver lobule during the 64 hr after partial hepatectomy. Mean values and range for four rats per interval.

at 42–50 hr, followed by a gradual decline to approximately 10% by 64 hr. The maximum mitotic indices occurred between 48 and 66 hr, i.e. approximately 8–10 hr following the peak of labeling; the numbers of mitoses, however, were small and estimates, therefore, unreliable. Labeled cells were distributed randomly throughout the liver lobule during regeneration, and mean grain counts varied widely. The first labeled littoral cell mitoses appeared 3 hr following TdR-^{3}H at 48 hr.

For a determination of whether littoral cell

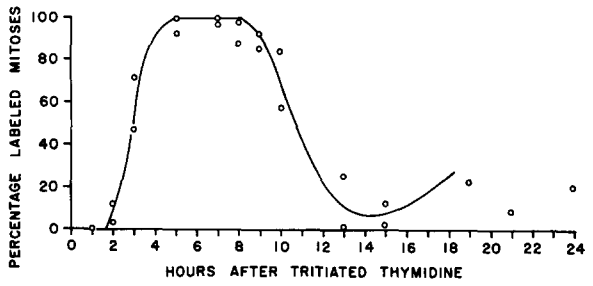

FIGURE 5 Percentage labeled mitoses of proliferating parenchymal cells scored against time after pulse-labeling with TdR-^{3}H (10). Zero hour, the time of injection, represents 20 hr after partial hepatectomy. Each point represents one rat. Only the values for $T_{g_2+m/2}$ and T_s could be measured with any precision; $T_{g_2+m/2}$ = 3.0 hr; T_s = 8.0 hr.

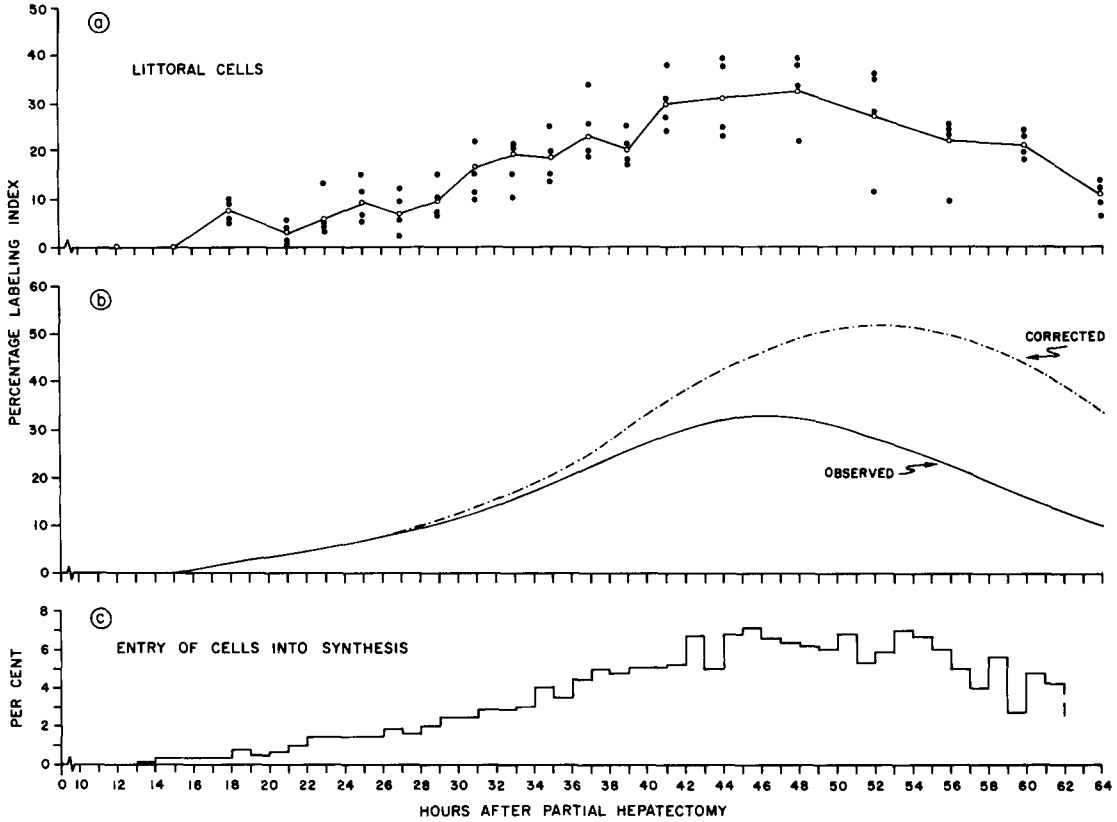

FIGURE 6 *a*, temporal pattern of incorporation of TdR-^{3}H into proliferating littoral cells during the 64 hr after partial hepatectomy. Mean values four rats per interval. *b*, temporal pattern of littoral cell proliferation during the 64 hr after partial hepatectomy. Solid line, observed percentage labeling index data plotted as a curve of best fit through the means; dashed line, corrected labeling index curve, assuming T_m = 1.0 hr and T_s = 8.0 hr. *c*, hourly rate of entry of littoral cells into DNA synthesis after partial hepatectomy determined from the corrected labeling index data.

migration may have occurred during regeneration, 28 rats were labeled with TdR-^{3}H at 20 hr after partial hepatectomy and sacrificed in groups of four 1 hr later and every 2 hr thereafter until 33 hr. Percentage labeling indices were compared to the 1-hr injection labeling indices (Fig. 7). The former group showed a slight rise in labeling index from 2 to 6% by 25 hr followed by a decline to 1% by 33 hr. The 1-hr injection group showed a continual rise from 3 to 22% during the same period.

PROLIFERATION OF INTERLOBULAR BILE DUCT EPITHELIUM: The first evidence of proliferative activity in the bile duct epithelium occurred at 23 hr (Fig. 8 *a*); the increase in labeling was gradual until 35 hr after which time the percentage labeling index rose to a peak of 19% at 48 hr, and then decreased gradually to 7% by 64 hr. Only few mitoses were observed, the maximum numbers occurring between 44 and 64 hr, i.e., up to 8 hr following the peak of labeling. The interval from the onset of synthesis to the onset of cell division was roughly 10 hr. Proliferating ductal cells were observed in clusters; whereas some ducts appeared quite active, others between adjacent lobules were quiescent. The first labeled mitotic figures in the interolobular bile duct epithelium appeared 3 hr following TdR-^{3}H at 48 hr.

PROLIFERATION OF MISCELLANEOUS NONPARENCHYMAL CELLS: Following partial hepatectomy, connective tissue was restored very slowly; labeled periportal fibroblasts appeared at 48 hr, and their numbers were greatest between 56 and 64 hr. Mitotic activity in the fibroblasts continued for more than a week, and following restoration of the parenchymal cell population, occasional fibroblasts in division were observed. Mesothelial cells of the peritoneum were labeled at 48 hr; the labeling index reached 20–40% by 56 hr and declined abruptly thereafter. No mitotic figures were observed. Occasionally, the vascular endothelium in the portal triads and in the large vessels was labeled between 48 and 56 hr.

DISCUSSION

Resting Liver

The data on percentage labeling indices are not sufficient for determining whether diurnal variation of DNA synthesis occurred in the different cell populations. In general, however, the findings do not disagree with observations (23) based on the more sensitive method of biochemical extraction of DNA and measuring TdR-^{3}H incorporation, that DNA synthesis in the liver was high at 6:00 a.m., and low at 12:00 p.m. and 6:00 p.m. Based on the labeling indices, if T_s were ~8 hr in parenchymal (7, 9, 19, 22, 30) and littoral cells, then the doubling times for the cell populations would be approximately 100 and 35 days, respectively. In the 6 wk old rat, the growing liver doubles its tissue mass, and thus, roughly, the number of hepatocytes, during the next 100 days (8). During this period, therefore, proliferation of

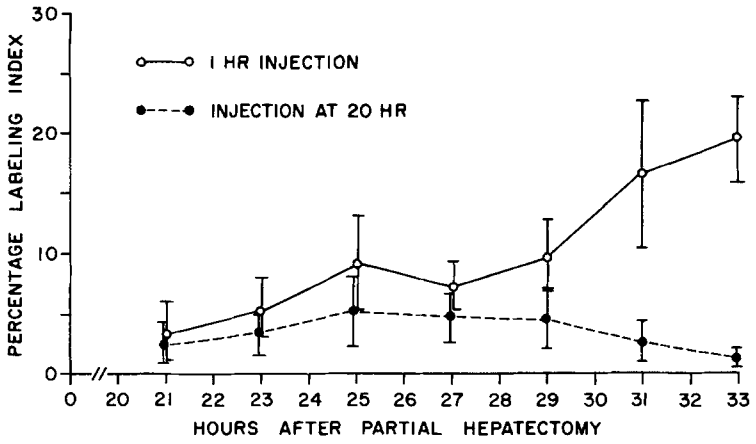

FIGURE 7 Littoral cell proliferation in the regenerating liver. Migration of littoral cells during liver regeneration. Solid curve, mean percentage labeling indices of proliferating littoral cells at intervals between 20 and 32 hr after partial hepatectomy; dash curve, mean percentage labeling indices at intervals after a single injection of TdR-^{3}H at 20 hr. Mean values and range for four rats per interval.

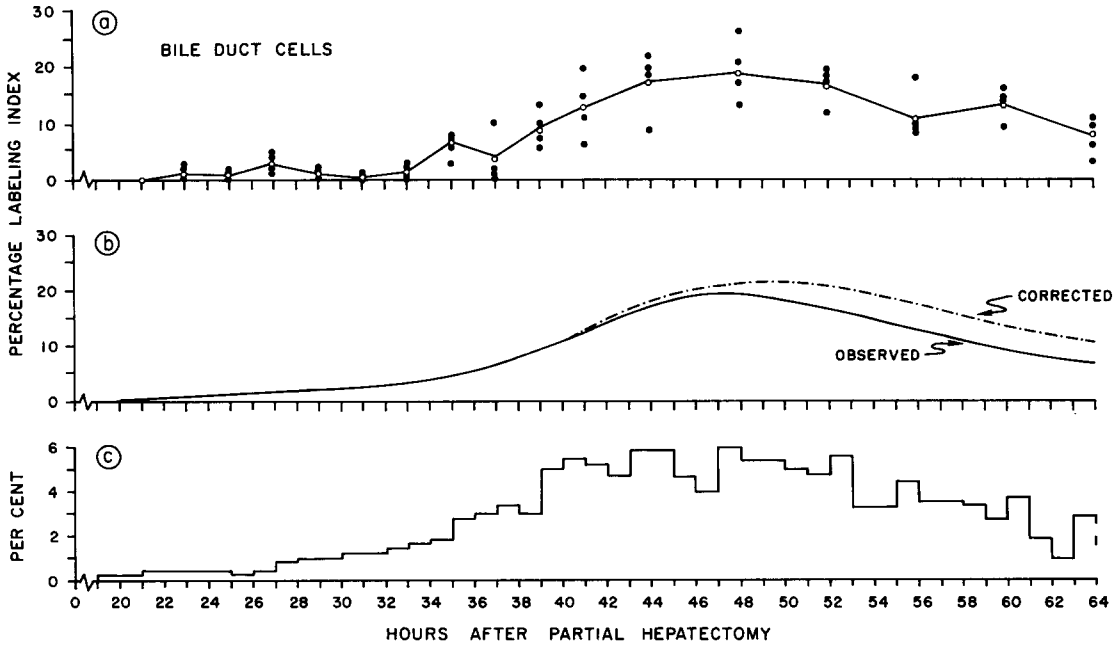

FIGURE 8 *a*, temporal pattern of incorporation of TdR-^{3}H into proliferating bile duct cells during the 64 hr after partial hepatectomy. Mean values for four rats per interval. *b*, temporal pattern of bile duct cell proliferation during the 64 hr after partial hepatectomy. Solid line, observed percentage labeling index data plotted as a curve of best fit through the means; dashed line, corrected labeling index curve, assuming T_m = 1.0 hr and T_s = 7.5 hr. *c*, hourly rate of entry of bile duct cells into DNA synthesis after partial hepatectomy determined from the corrected labeling index data.

parenchymal cells appeared to contribute primarily to tissue growth, with little or no renewal of dying or worn out cells.

Regenerating Liver

The burst of proliferative activity in the liver remnant during the first 64 hr results in a marked tissue expansion of cells with little or no cell loss. For accurate information for much of the quantitative analysis of the pattern of cell proliferation, the percentage labeling and mitotic indices must be corrected for the increasing mass of the liver in order to be a measure of the total number of cells in synthesis and division in the remnant at any given time. The direct measurement of changes in the number of parenchymal cells at frequent intervals presents some difficulties, but an estimate can be based on the temporal course of the mitotic index using methods of Smith and Dendy (28) and Goss (11). This was done for the mitotic index in the regenerating liver at hourly intervals based on a curve of best fit to the means (Fig. 9 *a*). From the labeled mitoses curve data, T_m was taken to be 1.0 hr, and T_s, 8.0 hr. This estimate of liver size, if the size of the remnant after partial hepatectomy is taken to be 1.0, is based on the hypothesis that at the end of each hour a number of cells equal to the number in mitosis during the hour is added to the cell population present at the beginning of the hour. This was determined progressively throughout the course of the regeneration, the number of cells in mitosis being calculated from the observed mitotic index and the estimated size of the liver at the beginning of the hour. At each subsequent determination there is a progressive, but very slight, underestimate of the size of the parenchymal cell population in the remnant which can be corrected by more tedious calculation (28).

If the *corrected mitotic index* (CMI_t) is defined as the total number of parenchymal cells dividing in the liver remnant at time t, expressed as a fraction of the number of cells in the remnant at the time of partial hepatectomy, then $CMI_t = MI_t \times H_t$, where MI_t is the observed MI at time t and H_t is the number of parenchymal cells in the liver

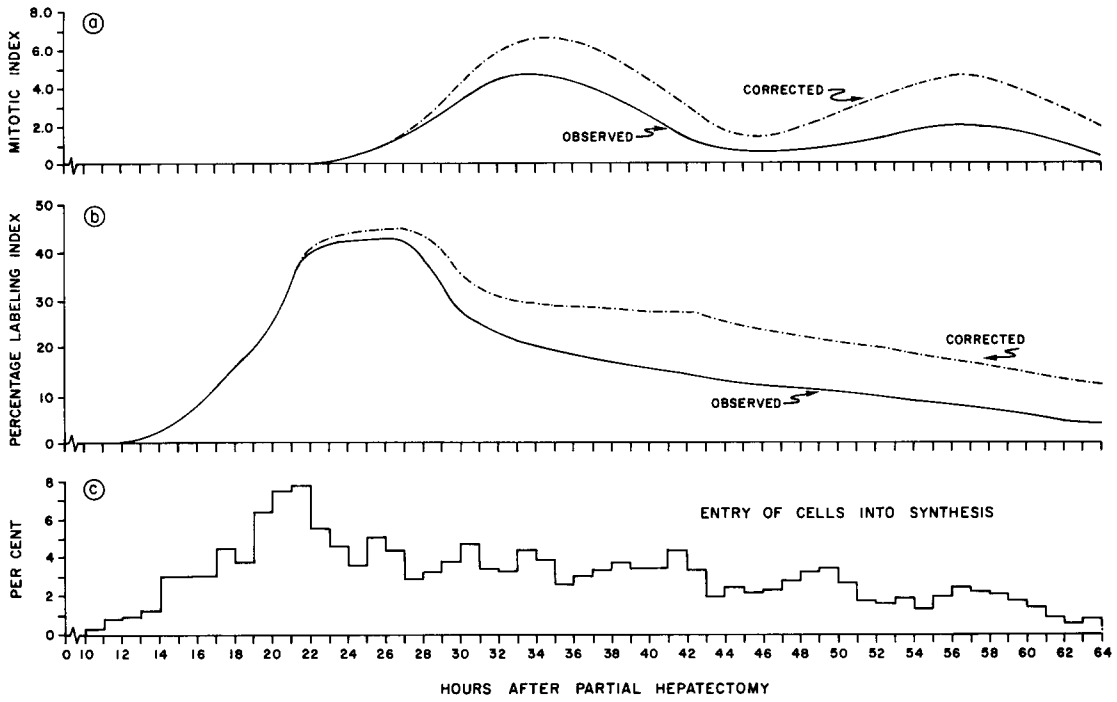

FIGURE 9 *a*, derivation of mitotic indices for parenchymal cells corrected for growth of the liver remnant at hourly intervals during the 64 hr after partial hepatectomy. Solid line, observed mitotic index data plotted as a curve of best fit through the means; dashed line, corrected mitotic index curve, assuming T_m = 1.0 hr. *b*, derivation of the percentage labeling indices for parenchymal cells corrected for growth of the liver remnant at hourly intervals during the 64 hr after partial hepatectomy. Solid line, observed percentage labeling index data plotted as a curve of best fit through the means; dashed line, corrected percentage labeling index curve, assuming T_s = 8.0 hr. *c*, hourly rate of entry of parenchymal cells into DNA synthesis after partial hepatectomy determined from the corrected labeling index data.

remnant at the time of observation t, expressed as a fraction of the cells in the remnant at the time of partial hepatectomy. H_t can be expressed in terms of the change in MI with time if T_m is known. If T_m is 1.0 hr, then $H_t = 1.0 + (MI_t \times H_{t-1})(MI_{t-1} \times H_{t-2}) + \cdots + (MI_{t-(n+1)} + H_{t-n})$ where 1, 2, $\cdots$ n, represent the number of hours prior to time t. Similarly, the *corrected labeling index* (CLI_t), the total number of parenchymal cells in synthesis in the liver remnant at time t, expressed as a fraction of the number of cells in the remnant at the time of partial hepatectomy, is given by $CLI_t = LI_t \times H_t$, where LI_t is the observed LI at time t (Fig. 9 *b*).

Comparison of the changing patterns of the observed and corrected labeling and mitotic indices during the 64 hr of regeneration indicates that the peaks of greatest synthetic and mitotic activity in the liver remnant reached higher levels and occurred later. Thus, the total number of proliferating cells was greater than estimates obtained from uncorrected curves of the observed data. Fig. 9 *c* is a histogram illustrating the rate of entry of parenchymal cells into DNA synthesis after partial hepatectomy determined from the corrected labeling index data. After a burst of synthesis in a relatively large population of hepatocytes (8% were labeled during the 22nd hr), there was a steady passage of cells through synthesis at a rate of 3–4%/hr until 50 hr, followed by a gradual decline. The efficiency with which the constant rate was maintained for some 24 hr indicated that following the initial wave of synthesis, mechanisms were operative to control the rates of synthesis and cell division in a manner similar to that of a steady-state renewal population, though without loss of cells through migration or death. By 64 hr, the cumulative hourly corrected labeling index and corrected mitotic index both increased by a factor of ~1.7, indicating an efficient synthe-

sis–mitosis sequence, and that a large proportion of the cell deficit was replaced within 3 days.

The present studies and repeated labeling experiments (1, 3, 4, 7, 9)[2] have shown that all but a few parenchymal cells in the region of the central vein of the lobule became labeled during regeneration; it appears, therefore, that most parenchymal cells of the liver comprise the potentially proliferative cell population. It is likely that all the cells are equally endowed with proliferative potential, and that they may constitute a resting reserve which could be in a dormant phase of the cell cycle until triggered-off to enter an active phase of the generative cycle. Further evidence for the extent of this proliferative pool have been provided from studies of repeated partial hepatectomies; Klinman and Erslev (16) demonstrated that regeneration resulted from proliferation of the general parenchymal cell population rather than multiple divisions of a small segment of that population, and Simpson and Finckh (27) have reported almost complete restoration of hepatic tissue following removal of up to 96% of the liver after five consecutive hepatectomies. It appears, therefore, that (*a*) most parenchymal cells in the normal liver are in a state of dormancy with respect to renewal and growth (probably G_0), (*b*) in this state, the hepatocyte performs the functions of the normal liver cell but retains its proliferative capacity, and (*c*) when the proper stimulus arises, in response to a demand situation, such as partial hepatectomy, the cell would be triggered-off to enter and proceed through a cell cycle. After division, the cell may enter a state of dormancy (again, probably G_0 or prolonged G_1 phase), indistinguishable from transitional or mature functional parenchymal cells, once again until triggered-off subsequently (Fig. 10). The experimental evidence indicates that following partial hepatectomy, few or no cells are lost from the proliferating population during regeneration, and most parenchymal cells divide only once and a few twice, and subsequently enter into the dormant population of cells with very low turnover. Eventually, almost all new hepatocytes enter into this population, since 3 wk following partial hepatectomy interphase labeling reverted to normal values.

SPATIAL DISTRIBUTION OF PROLIFERATING PARENCHYMAL CELLS: Morphologically and functionally, the liver lobule may be subdivided into peripheral, middle, and centrilobular zones, based on the length of the hepatic cords or sinusoidal paths, i.e., in relation to the sinusoidal blood flow from the portal vein and hepatic artery to the central vein. Evidence which links the anatomical pattern and biochemical anatomy of the liver lobule with sinusoidal blood circulation includes both altered gradients in blood composition along the sinusoid, particularly oxygen as well as certain enzymes and metabolites (1), and changing histochemical characteristics of the zones involving glycogen and lipid metabolism manifested as diurnal variation and during digestion (32). Oehlert et al. (21) and Fabrikant (9) have demonstrated a wave of RNA synthesis in parenchymal cells during regeneration which proceeded from the outer to the inner zones. Sulkin (29) observed morphological differences among hepatocytes of the three regions which indicated that the centrilobular zone contained cells with higher orders of polyploidy (up to 16 n) and the peripheral zone, primarily tetraploid and diploid cells.

It is likely that all parenchymal cells appear the same structurally, have equal functional capacity and can adapt depending upon the location of the cell in the lobule. Differences in metabolism of the three zones may help explain characteristic changes in the temporal and spatial patterns of parenchymal cell proliferation during regeneration and the utilization of available DNA precursor to

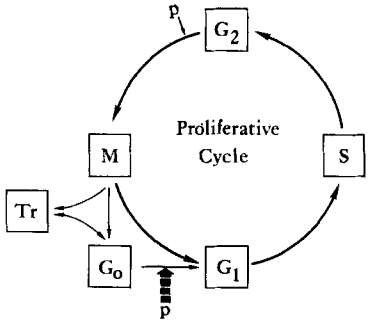

FIGURE 10 Scheme of the compartments of the parenchymal cell proliferation system as a conditional renewal system, based on a model of the cell renewal system suggested by Quastler (24). The proliferative cycle has four phases: M, G_1, S, and G_2. The system contains a population of potentially proliferative cells (G_0) which produce at a low rate or only on stimulation (p, partial hepatectomy). Additional phases of transitional and mature cells (Tr) yields a basic scheme with at least six compartments (see text).

[2] Brauer, R. W. Personal communication.

proliferating cells. RNA and DNA synthesis and cell division occur in sequence beginning in the outer zone and progressing in waves toward the central zone. Peripheral zone cells divide once, and a few twice, whereas some of the centrilobular zone cells do not proliferate at all. It therefore appears that the signal for proliferation does not affect all the parenchymal cells to the same extent. After regeneration, the degree of ploidy increases, particularly in the peripheral zone, and approaches the complex pattern characteristic of the centrilobular zone cells. The signal to enter synthesis is independent of and proceeds with greater efficiency than that to enter cell division, and such events which apparently initiate cell proliferation following partial hepatectomy are operative primarily on peripheral zone parenchymal cells.

ANALYSIS OF KINETIC PARAMETERS OF PARENCHYMAL CELL PROLIFERATION: Proliferative changes in the regenerating liver introduce an almost synchronous, but transient, pattern of birth of parenchymal cells in an expanding population with little or no cell loss through migration or death. Since the mean rate of emergence of cells from the S compartment is N_s/T_s, then $dN_t/dt = [(N_s)_{t-j}]/T_s$, where dN/dt is the rate at which cells divide, and j represents the interval between a given stage of S and the end of M, the stage of S being that at which the actual rate of passage of cells through S is equal to N_s/T_s. The value of j varies as a function of the rate of passage of cells through the S compartment, but as an approximation the required stage of synthesis may be designated as the midpoint of the S interval. Therefore,

$$\frac{1}{N_t}\cdot\frac{dN_t}{dt} = \frac{1}{N_t}\cdot\frac{(N_s)_{t-j}}{T_s} = \frac{1}{N_t}\cdot\frac{(N_s)_t}{T_s}\cdot\frac{(N_s)_{t-j}}{(N_s)_t}$$

$$= \frac{(N_s)_t}{N_t}\cdot\frac{1}{T_s}\cdot\frac{(N_s)_{t-j}}{(N_s)_t}.$$

Since $(N_s)_t/N_t$ is the labeling index at time t, then

$$\frac{T_s}{N_t}\cdot\frac{dN_t}{dt} = (LI)_t\cdot\frac{(N_s)_{t-j}}{(N_s)_t}.$$

Integrate

$$T_s\int_{N_0}^{N_f}\frac{dN_t}{N_t} = \int_0^{t_f}(LI)_t\cdot\frac{(N_s)_{t-j}}{(N_s)_t}\,dt$$

and

$$T_s\ln\frac{N_f}{N_0} = \sum_0^{t_f}(LI)_t\cdot\frac{(N_s)_{t-j}}{(N_s)_t}\Delta t,$$

where N_0 and N_f are the numbers of cells in the liver remnant at partial hepatectomy and at the time (t_f) when regeneration is complete, respectively. Since,

$$\frac{N_t}{N_{t-j}} = \frac{(N_s)_t}{(N_s)_{t-j}}\cdot\frac{(LI)_{t-j}}{(LI)_t}$$

then,

$$T_s\ln\frac{N_f}{N_{t-j}} = \sum_0^{t_f}(LI)_t\frac{(LI)_{t-j}}{(LI)_t}\cdot\frac{(N)_{t-j}}{(N)_t}\Delta t.$$

If j is 6 hr, and $N_f/N_0 = 3.0$ when regeneration is complete, then $T_s \ln 3.0 = 951\%$ labeled cells–hours, and $T_s = 8.6$ hr. A correction for the production of polyploid cells would decrease T_s by at least 10%, since polyploid cells progressively weight the percentage labeling index during regeneration. T_s, therefore, would be ~7.5–8.5 hr.

Since the mean rate at which cells divide is $dN/dt = N_m/T_m$ and N_m/N_t is the mitotic index at time t, then $(1/N_t)(dN_t/dt) = MI_t/T_m$. Integrating,

$$T_m\int_{N_0}^{N_f}\frac{dN_t}{N_t} = \int_0^{t_f}MI_t\,dt$$

and,

$$T_m\ln\frac{N_f}{N_0} = \sum_0^{t_f}MI_t\,\Delta t.$$

When regeneration is complete, $T_m \ln 3.0 = 97\%$ mitotic cells-hours, and $T_m = 0.9$ hr. The values for T_s and T_m are in close agreement with those obtained for normal, neonatal and regenerating ing liver by various investigators (Table II).

LITTORAL CELLS: Since the first labeled littoral cell mitoses appeared 3 hr following TdR-^{3}H, and since the numbers of mitoses were greatest 8–10 hr following the peak of labeling, the interval between the onset of DNA synthesis and cell division did not exceed 10 hr. Assuming $T_s = 7.5$ hr, the corrected labeling index data (Fig. 6 *b*) indicated that the period of greatest littoral cell

proliferation reached a higher level and occurred later than would appear from the observed percentage labeling index data. The rate of entry of littoral cells into synthesis during the 64 hr (Fig. 6 *c*), derived from the corrected labeling index data, slowly increased (0.2%/hr) during the first 25 hr, rapidly rose to a maximum of 7%/hr by 46 hr, and then declined gradually. Between 40 and 55 hr, the mean rate of entry was 6%/hr and by 64 hr the ratio of the number of cells which entered synthesis to the number originally present was ~ 1.9. Thus, by 64 hr, all but some 5% of the littoral cell population necessary to replace the cell deficit had entered synthesis.

There is evidence that extensive transformation and migration of littoral cells occurs during regeneration (3, 7, 22) and up to some 50% of the RES cell population in the liver remnant, particularly during the early period of regeneration, may migrate to the liver from the extrahepatic RES reserves to augment the deficiency. This mobilization would serve to protect the important functions provided by the RES, particularly when great stress has been placed on the liver. Evidence for such migration in the present studies is suggested by the appearance of a small number of labeled littoral cells 3–5 hr following a single injection of TdR-^{3}H 20 hr after partial hepatectomy (Fig. 7); the percentage labeling index rose far more than would be expected by division alone. In addition, an elevated 1 hr percentage labeling index persisted for more than a week and may have been due to the continued need for increased migration and replacement of cells during restoration of normal hepatic function, as well as the continued growth of the animal.

The delay in the onset of DNA synthesis in littoral cells for some 24 hr following the onset of parenchymal cell proliferation suggested that the signal for proliferation in the littoral cells may have been in response to growth of the parenchymal cell population. During the first 64 hr, the mean rate of entry of new cells into synthesis (and thus, the eventual birth rate) was over 4%/hr which was greater by a factor of 40 than the normal proliferation rate of the RES in the resting liver. At this increased rate, the doubling time was approximately 20 hr as determined by grain-count halving (9).

BILE DUCT EPITHELIUM: Since the interval from the onset of S to the onset of M was ~10 hr, and T_{g_2} = 3 hr, then T_s was ~7–8 hr. From corrected labeling index data, synthesis reached a higher level and occurred later (Fig. 8 *b*) than would appear from observed labeling indices. The rate of entry of cells into synthesis (Fig. 8 *c*), increased gradually between 20 and 39 hr at the rate of 0.1%/hr, and between 40 and 60 hr rose and remained between 2 and 3%/hr, after which time there was a gradual decline. It appeared, therefore, that for the interlobular bile duct epithelium, the rate of entry of cells into synthesis was in response to the expansion of the parenchymal cell population, and the system lagged well behind the parenchymal and littoral cell populations both in time and extent of proliferation.

Since proliferating duct cells occurred in clusters, it may be that budding to expand the duct wall was the method of growth of the interlobular bile duct system for the increased secretory function of the growing hepatic tissue. However, during parenchymal cell proliferation, the new bile

TABLE II

Values of T_s and T_m for Parenchymal Cells of Liver

Reference	Cell system	T_s	T_m
		hr	*min*
Brues and Marble (2)	regenerating rat		49
Landing et al. (17)	regenerating rat		45
Cater et al. (5)	regenerating rat		60
Looney (19)	regenerating rat	8.4	
Post et al. (22)	normal neonatal rat	9.0	180
Edwards and Koch (7)	normal rat	8.2	46
Edwards and Koch (7)	regenerating rat	8.5	52
Fabrikant (10)	regenerating rat	~8.0	~60
Fabrikant (10)	regenerating mouse	~8.0	~60

canaliculi forming within the anastomosing perforated plates of the new parenchyma were responsible primarily for the large increase in the biliary system associated with the increase in size of the liver lobules during regeneration. This would agree with the observation that following partial hepatectomy an increased flow of bile lagged some 24–36 hr behind the growth of the parenchymal cell population, and that an increased flow exceeding the control level continued for 15–20 days (3, 18, 31, 32). Thus, during regeneration, it may be that the response of growth extended over a longer period than for the parenchymal and littoral cells, and that the growth of the biliary system was effected primarily by an increase in the network of bile canaliculi, and only secondarily by proliferation of the interlobular duct epithelium.

CONNECTIVE TISSUE CELLS: The onset of DNA synthesis and cell division in the connective tissue cells, mesothelial lining cells, vascular endothelium, etc., during regeneration also appeared to occur in response to the rapid expansion of the parenchymal cell population. However, cell proliferation lagged behind the parenchymal and littoral cell populations both in time and extent of proliferation. Growth extended over a longer period during the regenerative process, thereby providing the architectural framework necessary for the subsequent remodeling of the organ.

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