The Rate of Incorporation of Labeled Thymidine into the Deoxyribonucleic Acid of Regenerating Rat Liver in Relation to the Amount of Liver Excised*

NANCY L. R. BUCHER AND MIRIAM N. SWAFFIELD

(The John Collins Warren Laboratories of the Huntington Memorial Hospital of Harvard University at the Massachusetts General Hospital, Boston, Massachussetts)

SUMMARY

The rate of incorporation of thymidine-2-C^{14} into DNA was determined at intervals during the first 2 days of hepatic regeneration following excision of varying amounts of liver in weanling, young adult, and older rats.

In young adults, extirpation of 9 or 34 per cent of the liver resulted in small increases in the respective 2-hour rates of DNA labeling to relatively constant levels, or "plateaus," over the 20- to 48-hour postoperative period. Removal of 43 or 68 per cent caused sharp bursts, or "peaks," in respective rates of labeling at 18–30 hours, followed by decreases to plateaus which persisted until 48 hours or more. The logarithms of the plateau rates of DNA labeling were directly proportional to the per cent of liver removed over the entire range of hepatic deficiency. The logarithms of the peak rates were similarly proportional—i.e., although the absolute rates of incorporation were increased, the percentage increments between these points were approximately the same as for the plateau rates.

In weanling and older rats peaks of DNA labeling occurred after removal of only 9 per cent of the liver, and the logarithms of the peak rates were proportional to the amounts of liver excised over the entire 9–68 per cent range, with percentile increments approximately the same as above. The plateau rates, estimated from 68 per cent liver removal only, exhibited a somewhat similar trend.

In radioautographs prepared from livers exposed to thymidine-H^3 throughout the 18- to 40-hour postoperative period (hence, including the peak plus transition to plateau) labeled nuclei appeared first at the periphery of hepatic lobules, especially when the deficiency was acute, whereas they were randomly scattered during normal growth. Smears of isolated nuclei supported the short-term data in showing a pronounced elevation in per cent of labeled nuclei in animals with 43–68 per cent removals compared with those with only 9–34 per cent.

Thus, in 1- to 15-month-old rats the rate of DNA labeling increases logarithmically in direct proportion to the amount of liver excised at the peak period (also, in young adults at least, at the plateau), the percentage increments in rate being approximately the same for all, except that, if the liver loss exceeds a certain critical amount, which differs with age, some additional mechanism comes into play, raising the baseline and resulting in higher levels of labeled thymidine incorporation.

The present study is an extension of previous work which dealt with the effects of age upon regeneration of the liver induced by partial hepatectomy in rats. Employing the incorporation of labeled thymidine into DNA as an index of proliferative activity, we found that as the animals grew older there was an increasingly longer lag in the initiation of DNA synthesis. Weanling rats manifested two consecutive waves of activity, which, with advancing age, merged into a single, progressively broadening peak (5). These studies have now been expanded to include the alterations in response that occur in rats of different ages when the size of the stimulus is reduced—i.e., when less than the usual 68 per cent of the liver is excised.

Few systematic studies have been conducted to deter-

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mine the effect of size of partial hepatectomy upon regeneration, although several groups of investigators have found that, when less than the usual two-thirds of the liver is removed, restoration of liver mass proceeds more slowly (6, 11, 12, 14, 18, 23). Recently, MacDonald *et al.* reported an extensive series of experiments in which fractions of liver ranging from 2.9 to 66.5 per cent of the total were removed, and the effects on nuclear labeling with tritiated thymidine were examined 21–23 hours later (17). They concluded, as had previous investigators using liver weight as the criterion of regeneration, that the response was directly proportional to the amount of liver excised (11, 12, 23). The evidence presented below suggests that, at least as regards rate of incorporation of labeled thymidine into DNA, this conclusion should be modified.

MATERIALS AND METHODS

Female rats of the Sprague-Dawley strain were obtained at 21 days of age from the Charles River Breeding Laboratories, Inc., North Wilmington, Mass., and maintained under the standard conditions previously described until used as weanlings at 24–28 days of age, as young adults at approximately 4 months of age, or as older rats at approximately 12–15 months of age (5).

Fractions of liver were removed by ablation of various lobes or combinations of lobes as follows: (*a*) caudate lobe, (*b*) left lateral lobe, (*c*) caudate plus left lateral, and (*d*) left lateral plus median,[1] the latter being the two lobes routinely excised in the almost universally employed operation first described by Higgins and Anderson (10). Under ether anesthesia the respective lobes were dissected free of anchoring ligaments or membranes where necessary, tied near the base with heavy silk thread (No. 2 twist), and excised just distal to the ligature. Intact rats were used as controls, since preliminary experiments showed good agreement with results from sham-hepatectomized animals.

At specified intervals after operation thymidine-2-C^{14} was injected intravenously. Two hours later the animals were killed. The DNA specific activity was determined as previously described (5).

Radioautography was employed in weanling and young adult rats to demonstrate the pattern of distribution within the liver lobule of nuclei undergoing DNA synthesis during the period of maximal activity. In one experiment tritiated thymidine (0.3 μc/gm of body weight; specific activity, 4000 μc/μmole, obtained from Schwarz BioResearch Inc., Orangeburg, N. Y.) was infused continuously during the 24-hour interval from 17 to 41 hours after operation. In subsequent experiments rats were given separate intravenous injections of 0.3 μc. of thymidine-H^3/gm of body weight at 20, 26, and 32 hours postoperatively and killed at 40 hours. Since each cell requires approximately 6–8 hours to duplicate its complement of DNA (8, 15), this method would be expected to label very nearly all the cells entering DNA synthesis during the final 24 hours and thus yield results comparable to the infusion procedure. At least three rats were examined for each fractional hepatectomy in each age group. At 40–41 hours blocks of liver were fixed in Tellyesniczky's or Carnoy's fluid, embedded,

[1] Nomenclature of lobes is according to Griffith and Farris (7).

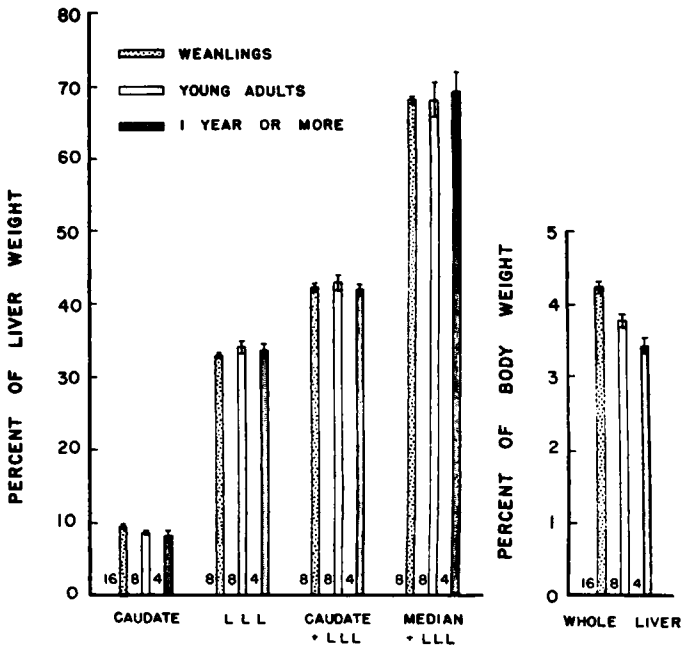


CHART 1.—Ratios of weights of various lobes to total liver and total liver to body weight in weanling, young adult, and older rats. LLL is left lateral lobe. Numbers near baseline indicate number of rats in each group and vertical lines are standard errors of the mean.

and sectioned at 6–8 μ. In one series of animals tissue was rapidly frozen in a dry ice-acetone bath, sectioned in a cryostat, mounted, and fixed in Carnoy's fluid. Radioautographs of both paraffin and frozen sections were prepared with liquid emulsion (Kodak NTB-2).

To survey the extent of nuclear labeling in the above animals, in addition to tissue sections, isolated nuclei were prepared from two rats in each category. After removal of a small block of tissue for fixation, the remainder of the liver was rapidly perfused *in situ* with cold 5 per cent citric acid, put through a chilled tissue press (Harvard Apparatus Co., Dover, Mass.), and shaken mechanically at 360 oscillations per minute in 2 volumes of the citric acid solution with twenty glass beads (6 mm. in diameter) for 15 minutes, a procedure previously found to disrupt the cells with negligible destruction of nuclei.[2] Smears were prepared from the resulting nuclear suspension, dried, fixed in Carnoy's fluid, stained by the Feulgen procedure, and covered with stripping film (Kodak AR-10). Radioautographs were developed at intervals until the percentage of labeled nuclei became constant, the values being based on counts of 2000 nuclei per animal by each of two observers.

RESULTS

Determination of per cent of liver excised.—To obtain reliable estimates of the fractions of liver removed, groups of rats were subjected to the various hepatic lobectomies described above, and the excised portions were weighed. Immediately afterward the remainder of the liver was removed and weighed. The relative amounts of liver thus resected did not vary significantly among age groups: ablation of the caudate lobe removed approximately 9 per cent; left lateral, 34 per cent; caudate plus left lateral, 43

[2] Unpublished observations of J. F. Scott and N. L. R. Bucher.

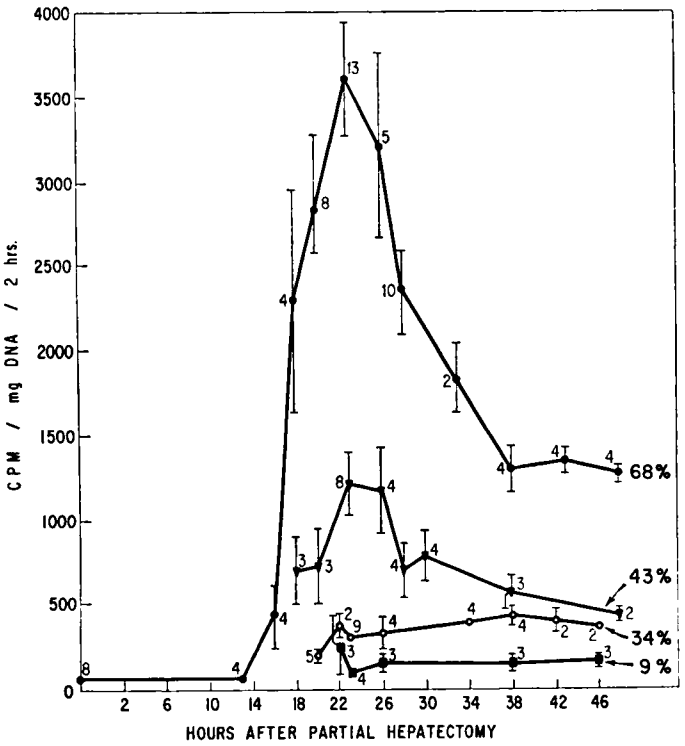


CHART 2.—Rates of incorporation of labeled thymidine into hepatic DNA of young adult rats at intervals after excision of various amounts of liver.

After resection of 9, 34, 43, or 68 per cent of the liver, rats received a single intravenous injection of 0.5 ml. of thymidine-2-C^{14} in physiological saline solution (0.8 μc.; specific activity, 2 μc./μmole) at the times plotted above and were killed 2 hours later.

Small numbers indicate the number of rats per point and vertical lines standard errors of the mean. When vertical lines are absent, standard errors lie within the area of the point itself.

The curve for 68 per cent hepatectomies is reproduced from the previous report (5).

per cent; and median plus left lateral, 68 per cent of the total liver, regardless of the age of the animal (Chart 1). These values were used in plotting the results of the ensuing experiments.

Alternatively it is possible to estimate the per cent of liver excised from the weight of the animal, the weight of the excised lobes, and a previously determined standard set of values for ratios of liver weight to body weight. However, the ratio changes as the animal grows (16, 24) and must be determined separately for each age group (Chart 1). An additional disadvantage is that experimental conditions often entail rapid deposition or withdrawal of hepatic glycogen or lipid which tends to distort liver to body weight ratios; lobar ratios are relatively unaffected, since the liver tends to enlarge or shrink as a unit.

Response to fractional hepatectomies in young adult rats.—The rate of incorporation of C^{14}-labeled thymidine into hepatic DNA in fractionally hepatectomized young adults at intervals up to 48 hours after operation is shown in Chart 2. By 23 hours after operation excision of 9 or 34 per cent of the liver resulted in respective rises in DNA-specific activity to approximately 2 or 5 times the non-hepatectomized control value. These elevations, or plateaus, persisted relatively unchanged for the duration of the experiment. When the fraction of liver excised was raised by a small increment to 43 per cent, instead of a plateau a well defined peak appeared, reaching a maximum of 17 times the control and corresponding in time to the usual peak following the standard 68 per cent hepatectomy. At 68 per cent the peak exceeded the control level by 50-fold. After 40 hours or more, when the peaks were passed, a graded level of activity persisted, resulting in plateaus which tended to parallel the slow replacement rates of the smaller deficiencies.

In Chart 3 the data for young adults, at the 25-hour interval from Chart 2, have been replotted to emphasize the relation between rate of DNA labeling and size of stimulus *at the time at which the rate of DNA synthesis is maximal.* It is obvious that the response is greater when more than a third of the liver has been excised. Thus, during the peak period the elevations in rate of thymidine-C^{14} incorporation are small yet proportional to the degree of liver deficiency as long as the deficiency is below 34 per cent. At this point there is a break in the curve, and DNA labeling becomes much more rapid—although it is still proportional to the liver loss (Chart 3, curve for young adults).

To rule out the possibility that the slightly more extensive operation involved in removal of two lobes instead of one could be a factor in producing the greater response, six animals were subjected to a procedure in which the entire

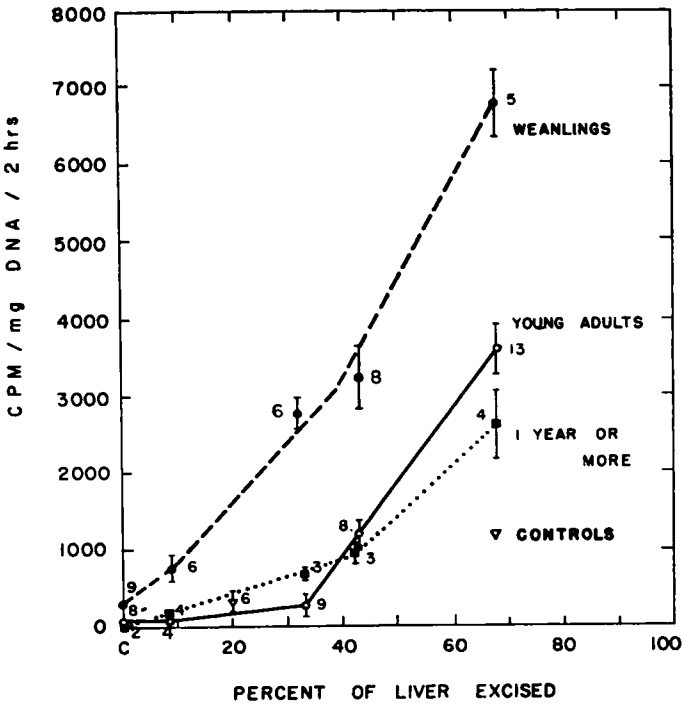


CHART 3.—Maximal rates of incorporation of labeled thymidine into hepatic DNA at various levels of liver deficiency in weanling, young adult, and older rats.

Adult rats received 0.8 μc. and weanlings 0.48 μc. of thymidine-2-C^{14} solution at the post-operative interval at which incorporation of labeled thymidine into hepatic DNA was at its peak for each age group (5)—i.e., at 20 hours for weanlings, 23 hours for adults, and 28 hours for older rats (see legend to Chart 2 for other details).

For point at 20 per cent, see text.

caudate lobe and the distal end of the left lappet of the median lobe were excised. It was estimated from the relative weights of the two pieces that approximately 20 per cent of the liver was removed in this way. The actual degree of liver deficiency was probably somewhat greater than this, since the necrosis adjacent to the ligatures tended to be more extensive when fractions of lobes were resected. The result demonstrated that the dual procedure did not produce a rise significantly above that caused by a single lobectomy (Chart 3, 20 per cent point).

Response to fractional hepatectomies in other age groups.—In addition to the curve for young adults, Chart 3 shows the results of fractional hepatectomies in weanlings given thymidine-C^{14} at 20 hours and in older rats at 28 hours after operation—the times at which their respective DNA synthetic rates are maximal (5). The curve for weanlings differs from the others in roughly paralleling the steep part of the young adult curve (Chart 3). Regeneration in weanlings starts from an already elevated base, since their livers are already growing prior to the partial hepatectomy. It is as if the adult curve had simply shifted to the left. The curve for older rats lies between the other two, showing a higher rate for low levels of deficiency than that for the adults. These interrelations will be discussed below.

Distribution of labeled nuclei.—Radioautographs were examined for changes in distribution of proliferating cells in response to different degrees of liver deficiency. The labeled precursor was administered over a prolonged period in an effort to bring out the nuclear labeling pattern in the animals with low levels of response. The pattern was found to be essentially the same regardless of whether the thymidine-H^3 was administered by continuous infusion or serial injections and whether the paraffin or frozen sectioning technic was employed (compare Figs. 1, 2, and 4 with Figs. 5, 6, and 8). Because of the prolonged period of labeling, the distribution of tagged cells reflects both the site of active proliferation and the movement of some of the new cells toward the center of the lobule when the growth process is extensive (8).

In young adults nuclear labeling was rare in intact animals and mainly confined to reticuloendothelial cells. Following 9 per cent hepatectomy DNA synthesis occurred in a few widely scattered parenchymal nuclei, especially in peripheral parts of the lobule (or in zone 1 according to the acinar concept of liver structure [21]). Labeled nuclei were altogether too few in number to form a distinctive pattern (Figs. 1, 5). After 34 per cent hepatectomy a few more became labeled, definitely concentrated in zone 1 (Figs. 2, 6). The number rose remarkably when the hepatectomy was increased to 43 per cent, the dense labeling of the peripheral zone then encompassing about half of the lobule or zones 1 and 2 of the acini (Figs. 4, 8). By 68 per cent, labeled nuclei extended all the way to the center (zones 1, 2, and 3), though they were less numerous immediately adjacent to the central vein (Fig. 3).

In weanlings, similarly treated, the livers of nonhepatectomized controls showed a random scatter of labeling commensurate with their active state of growth (Fig. 7). At the 9 and 34 per cent levels of liver deficiency, the concentration of radioactive nuclei increased preponderantly at the periphery, but labeled nuclei were also

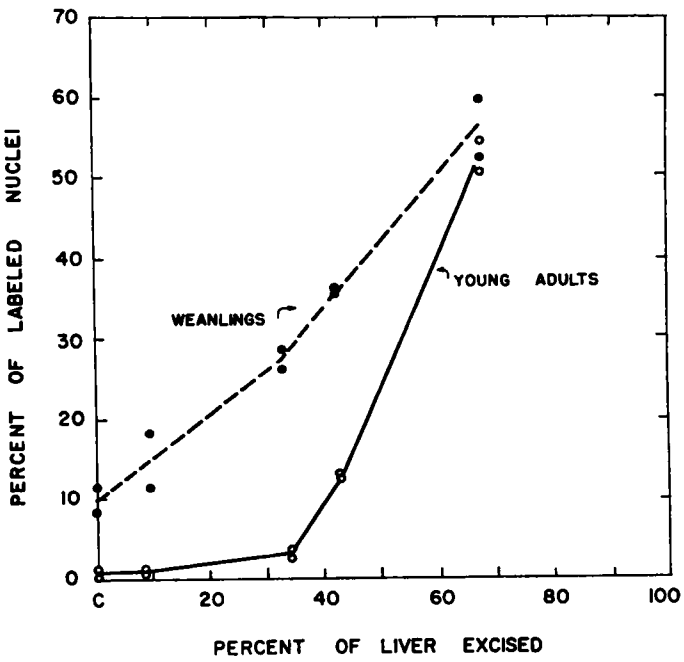


CHART 4.—Per cent of hepatic nuclei labeled after continuous (or repetitive) administration of thymidine-H^3 during entire period of most active DNA synthesis (approximately 18–40 hours) at various levels of liver deficiency in weanling and young adult rats.

Counts were performed on radioautographs of smears of isolated nuclei. Each point represents the value obtained from a single rat.

scattered throughout the lobule (Figs. 9, 10). By 43 per cent, the more active process had invaded the inner region and approached the central vein, although the periportal areas were still more heavily labeled (Figs. 12–14). By 68 per cent, this process had intensified, with labeled nuclei more numerous throughout the lobule (Fig. 11). The lesser labeling immediately adjacent to the central vein persisted, however (Figs. 15, 16). In general, the localized pattern of labeling was less pronounced in the weanling than in the corresponding adult livers.

Percentage of labeled nuclei.—Because the nonrandom distribution of proliferating cells in tissue sections could lead to sampling errors, we employed the smearing technic described above to determine the percentage of total hepatic nuclei that incorporated the label (Chart 4). The results are not strictly comparable to the analytical data, since the livers were exposed to thymidine-H^3 throughout the postoperative period, which includes the peak of DNA labeling plus transition to the plateau. However, the findings support the foregoing (cf. Chart 3) in showing the relatively linear character of the weanling curve compared with the pronounced change in slope of the adult curve at the 34 per cent point.

DISCUSSION

The finding that the main lobes (median plus left lateral) comprise an average of 68.3 per cent of the total liver in Sprague-Dawley rats recorded in Chart 1 is corroborated by our own earlier report (with a different operator) of 68.3 per cent in Wistar rats in 1950 (3), and of 68.4 per cent

in Slonaker rats by Brues *et al.* in 1936 (1). Chart 1 implies that excisions of left lateral and caudate lobes, separately or in combination, are similarly reproducible. A divergent view has been reported, however (17).

It should also be mentioned that, although the liver is made up of a number of cellular types, the measurements of DNA specific activity recorded above, in adults at least, reflect largely (but not exclusively) the rates of synthesis in parenchymal cells. This follows from (*a*) the observations of Grisham (8) who found that nonparenchymal cells did not achieve a maximal rate of thymidine incorporation until 42 hours postoperatively, which is toward the end of the period under study (see Chart 2); and (*b*) the occurrence of polyploidy in the parenchymal but not other cell types (especially in the adults), so that a high proportion of the newly formed DNA must belong to the hepatocytes. With respect to the radioautographic studies, it has been observed that, although proliferating hepatocytes exhibit a zonal pattern, littoral cells are randomly scattered (8, 9). Figs. 1, 2, 4–6, and 8 show how few labeled cells are present in the central region—a good indication of the relatively low contribution of littoral cells to the total numbers of labeled nuclei.

The present study covers the period during which the maximal proliferative response to partial hepatectomy takes place. The rate of DNA labeling in relation to amount of liver excised in the several age groups is examined in Chart 5, which is a semilogarithmic plot of data compiled from Charts 2, 3, and the previous paper (5). Chart 2 shows that when more than 34 per cent of the liver is excised in young adult rats the rate of DNA labeling reaches a peak at 23–25 hours after partial hepatectomy, then falls, leveling off in a temporary plateau after partial restoration has occurred; if the liver loss is smaller there is only a plateau—no peak. Thus, depending upon the degree of deficiency, there are two kinds of response—peaks and plateaus. Partial restitution following a major loss allows the rate of DNA labeling to fall from a peak to a plateau. Chart 5 demonstrates that the heights of the plateaus, whether resulting from small deficiencies (34 per cent or less) or from larger ones *after* some of the lost tissue has been replaced, are directly proportional on a logarithmic scale to the percentage of liver excised (Chart 5, solid lines: adults at peak but with liver loss of 34 per cent or less, and adults at 48 hours with all degrees of liver loss). In addition, Chart 5 shows that the heights of the adult peaks (which occur only during major deficiencies) are also proportional to the liver loss, but starting from a higher level; the abrupt upward displacement of the curve for the peak period of activity at around the 40 per cent point reflects the change in response from plateau to peak as the deficiency becomes major (Chart 5, curved solid line). It is as if during the peak period of activity the rate of incorporation of labeled thymidine is suddenly stepped up to a higher basal level, from which it continues to exhibit the same proportional increase as before, the curve running parallel to the ones for the lower levels of deficiency.

The maximum rate of thymidine incorporation occurs at 20 hours postoperatively in weanlings and at 28–30 hours in older rats (5). When these values are plotted against per cent of liver excised (Chart 5, dashed and dotted lines)

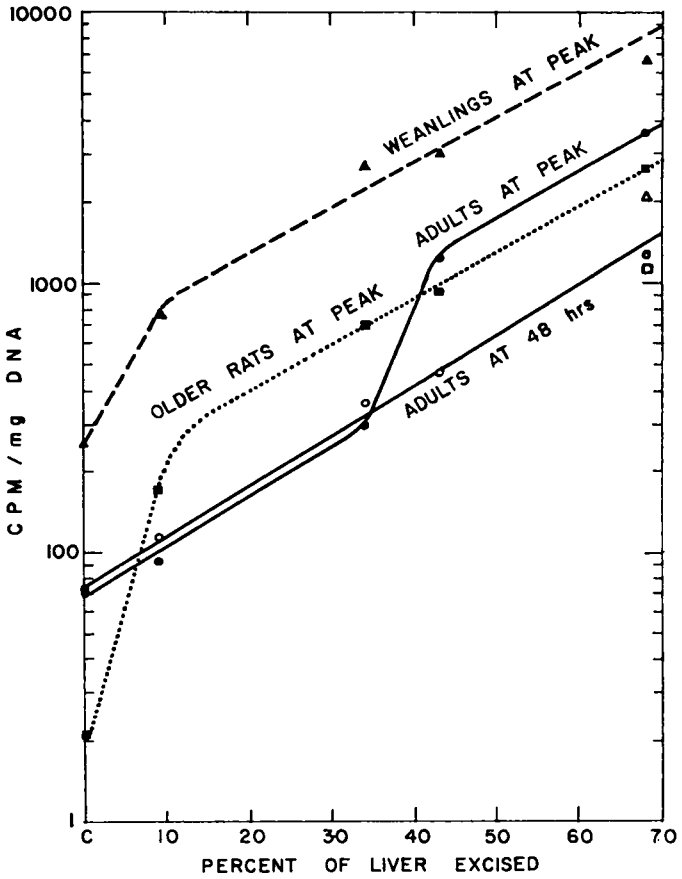


CHART 5.—Semilog plot showing rates of DNA labeling at various levels of liver deficiency in weanling, young adult, and older rats at times when synthesis is maximal, and also in young adults after rates have leveled off at 48 hours.

Open triangle above 68 per cent point is value for weanlings at 50 hours after operation and open square is corresponding value for older rats.

Data taken from Charts 2 and 3 and Chart 2 of preceding paper (5).

both curves show an immediate steep increase in rate, resembling the abrupt upward displacement on the young adult curve, and indicating that a peak type of response occurs in these animals at a liver deficiency of only around 9 per cent. Beyond this the curves are linear, implying that the logarithms of these peak rates are directly proportional to the amounts of liver excised over the entire 9–68 per cent range. The plateau rates for weanlings and older rats were determined only for 68 per cent excisions. If the remaining points are assumed to lie on a line connecting this 68 per cent value with the control or "zero per cent excised" point, the results suggest general conformity with the adult pattern, although the slope deviates slightly in the case of weanlings and somewhat more in the case of older rats from the seemingly parallel courses of the other curves (Chart 5, 68 per cent points, lines not drawn). The justification for the curves as drawn in Chart 5 rests upon the numbers of rats involved, the degree of reproducibility of the data, and the relative similarity of patterns among age groups. From these patterns it appears that, in terms of rates of DNA labeling during hepatic regeneration, a liver deficiency of around 10 per cent in weanlings or older rats may be equivalent to a 40 per cent liver loss in

young adults. Why peaks occur at much lower levels of liver loss in weanling and older rats than in young adults is open to conjecture; probably the kind of response reflects the reserve capacity of the liver which may be considerably greater in young adults than in older animals, or in weanlings which are actively growing. Further, although rat liver has a vast growth potential as evidenced by its massive reaction to 68 per cent hepatectomy, it reserves high rates of regeneration for acute deficiencies and repairs small losses gradually over a prolonged period. Similarly, it has been reported that mouse liver, following the removal of either one, two, or three lobes, requires 7 days to regain its initial weight, thus growing more slowly when the liver loss is smaller (23).

Along the same lines, rat kidney has been shown to exhibit a curvilinear response to varying partial nephrectomies, the increase in mitotic rate becoming greater in proportion to the increase in amount of the kidney complement removed (19).

The remarkable feature of Chart 5 is the extent of linearity and parallelism exhibited among the various curves. The implications are that in weanling, young adult, and older rats, at the peak period (and also at the plateau, in young adults at least), not only does the rate of DNA labeling increase logarithmically in proportion to the amount of liver excised, but the percentage increments in rate are approximately the same for all ages except when the deficiency becomes severe. Then the base level abruptly shifts upward, but beyond this point the percentile increments are again approximately the same. The upward shift in baseline could represent either (*a*) a true increase in the rate of DNA synthesis or (*b*) a seeming increase in this rate due to a sudden rise in the specific activity of the immediate labeled precursor of DNA, thymidine triphosphate. Thymidine is not on the normal metabolic pathway of thymidine triphosphate formation but rather on the so-called "salvage" pathway (13, 22). Certain alterations in enzymic activities are known to occur during regeneration that increase the flow of thymidine from the salvage pathway to the mainstream of DNA synthesis (2); a sudden rise in the specific activity of the triphosphate could result if one or more such alterations should occur only when the deficiency exceeds a certain critical level. Regardless of the explanation, it is clear that above a critical level of hepatic deficiency an altered response occurs, resulting in a sudden elevation in rate of DNA labeling. Although the per cent increases in labeled thymidine incorporation relative to liver deficiency are approximately the same in all three age groups, the timing of the maximum response varies with age and is independent of the size of the stimulus.

Concentration of labeled nuclei in the periphery of the lobule during the peak period of regeneration has been described in detail by Grisham (8). It appeared from his preliminary evidence that cellular reproduction occurred randomly throughout the lobule during normal growth and during regeneration when the liver loss was small or when the peak of activity was passed; accordingly, he suggested that the restricted type of proliferation might only occur when the deficiency exceeds 20–30 per cent. We found that in weanlings regeneration appeared to be superimposed upon an already active normal growth process; the less sharply defined localization observed in these animals reflected both the diffuse pattern characteristic of normal organ growth and the zonal pattern characteristic of regeneration. In the young adults, which were somewhat older than the rats studied by Grisham, we observed the pronounced zonal distribution typical of regeneration to be incipient at the 9 per cent and already well demarcated at the 34 per cent level where the deficiency is not yet acute. It thus appears possible that the patterns of regeneration and normal growth are distinctive at *all* degrees of liver loss, suggesting different kinds of regulatory mechanisms.

In a study similar to the present one in some respects MacDonald *et al.* (17) have defined a "threshold" of 9–12 per cent, which is the amount of liver that must be excised to elicit a statistically significant increase in nuclear labeling above the control level, as estimated by radioautography following injection of thymidine-H^3. This threshold reflects the sensitivity of the method used to measure the proliferative response; it may or may not be a physiological entity, since even smaller insults might be gradually repaired, even though undetected by present technics. It is altogether different from the critical level of liver deficiency described above, which determines whether the rise in DNA labeling will conform to a plateau or a peak.[3]

MacDonald *et al.* conclude that above the 9–12 per cent threshold the numbers of labeled nuclei are directly proportional to the amount of liver removed within a given group of rats. However, they observed three- to fivefold variations among comparable groups of animals, which they attribute primarily to nonreproducibility of the operative

[3] Unfortunately we too used the word "threshold" in preliminary reports of this work (2, 4). "Critical level" now seems preferable, since it both describes the phenomenon more correctly and avoids confusion with the terminology of MacDonald *et al.*

PLATES 1–4.—Radioautographs of liver sections from rats given thymidine-H^3 after excision of the fractions of liver indicated below. Hematoxylin stain. Long arrows indicate portal tracts, short ones central veins. Figs. 1–12, mag. × 140.

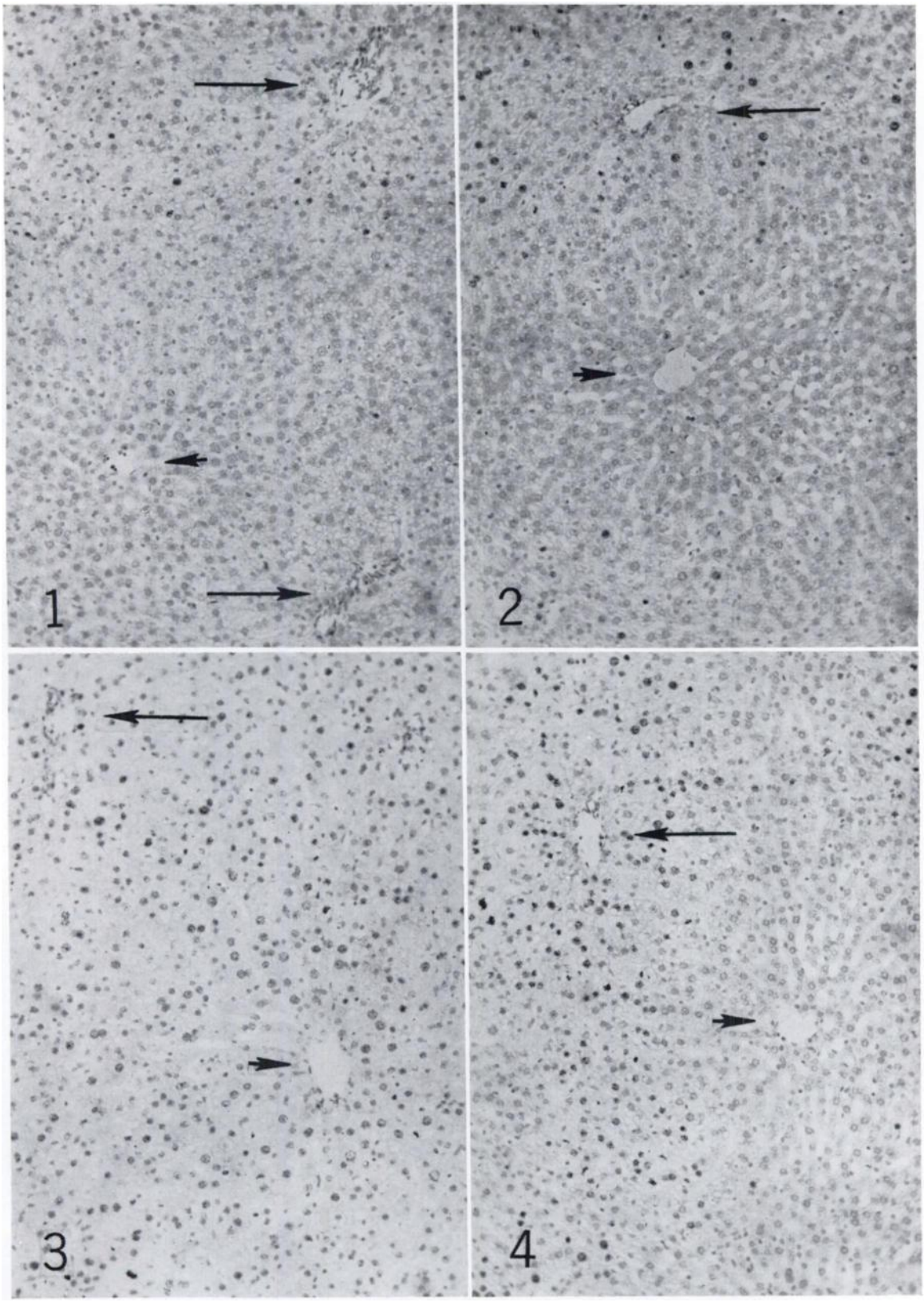
FIGS. 1–4: are paraffin sections from rats that received thymidine-H^3 by continuous infusion.

FIG. 1.—9 per cent hepatectomy, young adult rat.

FIG. 2.—34 per cent hepatectomy, young adult rat.

FIG. 3.—68 per cent hepatectomy, young adult rat.

FIG. 4.—43 per cent hepatectomy, young adult rat. Note pronounced increase in number of labeled nuclei compared with 34 per cent hepatectomy.



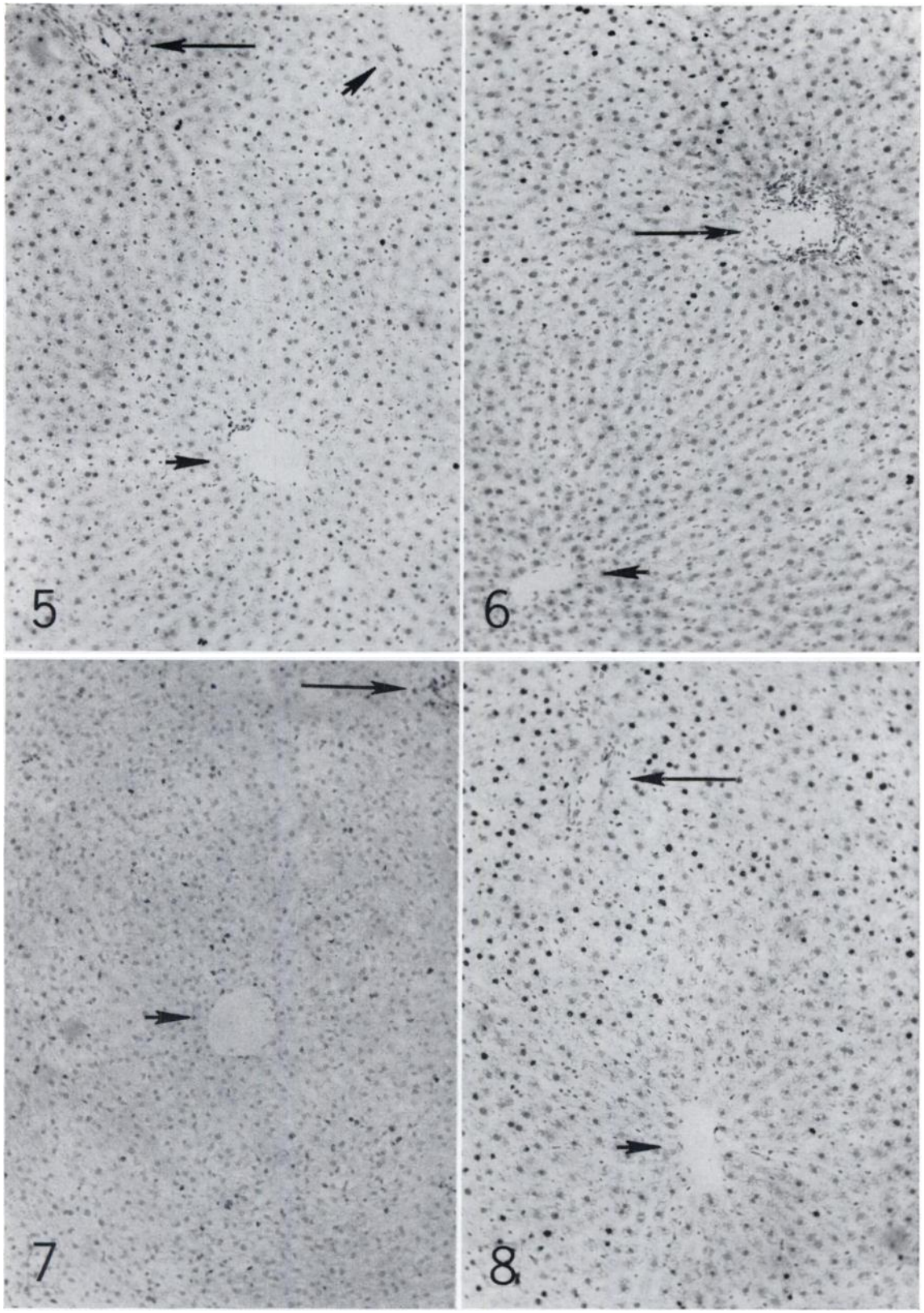
FIGS. 5, 6, and 8 are frozen sections from rats given thymidine-H^3 by serial injections. Compare with Figs. 1, 2, and 4. Fig. 7 is a paraffin section from rat given thymidine-H^3 by serial injections.

FIG. 5.—9 per cent hepatectomy, young adult rat.

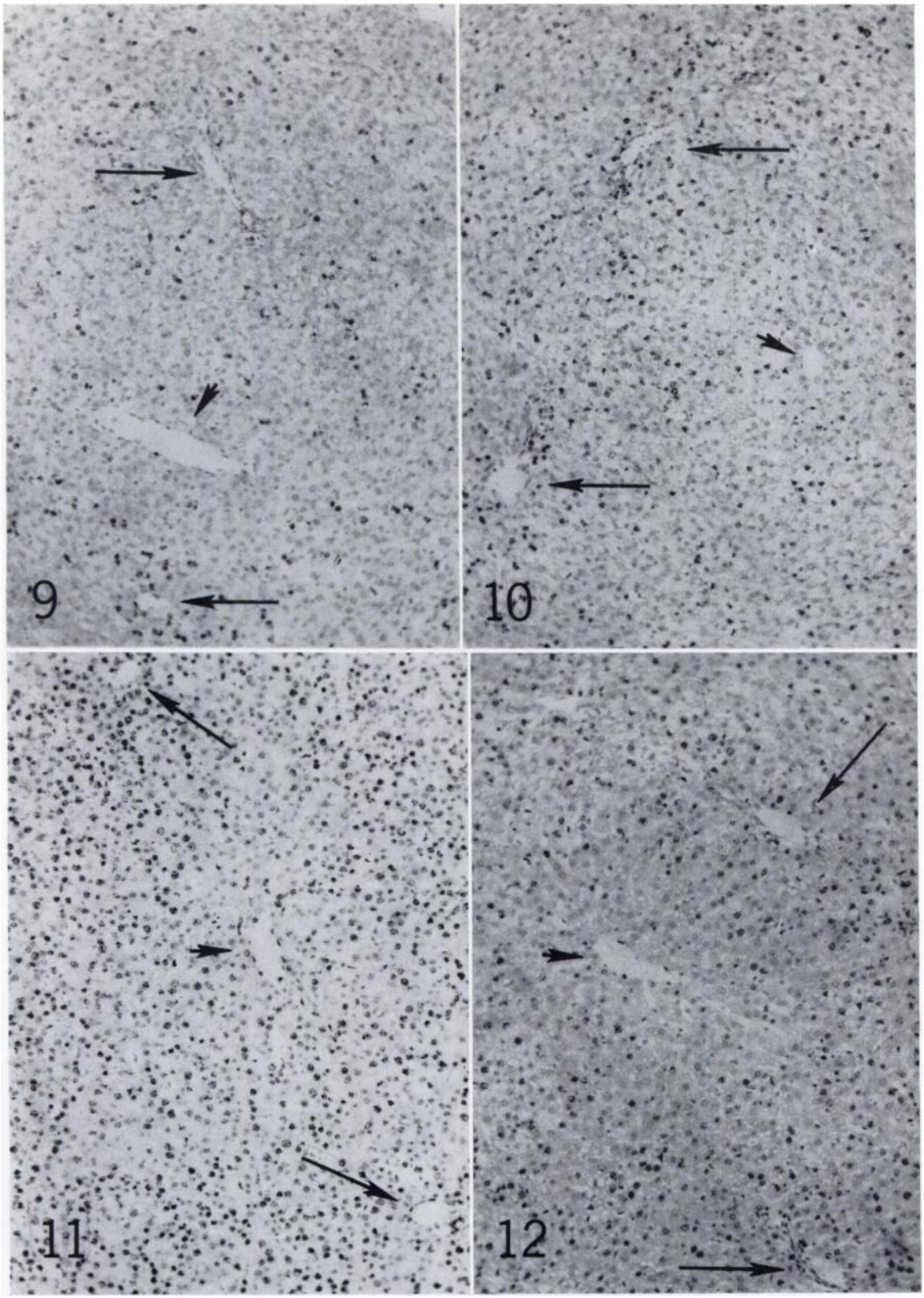
FIG. 6.—34 per cent hepatectomy, young adult rat.

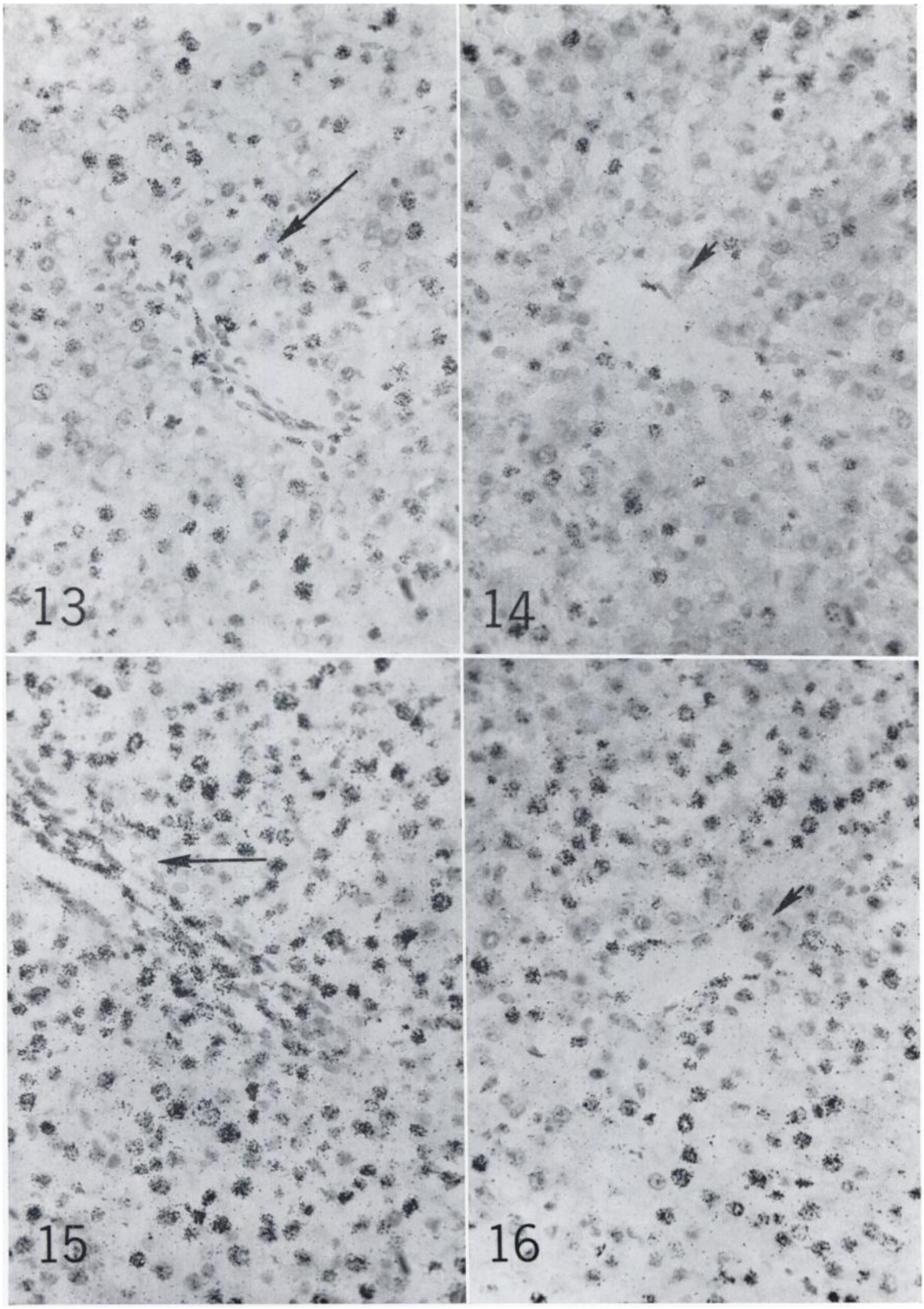
FIG. 7.—intact weanling rat.

FIG. 8.—43 per cent hepatectomy, young adult rat.



FIGS. 9–12.—Same procedure as for rat in Fig. 7.
FIG. 9.—9 per cent hepatectomy, weanling rat.
FIG. 10.—34 per cent hepatectomy, weanling rat.
FIG. 11.—68 per cent hepatectomy, weanling rat.
FIG. 12.—43 per cent hepatectomy, weanling rat.





technic. Our findings differ somewhat, as noted above, and further comparisons are difficult because of differences in experimental conditions and methodology.

The curves in Chart 5 attest to the existence of delicately balanced mechanisms for weighing the numerous metabolic demands upon the liver against the urgent need for replacement of lost tissue. Of special interest is the pronounced difference in response to minor and more acute levels of deficiency. This finding suggests that, above a certain critical level of liver loss, a new mechanism enters to allow the sudden stepping up in rate of DNA labeling; it offers an additional means of probing into the mode of regulation of the regenerative process.

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