Reutilization of 5-^{125}I-Iodo-2'-Deoxyuridine and ^{3}H-Thymidine in Regenerating Liver of Mice (35793)

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(Introduced by H. Meier)

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The reutilization of liberated DNA catabolites follows a similar or identical pathway to that incorporating exogenously administered thymidine into DNA-synthesizing cells (1, 2). Unlike thymidine, its analogue 5-iodo-2'-deoxyuridine (IUdR) labeled either with ^{125}I or ^{131}I, is poorly or not at all reutilized under physiological conditions (3, 4). Thus, the use of both ^{3}H-thymidine (^{3}H-TdR) and ^{131}IUdR or ^{125}IUdR allows for quantitative determination of thymidine reutilization; a number of such parallel studies have been made for normal, proliferating, and tumor tissue (4–7). We have used this technique in studies on regenerating liver with the hope of answering at least three questions: what is the site of thymidine reutilization, what is its extent, and at what molecular level does it occur.

Materials and Methods. We used 18 female Swiss albino mice (Texas Yale strain KFA), 12 weeks of age and weighing 21.5 ± 2.8 g. They were fed standard diet No. 1115 from Altromin GmbH, and kept on a 12-hr day–night cycle. Then 3 days before and during the experiments the animals received 0.1% NaI in the drinking water.

The mice received 5 intraperitoneal injections of labeled precursors[3] over a period of 40 hr and at 8-hr intervals. Nine mice each were given ^{3}H-TdR and ^{125}IUdR, respectively. Twenty to 24 hr after the last injection, the mice were partially hepatectomized; about 50% of the liver mass was removed. The excised liver pieces served as controls for isotope label at day 0. These excised liver pieces represent true "nonhepatectomized" controls at the time of hepatectomy; no differences exist between them and similarly pretreated mice killed instead of hepatectomized. Two, 4, and 8 days after partial hepatectomy, 2 to 3 mice of each "isotope"-group were sacrificed. Parts of the liver were immediately frozen for biochemical fractionation. Imprints of liver tissue on glass slides were made and processed for autoradiography as described previously (8).

For biochemical studies, pieces of fresh liver tissue were weighed, homogenized in ice-cold 1 *N* perchloric acid (PCA), and washed 3 times with 0.5 *N* PCA. The precipitate was then washed twice with absolute ethanol and once with ether to extract the lipid fraction. The remaining precipitate containing DNA, RNA, and protein fractions were digested by Hyamine-10×. ^{3}H-activity was determined by a liquid scintillation technique (4) and ^{125}I-activity was counted in a NaI-well-counter. In our calculations, absolute activity was expressed per gram of wet tissue weight.

For autoradiographic evaluation, the slides were developed for 36 days and stained with Giemsa stain, pH 6. The silver grains over hepatocyte nuclei were counted in 200 cells/slide, and the labeling index (LI) and mean grain counts (MGC) were determined. The activity per 100 cells was calculated by multiplying LI times MGC.

Results. We found that the amount of ac-

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[3] The DNA precursors, supplied by the Radiochemical Centre, Amersham, England, were used as follows: ^{3}H-6-thymidine (sp. act. 5 Ci/mmole), 1.0 μCi/g of body weight; ^{125}I-iododeoxyuridine (sp. act. 1.3 Ci/mmole) 1.0 μCi/g of body weight.

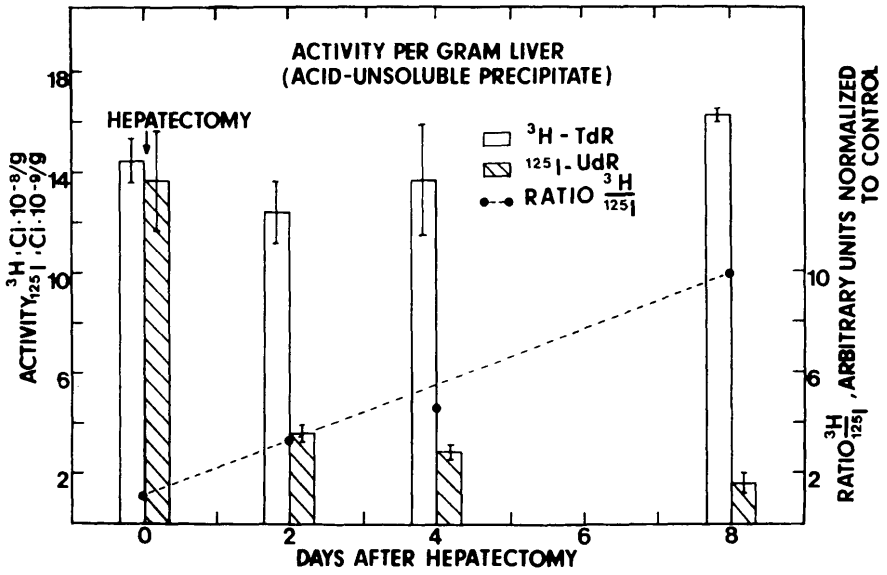

FIG. 1. Radioactivity per gram of liver (wet weight) in the acid- and alcohol-insoluble precipitate (means ± standard errors) containing DNA, RNA, protein. The ratio of ^{3}H to ^{125}I continually rises over the 8-day period of the experiment.

tivity incorporated per gram of organ weight at time of partial hepatectomy was 10-fold greater for ^{3}H-TdR than ^{125}IUdR (Fig. 1). Whereas, ^{3}H-activity remained almost constant, ^{125}I-activity decreased rapidly for the first 2 days following partial hepatectomy, but slowed considerably for the following 6 days. Thus, the ratio of ^{3}H/^{125}I continually rose to its highest value at day 8.

The relative amount, *i.e.*, percentage of all biochemical fractions, of activity present in the acid-soluble fraction of the excised liver piece at the time of hepatectomy (20–24 hr after last precursor injection) fall into the range reported previously (5, 11) for a single pulse injection (5–15%). Since attempts to cocrystallize this activity with cold IUdR (3) failed, the availability time of each dose of injected ^{125}IUdR must remain very short (3), and there is no evidence for a persistent acid-soluble pool. There is good reason to assume that the same applies to ^{3}H-TdR (2).

The LI of the hepatocytes in the controls reached 8% for ^{125}IUdR labeled cells and 14% for ^{3}H-TdR labeled cells (Fig. 2). From the day of hepatectomy (day 0) it rose to 24 and 64%, respectively, at day 4. Whereas, the ^{3}H-TdR-index continued to rise, reaching almost to 90% at day 8, the ^{125}IUdR-index fell sharply.

A somewhat similar behavior was reflected in the mean grain counts. For ^{3}H-TdR, MGC rose continually from 6 grains (day 0) to 28 grains/labeled cell. In the ^{125}IUdR labeled cells the values plateaued between days 2 and 4, and then fell to about 4 grains/labeled cell at day 8. The ^{3}H-activity increased steadily to a level about 40-fold higher than in the controls at day 8. In contrast, maximal ^{125}I-activity was only 4-fold greater than that of controls at day 4. It again decreased to the control value at day 8. Unlike hepatocytes, over 90% of the leukocytes present in liver imprints were labeled throughout the 8-day period (Table I). Although both the mean grain counts per cell and activity per 100 cells fell after 4 days, we had difficulty in obtaining accurate grain counts of single cells because of extremely heavy labeling.

Discussion. High regenerative or proliferative activity of liver occurs within 16 to 24 hr after partial hepatectomy (9–12). We found that ^{3}H incorporation as determined by grain counts increased almost 40-fold be-

TABLE I. ^{3}H-TdR Labeled Leukocytes in Liver Imprints.

After hepatectomy[a] (days)	Labeling index (% labeled cells)	Mean grain count (grains/cells labeled)	Activity/100 cells (grains)
0	93.4 ± 0.8	48 ± 5	4480 ± 485
2	85	51	4335
4	98.0 ± 0.2	35 ± 2	3430 ± 215
8	97	34	3298

[a] Time interval 0 is represented here by 3 animals, time interval 4 by 2 and time intervals 2 and 8 by 1 mouse.

tween the time of partial hepatectomy and 8 days postsurgery despite the fact that radioactivity would be expected to gradually decrease becase of "dilution" of label due to cell proliferation (13). Thus, there must be additional sources for ^{3}H-TdR reutilization to that operative *in situ*. These are the gut from which nucleosides can enter regenerating liver tissue via the portal circulation (3) and leukocytes which upon migration into the liver disintegrate and liberate DNA (14). Although Bryant (15) estimated that about 5 to 10% of leukocyte DNA-thymidine can be reutilized by proliferating hepatocytes, his estimates of the amount of TdR reincorporation would not suffice to explain the degree of increase in liver cell activity observed in our study. It has been shown previously that label from DNA of intestinal epithelium following sloughing is reabsorbed (16, 17) and enters first the liver (3). Indeed, periportal hepatocytes proliferate to a greater extent than other liver cells within an hepatic lobule (9, 18, 19), and thymidine kinase(s) is elevated in regenerating liver (20, 21).

An important finding of our studies is the reutilization of ^{125}I-UdR by regenerating liver. Although it is from 10 to 35 times less efficient than that of ^{3}H-TdR (^{3}H–^{125}I), it is nevertheless significant and perceptible in contrast to other proliferating tissues such as bone marrow (4–6), where cells proliferate at all times, and where the magnitude of IUdR reutilization is unrecognizable by autoradiography of single cells (6). We connot exclude the possibility that polynucleotide chains (22, 23), as well as nucleosides are incorporated into DNA of proliferating cells; yet, if this were the case, the discrimination between ^{3}H-TdR and ^{125}IUdR should be less obvious.

Summary. Parallel studies of ^{3}H-TdR and

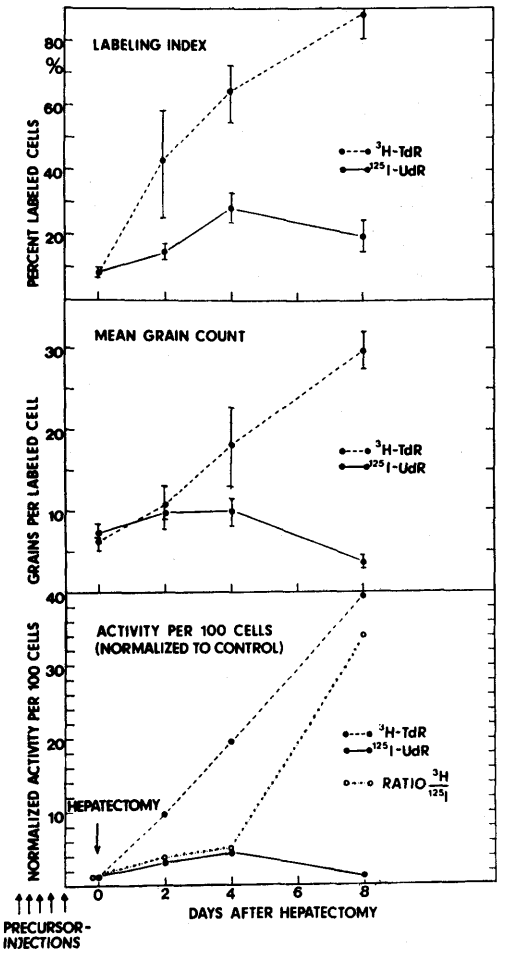

FIG. 2. Labeling indices, mean grain counts and total activity of hepatocyes in liver after partial hepatectomy (means ± standard errors).

^{125}I-UdR incorporation into regenerating liver following repeated injection into female Swiss albino mice yielded the following results: (i) both ^{3}H-TdR and ^{125}I-UdR are reutilized, although ^{3}H-TdR to a considerably greater extent; (ii) in addition to local reutilization, the data are explained by labeled precursors entering the regenerating liver from the intestine via the portal circulation. Precursor supply from immigrated leukocytes also occurs; and (iii) since a bypass of the thymidine kinase salvage pathway is unlikely, the incorporation of tracer into the regenerating liver must occur from the nucleoside level.

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