Protein Metabolism among Lobes of the Rat Liver in Relation to Site of Radioisotope Injection[1] (34137)

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(Introduced by J. N. Hayward)
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Our interest in the liver is concerned with possible differences of protein metabolism among liver parenchymal cells (1, 2) using the simple liver acinus as a reference point (3). However, we have routinely removed only one liver lobe for these studies, tacitly assuming that the removed lobe was representative of the entire liver. Yet there has been evidence for a bilateral or streamlined blood flow in the portal vein (4) which could influence protein metabolism among the various liver lobes. In view of this evidence, coupled with the enormous number of studies done on all aspects of protein metabolism in rat liver we decided to investigate the possibility that rat liver lobes may not be identical with respect to general protein metabolism.

Materials and Methods. Injection and perfusion. Under ether anesthesia male Sprague-Dawley rats (150–180 g) were injected via the external jugular vein with L-leucine-4,5-^{3}H (40 Ci/mmole, Schwarz BioResearch, Orangeburg, New York) at a dosage of 15 μCi/g. Most rats were injected between 10 a.m. and noon. Shortly before being dispatched the rats were reanesthetized and taken to a cold room (4°) where subsequent procedures were done. The portal vein was visualized by a midline incision and the liver was perfused free of blood with citrate–saline (1). Each lobe was dissected free and placed in a separate dish of citrate–saline. We used our own system of nomenclature,[2] which is essentially that of Gershbein and Elias (5). As a control the median lobe was severed along the falciform ligament into right median (RM) and left median (LM) lobes. These parts of the median lobe do receive their own branch of the portal vein (Fig. 4).

Homogenization and centrifugation. A small portion of each lobe was minced with a scalpel and weighed to the nearest 0.01 g. The minced liver was homogenized in 0.25 *M* sucrose–0.001 *M* EDTA (4 ml/g of wet wt) with a Teflon pestle for 6–10 rapid passes at 1000 rpm. Samples of the homogenate were placed in small plastic centrifuge tubes and centrifuged in a Beckman Minifuge at near 15,000 g for 10 min to sediment whole cells, nuclei, and mitochondria; allowing microsomes, soluble proteins, and free amino acids to remain in the supernatant fluid.

Fractionation and scintillation spectrometry. Precipitates were obtained from the supernatant fluid by addition of an equal volume of 10% TCA–0.5% sodium tungstate (TCA-T) (6). The tubes were allowed to stand at 4° for 10 min then the precipitate was centrifuged down. Samples (50 μl) of the resulting supernatant fluid (TCA-T soluble) were transferred to scintillation vials and the remaining sediment was washed once with TCA-T. After this second centrifugation the tip of the plastic tube which contained the washed sediment (TCA-T insoluble) was cut away and dropped into a scintillation vial. One ml of N.C.S. solubilizer (Amersham-Searle, Des Plaines, Illinois) was added to all vials. The TCA-T soluble samples were instantly solubilized but the TCA-T insoluble samples were incubated overnight at 50° to effect solution. Then 10 ml of scintillation fluid (7) were added to each vial and radi-

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[2] Abbrev. for the various lobes are: anterior caudate, AC; posterior caudate, PC; right posterior lateral, RPL; left median, LM; right anterior lateral, RAL; right median, RM; left lateral, LL.

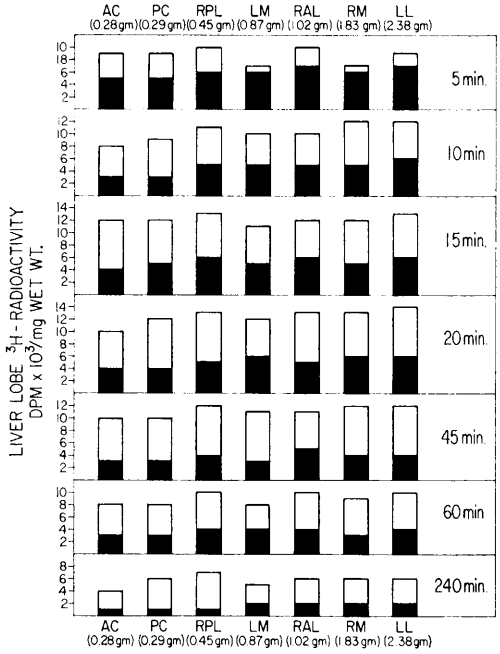

FIG. 1. Radioactivity in liver lobes at times after injection of leucine-^{3}H in the jugular vein. Values are averages of three determinations. The standard error of the mean was small in all cases and is omitted for clarity. Open block is the TCA-T insoluble fraction; solid block is the TCA-T soluble fraction. See footnote 1 for explanation of lobe abbreviations. Average lobe weights are in parentheses under the lobe abbreviations.

oactivity in the samples was estimated in a Mark I scintillation spectrometer (Nuclear Chicago, Des Plaines, Illinois) as described previously (7). Afterwards the appropriate calculations were done in order to convert cpm to dpm/mg of wet wt for each lobe at each time interval.

Liver lobe weights. The livers of six rats were perfused as described above. Each lobe was dissected free, blotted and weighed to the nearest 0.01 g.

Intraorgan injections. To test for the preferential flow of blood from various organs into specific liver lobes the following experiments were done. Only two animals were used for this study. One rat received 0.36 mCi of leucine-^{3}H (0.06 ml) directly into the spleen. The other rat was given 0.90 mCi of leucine-^{3}H (0.15 ml) into the lumen of the jejunum. The viscera in both animals were visualized by small incisions and were not handled at all during the injections. Both rats were maintained under either anesthesia in a prone position for 15 min when the livers were perfused and radioactivity in the various lobes was estimated as described above.

Results. Radioactivity after jugular injection. Figure 1 shows the radioactivity in TCA-T soluble and insoluble fractions from the various lobes at different times after radioisotope injection. Values of the TCA-T soluble fraction for all lobes at any time were quite similar. In general the radioactivity was already high by 5 min, decreased at 10 min, increased again at 15–20 min then diminished thereafter. Values for the TCA-T insoluble fractions were also very similar to each other, attaining peak levels at 15–20 min and then decreasing slowly during the rest of the experiment.

Correlation of radioactivity to lobar weight. The average weight of each lobe from the six determinations is seen under the abbreviation of each lobe in Fig. 1. When the values of dpm/mg of wet wt of the TCA-T insoluble fractions from each lobe were multiplied by their respective average weights in milligrams, an estimate of total radioactivity in the lobes at each time interval was obtained. Correlation diagrams were constructed by plotting total calculated lobar radioactivity against average lobar weight for each time interval. As seen in Fig. 2, a representative correlation plot for the 15-min values exhibited an almost perfect positive correlation between lobar weights and total lobar radioactivity.

Radioactivity after intraorgan injection. The results of the jejunum injection are depected in the top of Fig. 3. Contrary to the data from the jugular vein injection there was a difference in the amount of radioactivity in the liver lobes. The lobe with most TCA-T insoluble radioactivity was LM (not the heaviest) whereas the lobe with the least radioactivity was RPL (not the lightest). Data from the splenic injection seen in the bottom of Fig. 3 also show a difference in lobar radioactivity/mg of wet wt. In this case RAL was highest (not the heaviest) and AC was lowest in value (the lightest lobe). At

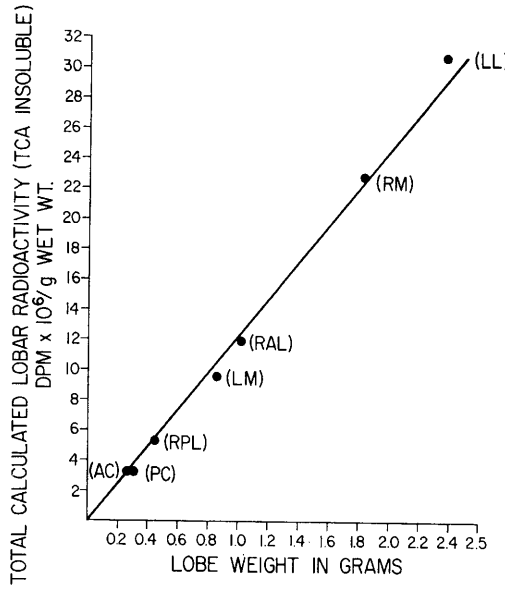

FIG. 2. Correlation plot between total calculated lobar radioactivity and lobe weight 15 min after jugular injection of leucine-^{3}H. Abbreviations as in footnote 1.

first glance there seemed to be no correlation in either case between lobe weight and TCA-T insoluble radioactivity. But on further study the data did fall into two groups, a right group (RPL and RAL) and a left group (the remaining lobes) (see Fig. 4 for portal vein branches to the lobes). With this grouping in mind it became apparent that the right group exhibited a correlation between lobar size and radioactivity content in both the jejunum and splenic injections. The left group showed this correlation also but only with the splenic injection (see Fig. 1 for lobe weights). The TCA-T soluble values were not as high and essentially mirrored the TCA-T insoluble amounts.

Discussion. The differences in radioactivity of the TCA-T insoluble fractions after the jugular vein injection (Fig. 1) are not considered significant since these variations could be due to numerous biological variables in addition to inherent error in the technical aspects of the experiment. The positive correlation between lobe weight and total calculated lobar radioactivity would, in simplistic terms, show that large lobes received more leucine-^{3}H than small lobes and in the correct proportion. Also since all of the lobes attained peak labeling at nearly the same time (15–20 min) we conclude that the various lobes of the rat liver conduct general protein metabolism in nearly identical fashion. Hence protein synthetic data obtained from any lobe of the rat liver can confidently be ascribed to the entire organ. This generalization cannot be applied to the cells *within* any lobe since one of us, LeBouton (1, 2) has demonstrated differences in protein metabolism among liver cells at the tissue level. However, other studies have shown uniformity among liver lobes with respect to water, oxygen, hematocrit, fatty acid, and cholesterol content (8–10).

Reviewing the data from the visceral injections we must conclude there is a streamlining of blood flow in the portal vein of anesthetized rats in a prone immobile position. Whether this also pertains to the conscious upright animal cannot be stated. This phenomenon of streamlining of blood flow in the portal vein has been reviewed (11). It ap-

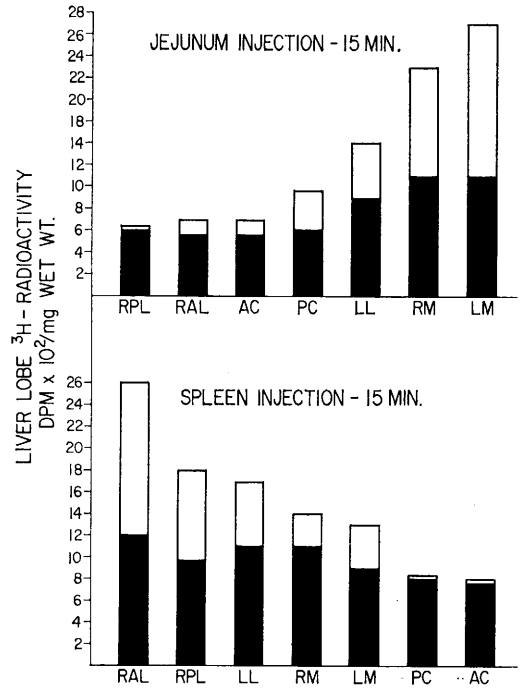

FIG. 3. Radioactivity in liver lobes 15 min after injection of leucine-^{3}H into the jejunum (top) and spleen (bottom). Expanations as under Fig. 1.

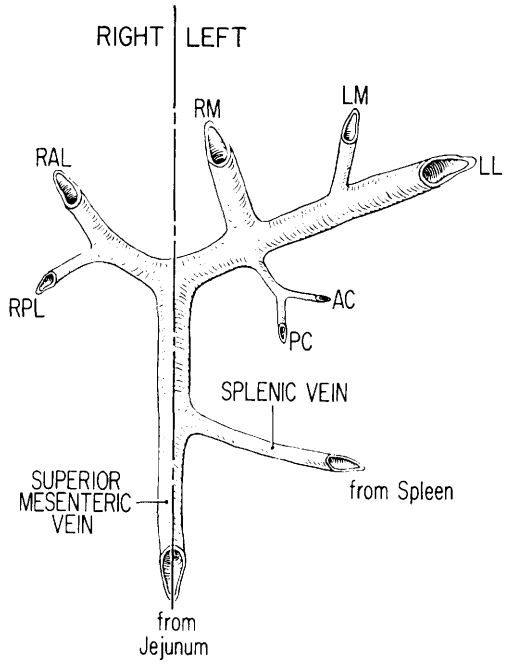

FIG. 4. Drawing of the two main tributaries of the rat portal vein and its subsequent lobar branches; this drawing is compiled from numerous personal dissections and published work by Gershbein and Elias (5).

pears that under the conditions of the present experiment blood from the spleen does preferentially enter the extreme right liver lobes (RAL and RPL) and does so in relation to lobe size. Reference to Fig. 4 would show that this requires "crossing-over" of blood streams, which has been shown to occur in experimental models (12). The study by Barnett and Cochrane (12) also showed that crossing-over was more likely to occur when the small tributary joined the larger one at near a right angle, which is the case for the splenic vein in the rat (Fig. 4). Conversely, blood from the jejunum enters primarily the left side of the liver but does not enter lobes in relation to their size, that is jejunum blood is not evenly distributed to lobes in the left side of the rat liver. In order to explain this discrepancy and since we have already assumed streamline flow in the portal vein, we would further assume there is a streamline flow in the superior mesenteric vein such that blood from the jejunum preferentially enters the LM and possibly RM lobes. Reference to Figs. 1 and 2 reveals that both these lobes are smaller than LL, yet they contained more radioactivity than LL after the jejunum injection. The fact that all lobes were eventually labeled in the visceral injection studies is not surprising since 15 min would allow adequate time for recirculation of the leucine-^{3}H.

The data presented here also emphasize that caution should be used when extrapolating animal data to the human. For example, it is well known that portal vein blood flow in the human is the exact opposite to that found here in the rat, *e.g.*, primary carcinomas of the small intestine usually metastasize to the right half of the liver.

Summary. Protein metabolism among rat liver lobes was studied by leucine-^{3}H injections coupled with liquid scintillation spectrometry of TCA-tungstate soluble and insoluble fractions. All liver lobes appeared to metabolize protein in identical fashion when the label was injected in the external jugular vein. On a per milligram of wet weight basis, the lobes exhibited similar values of radioactivity at the various experimental times. When this data was converted to total radioactivity per lobe a positive correlation was revealed between total lobe radioactivity and lobe weight. However, when the radioisotope was injected directly into the spleen, it was the right side of the liver which contained most radioactivity 15 min later. Whereas leucine-^{3}H injection into the lumen of the jejunum resulted in lobes of the left side having most radioactivity 15 min later. This was interpreted as a crossing-over of streamlined blood flow in the rat portal vein under these experimental conditions.

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