ASSESSMENT OF LIVER REGENERATION IN THE RAT USING THE GAMMA CAMERA

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A method of imaging the liver of the rat using a pinhole collimator and a gamma camera is described. The method allows imaging of other organs in small animals. Radioisotopic scintigraphs of the rat liver using ^{99m}Tc-sulfur colloid and ^{75}Se-L-selenomethionine have been obtained before and at intervals after partial hepatectomy. The sizes of the scintigraphs of the two compounds were closely similar and there was no obvious difference in their distribution within the liver, either before or at any time after partial hepatectomy. A close correlation was found between the mean area of the lateral scintigraphs of the liver and liver weight ($r = 0.95$), and a formula was derived whereby liver weight could be calculated from the size of the scintigraphs. Following two-thirds partial hepatectomy in the rat the remnant was found to regain its normal mass within 7–10 days. The results support the use of technetium-sulfur colloid in the clinical assessment of liver regeneration after hepatectomy.

This paper reports the development of a technique for obtaining scintigraphs of organs in small animals and a subsequent study of the uptake of the radiopharmaceuticals ^{99m}Tc-sulfur colloid (TSC) and ^{75}Se-L-selenomethionine (SM) by the regenerating rat liver. TSC is concentrated by the von Kupffer cells of the reticuloendothelial system whereas SM, being an amino acid analog, is concentrated by the hepatocytes (*1*). The suggestion has been made that differences might occur in the uptake of these two compounds in regenerating liver tissue (*2*). We have therefore been interested in developing a means of investigating the uptake of these substances by the liver both before and after partial hepatectomy. From scintigraphs of the rat liver we have obtained measurements which allow calculation of the mass of the organ and thus in vivo assessment of liver size after partial hepatectomy is possible.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing between 172 and 550 gm were used. The animals were bred in the Animal Unit at the Welsh National School of Medicine, weaned at 3 weeks, and subsequently maintained on Spillsbury's breeding diet. All experiments were carried out under general anesthesia induced with ether and continued with intraperitoneal nembutal (60 mg/ml 0.07 ml/100 gm body weight).

Partial hepatectomy was performed by the method of Higgins and Anderson (*3*). In order to avoid superimposition of the splenic and hepatic TSC scintigraphs, splenic mobilization (*4*) was carried out. Splenectomy was not performed in order to disturb the portal circulation as little as possible.

TSC scans were obtained after the isotope had been injected into the inferior vena cava at the time of splenic mobilization (prehepatectomy scintigraphs, Fig. 1A). This was carried out 1–3 days before partial hepatectomy. Immediately after partial hepatectomy, TSC was again injected into the inferior vena cava and the scintigraphs repeated (posthepatectomy scintigraphs, Fig. 1B).

Animals were allowed to survive for intervals up to 44 days after partial hepatectomy. A mixture of SM and TSC was then injected into the internal jugular vein and scintigraphs of both compounds obtained (antemortem scintigraphs, Figs. 1C and 2). The animals were then killed and the liver removed, weighed, laid on the cork-board, and scintigraphs of both compounds again obtained (postmortem scintigraphs, Figs. 2 and 3). In two animals the other

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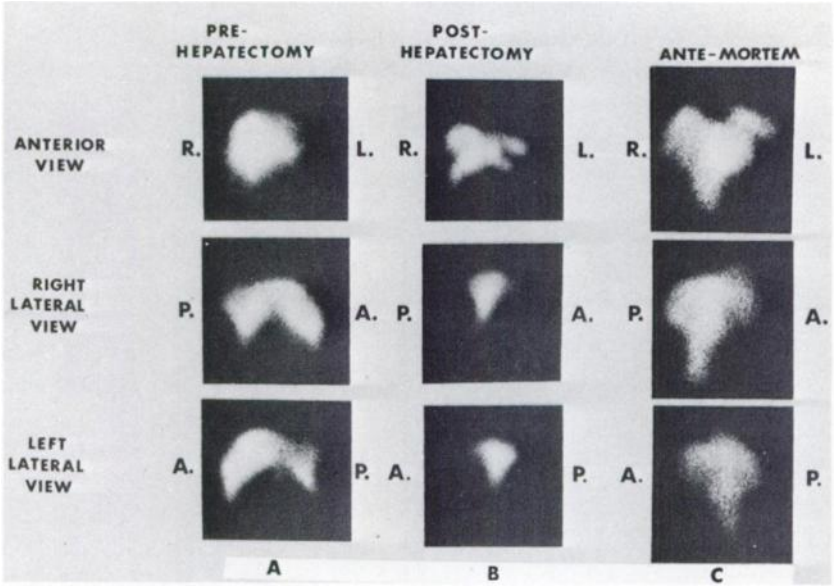

FIG. 1. Typical technetium-sulfur colloid scintigraphs. Three views obtained (A) before partial hepatectomy. (B) immediately after partial hepatectomy, and (C) before sacrifice (in this case 22 days post-hepatectomy). Note removal of anterior lobes is best seen in lateral views. R = right; L = left; A = anterior; P = posterior.

abdominal viscera were also removed, displayed alongside the liver, and scintigraphed (Fig. 2).

In two further animals pre- and posthepatectomy scintigraphs were obtained and the animals were then scintigraphed sequentially with TSC which was injected into the jugular vein at intervals from 1 to 99 days after operation.

It was found that, if both pre- and posthepatectomy scintigraphs were attempted on the same day, some animals died. A minimum of 24 hr was therefore allowed between the procedures.

Scintigraphic techniques. It is not possible to obtain well-defined scintigraphs of organs as small as the liver of the rat using a gamma camera and a parallel-hole collimator. However, adequate magnification was obtained using a simple pinhole collimator constructed from lead blocks (Fig. 4). The gamma camera used was a Nuclear-Chicago Pho/Gamma III. The pinhole in a lead block 2.5 cm thick had a minimum diameter of 4.4 mm and was 37 cm from the crystal face and 12 cm from the surface on which the animal was positioned.

Technetium-99m-sulfur colloid was prepared by the method of Larson and Nelp (*5*). An activity of 500 μCi in a volume of 1 ml or less was routinely given so that there was a relatively low background count compared with counts obtained from the liver, and 200,000 counts were obtained within 3 min. Rapid concentration of the compound in the liver enabled scintigraphs to be commenced within 2–3 min of injection. Since ^{99m}Tc has a half-life of 6 hr, after 24 hr the residual activity is very small.

Selenium-75 has a half-life of 121 days and, although biological excretion of L-selenomethionine occurs, there was significant residual activity in the liver even a week or more after a previous injection. This made interpretation of repeat scintigraphs difficult. The time taken for each view (100,000 counts) usually exceeded 10 min because of the low counting rate from the activity of 100 μCi (approximately 100 μCi/ml) that was given. The background count was therefore relatively higher than for ^{99m}Tc and the definition of the ^{75}Se scintigraphs was consequently poorer.

Because of the wide difference in the energy of the gamma rays emitted by the two compounds, it was found possible to obtain separate scintigraphs despite simultaneous injection. TSC made no contribution to the SM scintigraphs and the SM contributed no more than approximately 10% to the TSC scintigraphs. The TSC scintigraphs were ob-

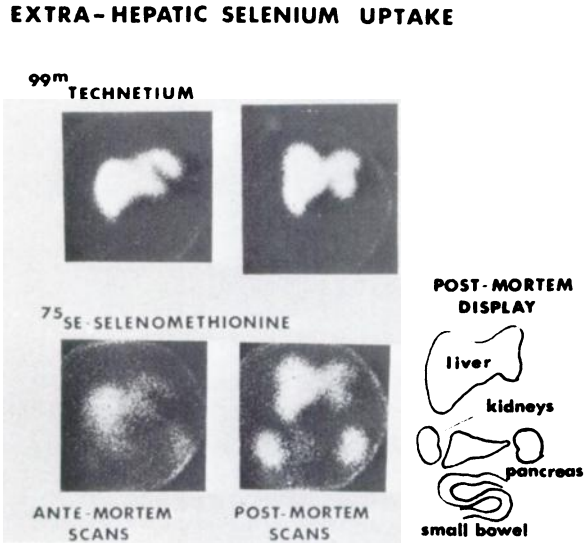

FIG. 2. TSC and SM scintigraphs in one animal immediately before sacrifice (left), showing distortion of hepatic image caused by uptake of selenium in other organs. These are shown separated in postmortem scintigraphs (right).

tained first because the much higher counting rate made it easy to locate the liver and thus position the rat under the collimator. Anterior (supine), right, and left lateral views were taken by rotating the animal on the corkboard. One view only, an approximate anterior view, was obtained for the postmortem scintigraphs because of the difficulty in fixing the removed liver for other views. All the scintigraphs were recorded on Polaroid film.

Size of the scintigraphs. The outline of the scintigraphs was traced by one of us (KGL). The photographic exposure influences the size of the scintigraph so that it must be neither under- or overexposed. A two-lens camera was used, and the exposure nearer the correct value was chosen. The edge of the organ image was most often quite definite, and its outlining was accomplished without difficulty. The area was then measured by another of us (SJK) by counting the number of square millimeters within the outline without knowledge of the animal from which the scintigraphs had been obtained. A number of variables obtained from the scintigraphs were evaluated. These were the area of the anterior view (A_a), the mean of the two lateral areas (A_L), and a volumetric figure (V) calculated from these figures in the following manner. The complete calculation of the volume of an irregularly shaped organ is complex, but an attempt was made to combine the information available from the two areas, A_a and A_L, in a relatively simple way. For a sphere the true volume may be calculated by multiplying the area (A_a) obtained from one view by the square root of the area (A_L) obtained from a view at right angles. If the volume is elongated, for example, as in the case of an ellipsoid, an error is introduced which, provided the appropriate views are obtained, may be corrected by dividing by the square root of the ratio of the axes of the ellipse area A_L. For other volumes in general this is not strictly true, but the calculation does yield a parameter which is dimensionally correct and which it was felt could be nearer the true value than either area alone. Hence, V was calculated using the following formula:

$$V = A_a \times A_L^{1/2} \div R^{1/2}$$

where R is the inferior-superior diameter divided by the anterior-posterior diameter. (Both diameters were the mean values for the two lateral images.)

Differences in image size because of the difference in gamma-ray energies of ^{99m}Tc and ^{75}Se are possible. To check this and obtain correction factors, circular phantoms of areas between 100 and 600 mm^2 were imaged for both isotopes and the image areas compared.

TABLE 1. PERCENTAGE RESECTION IN 81 ANIMALS USING TECHNIQUE OF HIGGINS AND ANDERSON

Range of body weight	73–564 gm
Mean % resection	66.8
s.d.	2.2

Magnification was checked each time an experiment was performed using a radioactive line source and at no time did differences exceed ±5% of the mean value. All measurements were corrected for any variation in the magnification.

Liver weight. The weight of the liver resected at partial hepatectomy and that of the regenerated liver removed at sacrifice were measured directly after washing and removal of surplus moisture. The weight of the liver both before and after partial hepatectomy could not be measured directly. The methods for obtaining these liver weights were as follows. The resected anterior and left lateral lobes of the rat liver constitute 67% of the total liver weight (*3*), and this relationship has been confirmed in our laboratory (Table 1).

From this information:

Total liver weight =
Resected liver weight × 1.49

and

Liver remnant weight =
Resected liver weight × 0.49.

Many of the animals increased considerably in body weight after partial hepatectomy, and it proved necessary to allow for this growth (*6*). The total liver weight at the time of partial hepatectomy was calculated as described above, and the liver weight at any time later was calculated according to the increase in body weight. This was done after measurements had been obtained in 56 animals in our laboratory and the liver weight to body weight ratio shown by direct measurement to be:

Liver weight/body weight =
5.91 − 0.0028 body weight

(Liver weight/body weight in percent
and body weight in grams).

RESULTS

The magnification achieved by using a pinhole collimator with a gamma camera gave scintigraphs of the rat liver of similar size and definition as those

TABLE 2. COMPARISON OF AREAS OF SM AND TSC SCINTIGRAPHS

Time after partial hepatectomy (days)	Percentage difference*
0.75	+7
0.75	+9
0.75	+4
1	+10
2	−6
2	−1
3	−10
3	−3
4	−1
5	−3
7	−1
9	+6
15	0
22	−1
28	−7
44	−6

* $\left(\frac{^{75}\text{Se area} - ^{99m}\text{Tc area}}{^{99m}\text{Tc area}}\right) \times 100.$

obtained in gamma camera studies of the human liver.

The TSC scintigraphs were of better definition and were more quickly obtained than those obtained after injection of SM (Fig. 2). In addition, TSC scintigraphs could be repeated within 24 hr because of the low residual activity. Although TSC was concentrated by the spleen, splenic mobilization separated its image from that of the liver. SM was concentrated not only by the liver but also by the small bowel, kidneys, and pancreas so that the outline of the antemortem SM hepatic scintigraphs was distorted by the uptake in these organs.

The TSC and SM scintigraphs obtained at postmortem were compared. Allowance was made for the effect on image size of the different gamma-ray energies of the two isotopes. The scintigraphs of the circular phantoms revealed that above 250 mm², the technetium scintigraphs were a constant 2.5% larger in area than the selenium scintigraphs. Of the 16 postmortem scintigraphs compared, 13 were 250 mm² or larger. For the other three, the largest correction factor was 1.075. Although there were small differences in the areas of the TSC and SM postmortem scintigraphs especially in the early stages (Table 2), these may be due to the limitations of the method. No differences were visible in corresponding parts of the TSC and SM scintigraphs (Fig. 3).

Information derived from the antemortem TSC scintigraphs was compared with directly measured liver weight (W). The correlation coefficient between A_a and W was 0.88 and that between the calculated volume V and W was 0.90 whereas that between $A_{I,}$ and W was 0.95. Of these variables it was found that not only did $A_{I,}$ correlate best with W but also it was easily obtained. $A_{I,}$ was therefore chosen as being the most convenient and accurate index of liver size derived from the scintigraphs.

It was for the group of antemortem scintigraphs alone that it was possible to obtain the weight of the liver by direct measurement whereas the liver mass at the time of pre- and posthepatectomy scintigraphs could be obtained only by indirect estimations (vide supra). Not surprisingly, the correlation between $A_{I,}$ and W for these latter groups was markedly inferior (prehepatectomy scintigraphs $r = 0.47$, $p < 0.01$; posthepatectomy scintigraphs $r = 0.72$, $p < 0.001$).

From the values of $A_{I,}$ for the antemortem scintigraphs and the directly measured liver weight (W) in 21 animals (Fig. 5), the linear regression equation was derived:

$$W = 0.026\, A_{I,} - 0.48$$

$$(W = \text{gm} : A_{I,} = \text{mm}^2).$$

It was thus possible, knowing the values of $A_{I,}$, to calculate the weight of the liver.

The weight of the liver at various times after par-

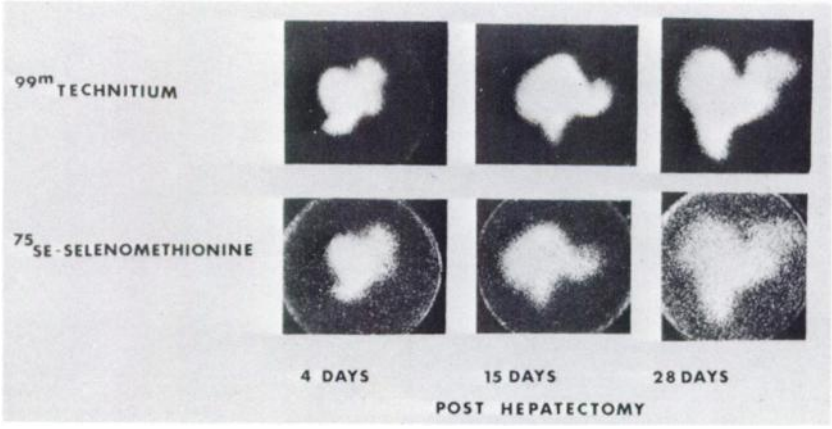

FIG. 3. Postmortem TSC and SM scintigraphs showing close similarity of shape and size and distribution of compounds.

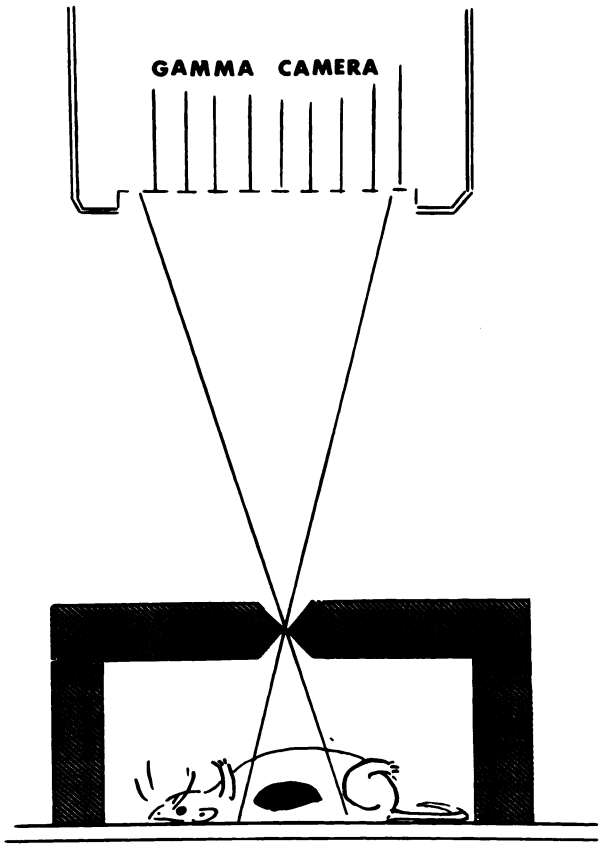

FIG. 4. Diagram illustrating magnification achieved with pinhole collimator.

tial hepatectomy was calculated from the antemortem scintigraphs and compared with the original weight of the liver calculated from the percentage resection. The resultant percentage was plotted against time. This showed that the liver returned to its preoperative size in approximately 7–10 days. This observation was confirmed in the same animals by comparing the directly measured postmortem liver weight with the calculated prehepatectomy weight. The resultant percentage is plotted against time in the same way (Fig. 6). In these calculations, allowance was made for any change in liver weight resulting from change in body weight, using the methods described above.

In the two animals which were scintigraphed repeatedly with TSC following partial hepatectomy, weights calculated from the scintigraphs were compared with the prehepatectomy weight. The resultant percentages, after correction for changes in body weight, were plotted against time (Fig. 7). No increase in this percentage was observed after 7 days. This is in agreement with the results found in the animals killed at varying intervals after partial hepatectomy.

DISCUSSION

Much interest has been shown recently in the use of radioisotopic imaging in the diagnosis of liver disease and also in the assessment of the size of the liver after hepatic resection (*7–10*). Several authors (*11,12*) have shown reasonable correlation between scan size and liver weight.

This study was undertaken to develop an experimental method for the radioisotopic imaging of the liver in the rat, first to assess the possibility of following the process of liver regeneration by such methods and second to compare the hepatic scintigraphs of TSC and SM before and after partial hepatectomy.

The technique has shown that it is possible to obtain well-defined scintigraphs of the rat liver and demonstrate the morphology of the organ sufficiently

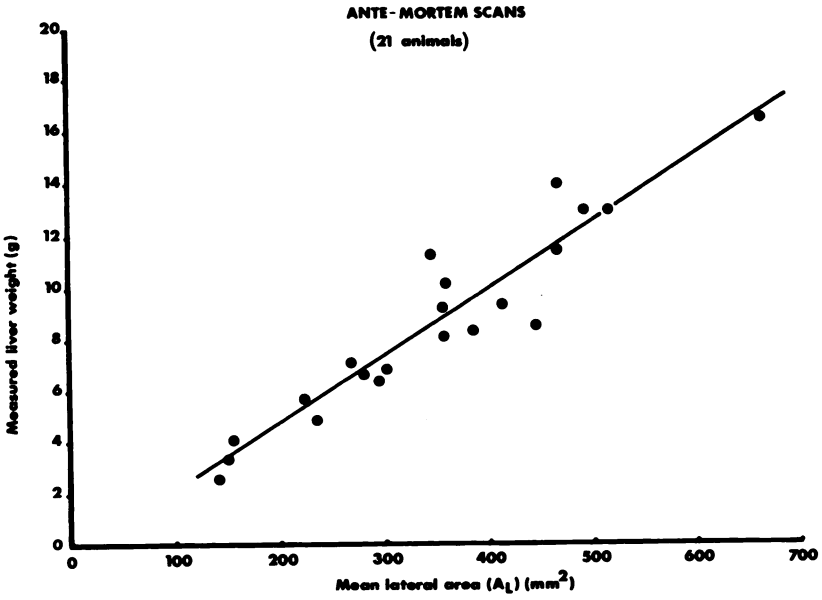

FIG. 5. Graph showing correlation between antemortem scintigraph size and liver weight from 5 min to 44 days after partial hepatectomy ($r = 0.95$, $p < 0.001$).

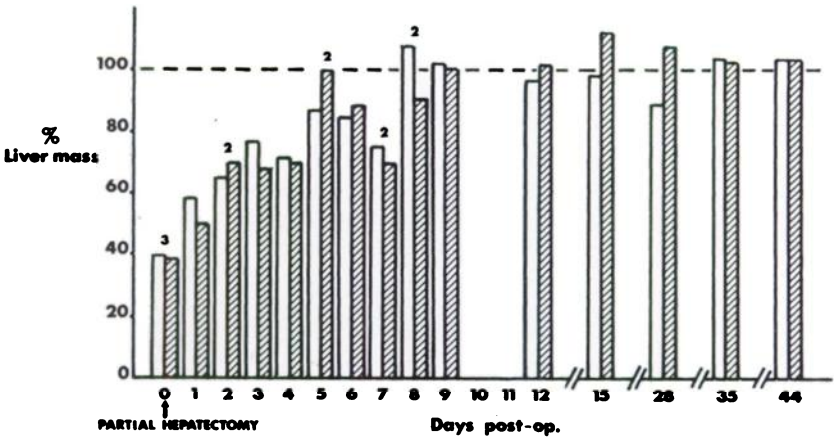

FIG. 6. Histogram illustrating growth of liver remnant after partial hepatectomy. Size of liver remnant is expressed as percentage of calculated prehepatectomy value. Hatched columns represent percentage calculated from weight measurements. Open columns represent percentage calculated from scintigraphs. Columns under figures represent mean values for numbers of animals.

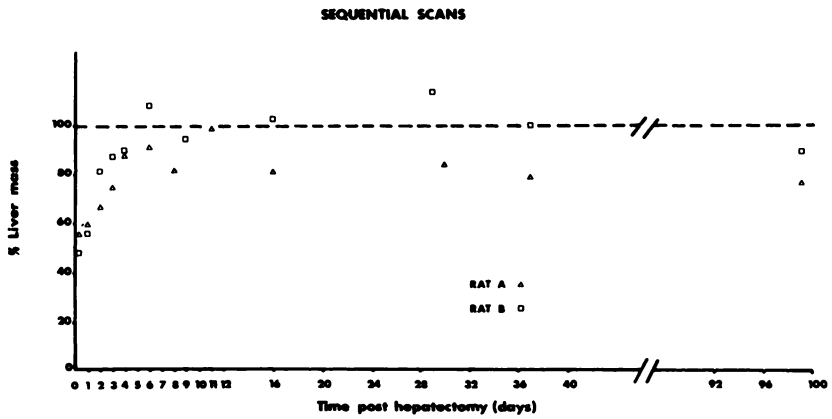

FIG. 7. Graph showing growth of liver remnant after partial hepatectomy in two animals scintigraphed sequentially. Size of liver remnant is expressed as percentage of calculated prehepatectomy value.

accurately to allow estimations of its size. In particular, the anatomical changes resulting from the removal of the anterior two thirds of the liver were best reflected by the lateral scintigraphs, and a close relationship was found between the mean area of the lateral scintigraphs and liver weight. The correlation was found to be closer for the antemortem scintigraphs ($r = 0.95$) than for either the posthepatectomy or preheptatectomy scintigraphs. This is not surprising since not only was the correlation with the antemortem scintigraphs based on actual liver weight but the pre- and posthepatectomy scintigraphs were obtained over a much smaller liver weight range.

It is interesting, that although there were obvious differences in the quality of the scintigraphs produced by the colloid and the amino acid, the size of the scintigraphs obtained with either compound were very similar. There were no obvious differences in the distribution of the compounds within the liver either before or at any time after partial hepatectomy. This is noteworthy, particularly in the immediate posthepatectomy period when much of the cellular energy is occupied with division and less is available for normal liver function (*13*). It might be expected that the hepatocytes would not take up selenomethionine so readily at this time. There was, however, no apparent delay or diminution in the uptake of this compound. Further, the accurate reflection of liver size by technetium-sulfur colloid, even in the initial postoperative period, and the clarity of the scintigraphs produced by this substance showed it to be ideal for assessing liver growth after hepatectomy.

Rapid regeneration of the liver remnant after two-thirds partial hepatectomy in the rat is known to take place (*3,13,14*). Previous studies have been dependent on measurement obtained by killing the animals at intervals after operation. The technique we have evolved offers an accurate and reliable nonsacrificial method of assessing liver size in the rat and may be of value in the study of liver regeneration since it allows sequential analysis in the same animal. It has been estimated that complete replacement of liver mass occurs within 10–20 days (*15*). It is of interest that our studies indicate that following two-thirds partial hepatectomy in the rat, the liver remnant grows so that its weight approximates the preoperative liver weight within 7–10 days.

The pattern of rapid growth was not influenced by repeated interference with the animals nor by the radiation received by the liver. A 12-gm liver receives an absorbed dose of approximately 13 rads

on each occasion it receives 500 μCi of TSC. The liver remnant following partial hepatectomy receives an absorbed dose of about three times this magnitude, and the dose delivered to the regenerating liver is approximately proportional to the inverse of the size to which it has grown. Pizzarello and Witcofski (*16*) have shown that 500 rads given just before partial hepatectomy was necessary to prevent mitosis and that 250 rads was only partially effective.

Finally, it is clear that the use of a pinhole collimator with the gamma camera will produce scintigraphs of small organs and we would suggest that this technique holds promise for similar studies of other organs in small animals.

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REFERENCES

1. CROFT DN: *Diagnostic Uses of Radioisotopes in Medicine.* London, Hospital Medical Publication Ltd, 1969, pp 61–71

2. FLEISCHER MR, SHARPSTONE P, OSBORNE SB, et al: Liver scintiscanning in acute hepatic necrosis. B.I.R. Meeting "Investigations of the Liver, Spleen and Pancreas using Radionuclides", 1970

3. HIGGINS GM, ANDERSON RM: Experimental pathology of the liver: Restoration of liver of the white rat following partial surgical removal. *Arch Pathol* 12: 186–202, 1931

4. BLUMGART LH, LEACH KG, MCLACHLAN MSF, et al: Portal venous injection in the rat. *Gut* 12: 585–591, 1971

5. LARSON SM, NELP WB: Radiopharmacology of a simplified technetium-99m-colloid preparation for photoscanning. *J Nucl Med* 7: 817–826, 1966

6. LEACH KG, KARRAN SJ, BLUMGART LH: In vivo estimations of liver mass in the rat and the effect of change in body weight. In preparation

7. PARKER JJ, SIEMSEN JK: Liver regeneration following hepatectomy evaluated by scintillation scanning. *Radiology* 88: 342–344, 1967

8. DELAND FH, WAGNER HN: Regeneration of the liver after hepatectomy. *J Nucl Med* 9: 587–589, 1968

9. SAMUELS LD, GROSFELD JL: Serial scans of liver regeneration after hemihepatectomy in children. *Surg Gynecol Obstet* 131: 453–457, 1970

10. BLUMGART LH, LEACH KG, KARRAN SJ: Observations on liver regeneration after right hemi-hepatectomy. *Gut* 12: 922–928, 1971

11. FISCHER MR, WOLF F: Quantitative estimation of spleen size by scintigraphy. *German Medical Monthly* 9: 63–68, 1964

12. HOLZBACH RT, CLARK RE, SHIPLEY RA: Evaluation of spleen size by radioactive scanning. *J Lab Clin Med* 60: 902–913, 1962

13. WEINBREN K: Regeneration of the liver. *Gastroenterology* 37: 657–667, 1959

14. BRUES AM, DRURY DR, BRUES MC: A quantitative study of cell growth in regenerating liver. *Arch Pathol* 22: 658–673, 1936

15. HARKNESS RD: Regeneration of the liver. *Br Med Bull* 13: 87–93, 1957

16. PIZZARELLO DJ, WITCOFSKI RL: Regenerating rat liver: A good system for radio-biological studies? *Radiology* 100: 163–167, 1971