IN VIVO ASSESSMENT OF LIVER SIZE IN THE RAT

K. G. Leach, S. J. Karran, M. L. Wisbey, and L. H. Blumgart*

Welsh National School of Medicine and University Hospital of Wales, Cardiff, Wales, U.K.

In vivo assessment of liver mass in the rat was achieved using several parameters obtained by scintigraphy, including one computed using a Nuclear Data 50:50 analysis system. All correlated well with the directly measured liver weight. The simplest parameter, the area of the anterior view measured from the Polaroid scintigraph, was therefore the one preferred. The results so obtained were compared with those derived from the relationship between liver and body weights. It is suggested that this accurate in vivo measurement of liver mass is of value in the study of the pathophysiology of this organ.

The phenomenon of liver regeneration has been extensively investigated in the rat (*1*). In such studies the assessment of liver size has hitherto depended on either direct measurement necessitating sacrifice of the animal or indirect calculations.

We have previously described a method using the scintillation camera and a simple pinhole collimator for the in vivo determination of liver mass (*2*). We believe that this technique, which allows sequential assessment of liver size, is of value in studies of liver regeneration. Our previous observation did not permit either the accurate estimation of the size of the normal liver or of the liver remnant following resection. In those studies the size of the remnant was calculated using the relationship between its weight and that of the resected lobes (*2,3*). This relationship, however, is subject to considerable individual variation.

We have therefore extended our previous study, first to improve the accuracy of the determination of the liver remnant mass and second, to assess, in vivo, the mass of the normal rat liver. In a comparative study a separate group of animals was sacrificed and liver-to-body weight ratios obtained. A computer method of calculating liver mass has also been introduced.

METHODS AND MATERIALS

Male Sprague-Dawley rats were used throughout. The animals were bred in the Animal Unit of the Welsh National School of Medicine, weaned at 3 weeks, and subsequently maintained on Spillsbury 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). The animals used for scintigraphy weighed between 60 and 383 gm and those sacrificed to obtain a liver-to-body weight ratio between 60 and 370 gm.

Technetium-99m-sulfur colloid was used as the liver-imaging agent and was prepared by the method of Larson and Nelp (*4*).

In the normal animals the ^{99m}Tc-sulfur colloid (2 mCi) was injected into the inferior vena cava at the time of splenic mobilization (*5*). The spleen was mobilized in order to avoid superimposition of the spleen on the liver image. Splenectomy was not performed so as to disturb the circulation as little as possible.

Partial hepatectomy was in every case performed by the method of Higgins and Anderson in which 67% (s.d. 2.2%) of the liver is removed (*2,3*). Scintigraphs were obtained using a Searle Radiographics Pho/Gamma III scintillation camera. In our previous work (*2*) a pinhole collimator constructed from lead blocks was used but for this study a more conventional device was constructed (The Cardiff Sheet Metal and Engineering Co., Ltd., Cardiff, Wales, U.K.) The pinhole-to-crystal distance of 37 cm is the same as for the lead block system and is longer than in commercially available apparatus. This minimizes the problem of distortion and varia-

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For reprints contact: K. G. Leach, Dept. of Medical Physics, University of Hospital of Wales, Heath Park, Cardiff CF4 4XW, Wales, U.K.

* Present address: Dept. of Surgery, Royal Infirmary, Glasgow G4 OSF, Scotland, U.K.

tion in magnification due to positioning errors and differences in organ depth. The minimum diameter of the pinholes of both collimators was 4 mm.

The scintillation camera pulse-height analyzer was adjusted to 133–161 keV. To obtain the scintigraphs the animals were taped with limbs extended to a cork board. The surface of the board was 11.4 cm from the pinhole. The anterior as well as lateral views were obtained by turning the rat onto its side and retaping it to the cork board. Two hundred thousand counts were collected in about 40 sec for each view. The resultant scintigraphs were recorded on Polaroid film using a two-lens photographic camera. Thus two photographic exposures were obtained enabling the correctly exposed one to be used for the measurement of area.

For data collection for the computer calculation of volume, the animals were placed in a "tufnol" tube of 7.6 cm o.d. and 0.5 cm wall thickness. Cotton wool padding was used to position and maintain the animal in the center of the tube which was 8.8 cm from the pinhole. The tube was then positioned so that the liver image was visible on a variable persistance oscilloscope and the tube rotated to obtain the minimum diameter at right angles to the inferior–superior diameter. Data were then accumulated in the multichannel analyzer memory of a Nuclear-Data 50:50 analysis system. The matrix comprises a 64 x 64 array and counts were acquired until the maximum content of a channel was 1,000. The memory contents, termed a "frame," were then recorded on magnetic tape. The tube was rotated through 90-deg keeping the direction of its axis constant and the animal was rescintigraphed. Four views were obtained in this manner. The multichannel analyzer was interfaced to a PDP8/L computer that was used for the calculation employing for the most part standard programs supplied by Nuclear Data.

Scintigraphs obtained. Immediately following splenic transposition, the animals were scintigraphed both on the cork board (prehepatectomy scintigraphs) and in the tufnol tube (prehepatectomy frames). Partial hepatectomy was performed as soon as the prehepatectomy imaging was completed and the resected liver was washed, dried, and weighed. The animals were rescintigraphed by both methods (posthepatectomy scintigraphs and posthepatectomy frames) without further injection of ^{99m}Tc-sulfur colloid. Only 100,000 counts were collected for the Polaroid scintigraphs since this gave approximately the same data density over the reduced area of liver image as for the prehepatectomy scintigraphs. The maximum count collected in the analyzer memory was unchanged. Finally the animals were sacrificed and the remaining liver weighed.

Magnification. In order to allow for variation in image size, a line source phantom was scintigraphed each time an experiment was performed. To correct the areas measured from the scintigraphs only a relative value for the magnification is required. The phantom was therefore simply placed on the table top on which the cork board was laid. The most likely source of variation in magnification is the setting of the pinhole source distance and for this reason the line source was used in one orientation only and the magnification at right angles was assumed to vary in the same manner. The magnification was calculated by measuring the separation of the lines on the scintigraph and relating it to a standard value.

For the computation of the volume using the computer, an absolute measurement is necessary so that the matrix unit size may be determined. A mean value of magnification was therefore obtained by positioning the phantom at the same distance from the pinhole as the center of the tufnol tube. The lines of activity were positioned parallel to the rows of points in the memory array, a frame was obtained and, because the true area per matrix point was needed, the phantom was turned through 90 deg and data again collected. The size of the matrix units was obtained by dividing the known distance on the phantom by the number of spaces between the matrix points containing the maximum numbers of counts. This was repeated for three parallel rows of points in each direction and the mean values calculated.

Measurement of area of scintigraphs. An outline was drawn around the exposure which was considered to be the nearer to the correct density and its area measured. The anterior area (A_a) and the mean lateral area (A_l) were obtained and corrected for variation in magnification. In addition, a volumetric quantity was calculated by combining these two areas, thus

$$V = A_a \times A_l^{1/2}/R^{1/2},$$

where R is the inferior–superior diameter divided by the anterior–posterior diameter. Both values were obtained from the lateral views and are the mean values. R is introduced in an effort to correct for elongation of the liver. Multiplying $A_a \times A_l^{1/2}$ results in a quantity which has the dimensions of volume but is only proportional to the actual volume if the liver is spherical. If all the axes of a volume are different such as for an ellipsoid, a correction is necessary if the volume is to be calculated from the areas. Thus for an ellipsoid with axes a,b,c;

$A_a = \text{constant} \times ab$, $A_l = \text{constant} \times ac$, $R = a/c$

and hence: $V = \text{constant} \times ab \times (ac)^{1/2}/(a/c)^{1/2}$ $= \text{constant } abc$. Thus, provided the right views are obtained, V is proportional to the true volume. This

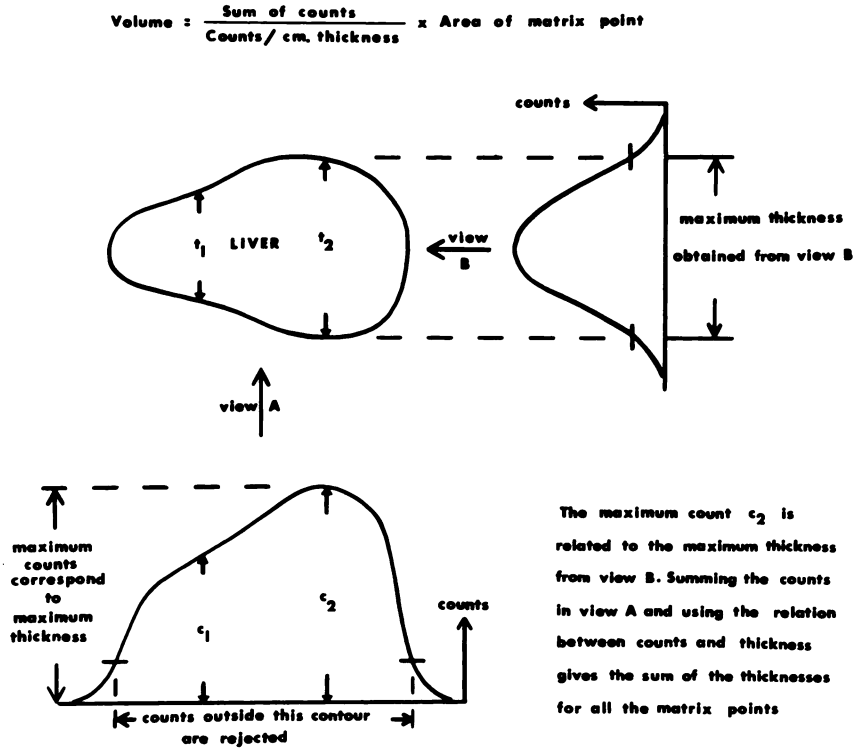

FIG. 1. Computer calculation of liver volume. Cross section of frames obtained from two projections at right angles are shown (c, count; t, thickness).

will not, in general, be true for a complex volume but it is an attempt to combine in a relatively simple manner the information from both areas.

Evaluation of volume by computer. The purpose of this calculation is the absolute derivation of the liver volume. Before any computation was undertaken, in order to reduce the effect of statistical variation, all the frames were smoothed by a four-point smoothing program (*6*).

The basis of the method is illustrated in Fig. 1. Two views only are shown in order to maintain clarity although four views were obtained. Similarly for simplicity, the illustration is drawn as if for a parallel-hole collimator. From view B, in which the liver has been turned to present a minimum thickness to the camera, a cross section is taken to give the value of the maximum thickness for that frame (t_2 in matrix units) at a previously determined isocount level (25%).

In view A, neglecting the effect of absorption, the contents of each matrix point may be considered to represent the thickness of the organ in "counts" at that particular point. In particular, the maximum count (C_2) may be equated with the maximum thickness obtained from view B and the counts per matrix unit thickness calculated (C_2/t_2).

Summing all counts above the 25% level gives the "total thickness" of the organ in "counts" for a cross-sectional area of one matrix unit. Thus the volume of the organ in matrix units is given by:

$$\text{Volume} = \frac{\text{sum from view A}}{C_2/t_2} \times (\text{area of matrix point})$$

This is converted to cm^3 by the magnification factors obtained with the line source.

Values were obtained as described using the two minimum-thickness views to give a mean value of the thickness. Totalization was then performed for both of the other views. Similar calculations were performed reversing the roles of views A and B. The mean of the four values thus calculated was used for the correlation calculation.

Phantom measurements. Measurements were made on a number of phantoms of known volume using a parallel-hole collimator and the volumes computed in the manner previously described. A number of different isocount levels were used in the computation and this indicated that the 25% isocount gave the optimum result (*7*).

Liver weight. (A) Total liver weight for the prehepatectomy animals was calculated as the sum of the resected liver weight and the liver remnant weight, both measured directly. (B) Liver remnant weight for the posthepatectomy animals was measured directly. (C) Fifty-six other animals were sacrificed, the livers were removed, weighed as already described, and the liver weight calculated as a percentage of body weight.

Statistical correlation. Correlation coefficients and regression lines were obtained using the BMD statis-

TABLE 1. PREHEPATECTOMY REGRESSION AND CORRELATION

Weight (gm) versus	Correlation coefficient	Slope (m)	Intercept (c)
Anterior area (mm^2)	0.973	0.0292 (5.5)*	−1.37
Mean lateral area (mm^2)	0.964	0.0336 (6.3)	−1.92
Volume (V)†	0.970	0.933 (5.5)	1.75
Computed volume (cm^3)	0.973	0.892 (5.5)	0.80

* Percentage s.e.
† Arbitrary units.

TABLE 2. POSTHEPATECTOMY REGRESSION AND CORRELATION

Weight (gm) versus	Correlation coefficient	Slope (m)	Intercept (c)
Anterior area (mm^2)	0.919	0.0156 (9.6)*	−0.21
Mean lateral area (mm^2)	0.936	0.0207 (8.7)	−0.40
Volume (V)†	0.937	0.738 (8.5)	0.86
Computed volume (cm^3)	0.957	0.873 (7.3)	0.18

* Percentage s.e.
† Arbitrary units.

tical programs (Health Sciences Computing Facility UCLA) and an ICL 470 Computer at the Cardiff Joint Computer Center.

RESULTS

Prehepatectomy scintigraphs and frames. The anterior area, the mean lateral area, the volumetric figure (V) obtained from the scintigraphs, and the volume computed from the frames were all correlated with the directly measured liver weight. The correlation coefficients and regression line slope and intercept are given in Table 1. In addition, the computed volume was plotted against the measured weight and the result is shown in Fig. 2. The other parameters show a similar variation. Twenty-one animals were used in this study.

Posthepatectomy scintigraphs and frames. The same correlations were made as for the prehepatectomy scintigraphs and frames and the results are given in Table 2. In addition the computed volume was plotted against the measured weight and the result is shown in Fig. 3. The other parameters show

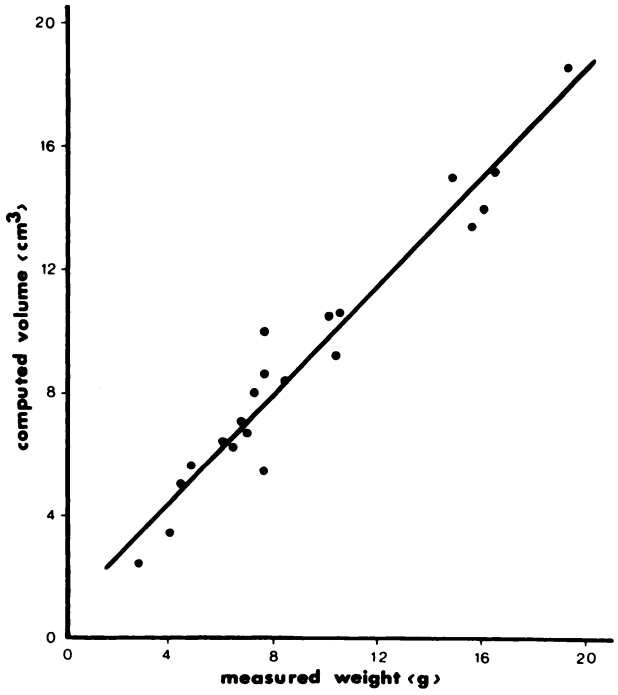

FIG. 2. Correlation of computed liver volume with liver weight for 21 prehepatectomy animals.

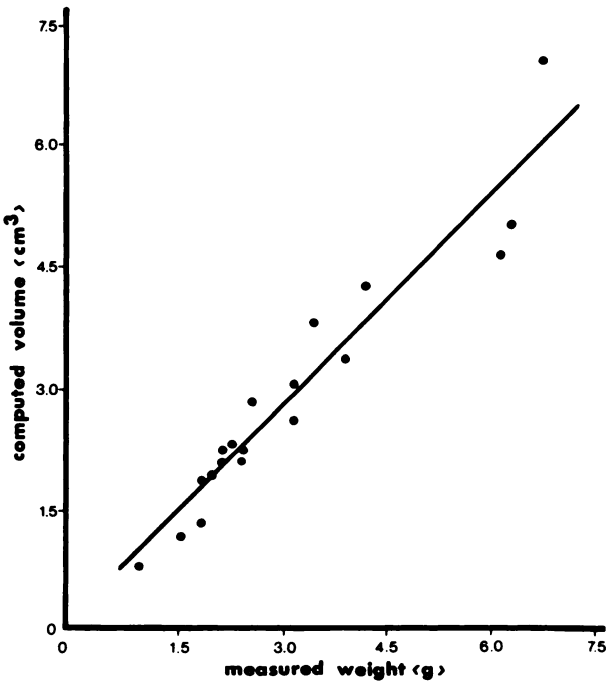

FIG. 3. Correlation of computed liver volume with liver weight for 19 posthepatectomy animals.

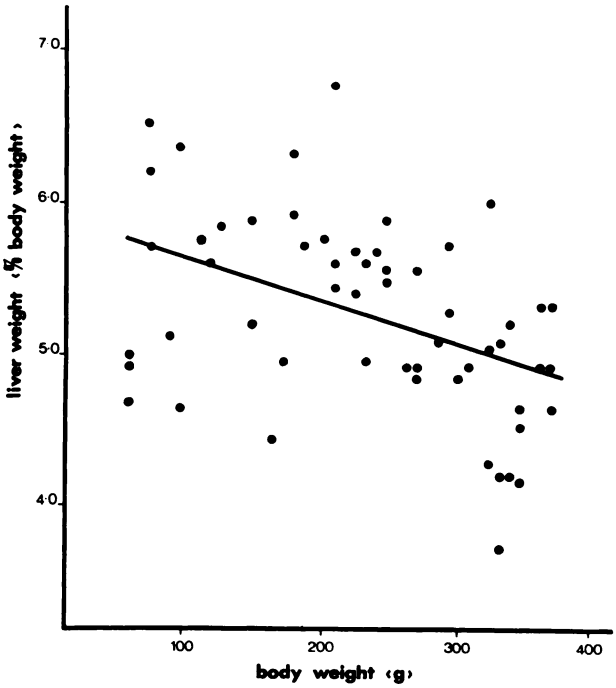

FIG. 4. Correlation of liver weight with body weight (56 animals). L.W. × 100 ÷ B.W. = 5.92 — 0.00286 B.W. r = 0.43.

a similar variation. Nineteen animals were used in this study.

Liver weight as a percentage of body weight. The liver weight as a percentage of body weight was correlated with body weight. In addition the percentage was plotted against body weight and the result is shown in Fig. 4. This revealed that, as body weight increases, the ratio of liver to body weight decreases. The correlation coefficient and regression line constants are given in Fig. 4.

DISCUSSION

The correlation between the measured parameters and the directly measured liver weight was excellent in every case. No one method was significantly better than any other ($p = 0.08$ for the differences between the highest and lowest correlation coefficients). In the absolute assessment of liver mass, the actual liver weight was underestimated by approximately 10%. The density of the liver is not sufficiently different from unity to account for this. It is more likely that it results from an error in the choice of isocount level, especially as this choice was dependent upon results obtained with the parallel-hole collimator.

Since the correlation of the more simply obtained anterior or mean lateral areas is as good as that of the more complicated volume calculated from either the scintigraphs or by computer, the method of choice rests between these simpler parameters. These results are in agreement with our previous ones for regenerating rat liver (*2*) for which the mean lateral area was chosen to assess regeneration. However, it appears in the present studies that a further simplification may be made in that only one area, the anterior, and not the two lateral areas, need be obtained.

When a 67% hepatic resection is performed, the accuracy of scintigraphy in assessing the mass of the remnant is comparable with the assessment from the known relationship of the remnant to the resected lobes. This is particularly so when the remnant is large. The standard deviation of the estimate by scintigraphy for a 6-gm remnant is 0.43 gm whereas, by the method using the percentage resection, the standard deviation is 0.40 gm. The main advantage of scintigraphy, however, lies in its applicability to situations where the method of percentage resection cannot be used, as for example when a different extent of resection is performed.

In the normal animal increased accuracy may be obtained by using scintigraphy to estimate liver mass instead of using a liver-to-body weight percentage. The standard deviation of the estimate of a 17-gm liver by scintigraphy is 0.96 gm whereas, using the liver-to-body weight ratio, the standard deviation is 1.9 gm. Although scintigraphy has no advantage in assessing the mass of a normal liver weighing less than 10 gm, it should be invaluable in disease states when the use of normal liver-to-body weight ratios is invalid and also in sequential studies of a changing liver mass. Among factors which influence the accuracy of assessment by scintigraphy must be the complex structure of liver, especially in the prehepatectomy animals. It is interesting that the computer method which might be expected to be less sensitive to geometry was no more accurate than the simple area measurements.

Linear dimensions (*8*) and scintigraphic area raised to a power (*9*) have been suggested as factors that should correlate with organ weight but there is no indication in our results that either of these would have been better. If the liver increases in size uniformly in all directions, then the mass of the liver would be expected to be proportional to the area of the scintigraph to the power of 3/2. That this is not so may be due partly to compensation because of increased magnification of the larger livers which tend to be closer to the pinhole collimator.

A 12-gm liver receives an absorbed dose of approximately 50 rads from an activity of 2 mCi. This is below the level reported necessary to prevent mitosis following partial hepatectomy (*10*). However, if the methods were used for determination of liver mass, the activity could be reduced to at least one quarter the level used. A high activity was employed because the absorbed dose was of no concern since the

animals were sacrificed immediately after scintigraphy. Hence a number of measurements could be made in a minimum of time. In addition, the animals were rescintigraphed after partial hepatectomy without additional activity being administered.

The scintigraphic method reported offers considerable promise in some situations. For example, not only may in vivo assessment of liver regeneration be made (*2*) but the method offers a possible means of assessing the viability and size of experimental liver transplants. Furthermore it may be of value in the study of other pathophysiologic states of the liver and in particular for sequential assessment of change in liver mass which is not possible by other methods.

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