In Vivo Measurement of Liver Perfusion in the Normal and Partially Hepatectomized Rat Using Tc-99m Sulfur Colloid

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A nonsacrificial technique for measurement of liver perfusion in rats using Tc-99m sulfur colloid and gamma camera with computer system is described. The results of measurement in normal rats and at various stages after partial hepatectomy are presented and are compared with results obtained by workers using other techniques. The method reported here is simpler to perform than those previously reported and has the additional advantage that frequent sequential determinations of liver perfusion can be made.

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Many pathophysiologic conditions affect hepatic perfusion. Measurement of vascular redistribution can assist in the understanding of the functional changes that take place in regeneration following partial hepatectomy, in cirrhosis with portal hypertension, and in hepatic malignancy.

In the past, measurement of hepatic perfusion has usually required the killing of animals (*1*, *2*), and sequential estimations in the same animal have not been possible. More recently we have described a technique that uses sequential quantitative gamma camera imaging. This has been used to assess changes in liver mass and structure following partial hepatectomy (*3*, *4*).

In the present paper we describe an extension of this work. A compartmental mathematical model is presented to describe the changes in uptake of radioactive colloid by the macrophage system in the rat, and is used to estimate changes in liver perfusion following partial hepatectomy.

Theory of mathematical model. A radioactively labeled colloid injected intravenously will spread rapidly throughout the blood volume and then be removed from this compartment by the cells of the reticuloendothelial system, primarily in the liver but also in the spleen and bone marrow. The dynamics of colloid movements may be represented by the compartment model shown in Fig. 1, where K_1 and K_2 represent the effective perfusion rates (ml/min) of the liver and extrahepatic reticuloendothelial sites, respectively. In each case the effective perfusion rate is given by the product of the true perfusion rate and the extraction efficiency, E, of the colloid, where E is the probability that a colloid particle entering the liver will be trapped in a single passage. The compartmental model illustrated is slightly simplified from the actual situation, since

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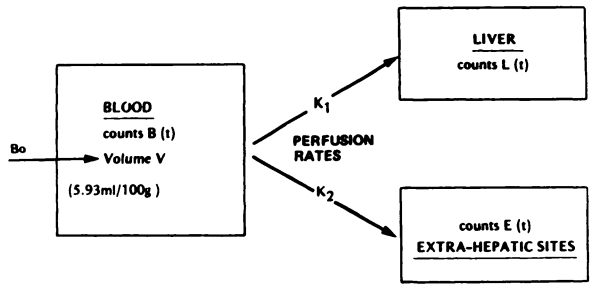

FIG. 1. Compartmental model for dynamics of sulfur colloid.

the fraction of hepatic portal supply arriving through the spleen has a reduced concentration of colloid due to splenic trapping. Since the spleen's effective perfusion rate represents only about 3% of the total reticuloendothelial effective perfusion rate in the rat (Table 1), this simplification is considered valid. The differential equations describing the variation of amounts of colloid, in terms of counts/sec, in the blood $[B(t)]$, liver $[L(t)]$, and extrahepatic sites $[E(t)]$, are then given by

$$\frac{dB(t)}{dt} = -\frac{K_1 + K_2}{V}\cdot B(t), \quad (1)$$

$$\frac{dL(t)}{dt} = \frac{K_1}{V}\cdot B(t), \quad (2)$$

and

$$\frac{dE(t)}{dt} = \frac{K_2}{V}\cdot B(t), \quad (3)$$

respectively, where V is the blood volume (ml).

These are easily solved to give

$$B(t) = Bo\ \exp[-(K_1 + K_2)t/V], \quad (4)$$

$$L(t) = \frac{Bo\ K_1}{K_1 + K_2}\{1 - \exp[-(K_1 + K_2)t/V]\}, \quad (5)$$

and

$$E(t) = \frac{Bo\ K_2}{K_1 + K_2}\{1 - \exp[-(K_1 + K_2)t/V]\}, \quad (6)$$

where Bo is the amount of colloid injected.

The rate constant $(K_1 + K_2)/V$ can be obtained from any of these equations. Since radioactivity measurements by external counter were used in this study, the liver curve was selected because the count rate was considerably higher than in the other two curves. Thus both statistical variations and possible false contributions to the curve are less important.

From Eqs. 5 and 6 it follows that

$$\frac{L(\infty)}{E(\infty)} = \frac{K_1}{K_2},$$

where $L(\infty)$ and $E(\infty)$ are the plateau values of the liver and extrahepatic decay-corrected count rates.

Thus, assuming that the colloid has been completely removed from the blood at the end of the imaging procedure, measurement of the ratio of the count rate from the liver to that from the rest of the body enables the ratio K_1/K_2 to be found.

Since $(K_1 + K_2)/V$ and K_1/K_2 are then both known, and assuming a value for the blood volume V of 5.93 ml/100 g body weight (5), K_1 can be found.

TABLE 1. LIVER BLOOD PERFUSION FOLLOWING PARTIAL HEPATECTOMY IN THE RAT

Group	No. of rats	Total counts (%)			Efficiency (%)	Total liver perfusion (ml/min)	Specific liver perfusion (ml/g-min)
		Liver	Spleen	Carcass			
Normal	11	89.7	2.4	7.8	81	25.1 ± 1.7*	2.2 ± 0.1
Following partial hepatectomy							
Immediate	8	87.3	2.5	10.3	61	22.5 ± 2.2	5.3 ± 0.4
4 hr	6	84.9	4.0	11.0	62.5	23.6 ± 1.7	4.8 ± 0.4
18 hr	6	83.9	4.0	12.1	64	23.7 ± 1.5	4.0 ± 0.2
24 hr	8	87.8	3.2	9.0	65.5	20.9 ± 1.9	3.6 ± 0.2
2 days	10	83.9	3.9	11.6	75.5	17.7 ± 1.0	2.7 ± 0.1
3 days	14	87.8	4.1	8.1	79	22.0 ± 1.2	2.1 ± 0.1
4 days	6	89.4	4.5	6.2	81	16.9 ± 0.8	1.9 ± 0.1
6 days	3	81.0	3.3	15.7		22.0	2.0
7 days	4	90.8	4.0	5.8		22.4	2.1
9 days	7	88.5	4.3	7.3		32.5 ± 2.5	2.6 ± 0.2
10 days	8	87.6	2.6	9.8		28.1 ± 1.4	2.4 ± 0.2
12 days	8	90.8	3.3	5.9		27.6 ± 1.0	2.3 ± 0.1
14 days	8	87.0	3.8	9.3		26.9 ± 1.5	1.9 ± 0.2

* Standard error of the mean is shown by the figures following ± sign.

MATERIALS

Male Wistar rats weighing 150–400 g were used. They were permitted unlimited access to a standard laboratory diet.

Technetium-99m sulfur colloid (TSC) was administered intravenously in doses of 1 mCi in a volume of 0.2 – 0.7 ml. The TSC quality was tested by routine chromatography and found to have about 98% binding efficiency. Colloid particle size variations affect the liver's extraction efficiency, E, for the colloid (*10*). Thus, evaluation of extraction efficiency variation in normal rats, which was found to be 81% ± 3% (1 s.d.) gave a relevant measure of the particle-size variation. Imaging was performed using a gamma camera with a pinhole collimator (*3*, *4*) and digital storage.

METHODS

Operative procedures. Partial hepatectomy, removing 67% of the liver, was performed under light ether anaesthesia (*6*). The excised lobes were washed, blotted dry, and weighed. The spleen was mobilized to a subcutaneous pouch (*7*) in order to obtain separation between liver and spleen images.

Radioisotope imaging. Animals were anesthetized with ether followed by intraperitoneal pentobarbital (4.2 mg/100 g). The animal was lightly taped to a flat surface and positioned under the gamma camera so that the heart, liver, and spleen could be imaged. TSC was injected into the tail vein and sequential digital images were stored at 5-sec intervals for a period of 15 min. Approximately 4000–5000 counts were obtained in a typical liver image when a plateau value had been reached.

In the initial experiments the animal was killed after completion of imaging and the liver remnant and spleen were removed and weighed. Their activities were measured separately under the gamma camera using a parallel-hole collimator to ensure similar counting geometry. The carcass activity was also measured after removing maximal volume of blood by cardiac puncture.

Subsequent experiments were carried out to assess perfusion in living animals. Studies of TSC uptake were repeated daily. No difficulty was encountered from residual activity remaining from a previous day's study; any residual activity was measured before the next study and subtracted from the new measurement. This was found to be a valid procedure, since the previous day's activity had reached a plateau value in each organ. Liver mass was estimated in living animals from the anterior and lateral gamma camera images (*3*).

At the conclusion of each dynamic measurement a digital image was summed from the individual images obtained during the 15-min investigation. From this integrated image, hepatic and splenic regions were selected by light pen. Activity in each region was then measured for each 5-sec interval and plotted against time (Fig. 2).

Efficiency measurements. In order to measure the liver's extraction efficiency for the colloid, the following procedure was carried out. The portal vein was cannulated and, with the rat under the gamma camera as described above, approximately 0.5 mCi of Tc-99m sulfur colloid was injected over a period of 15 sec. Sequential digital images were stored at 0.5-sec intervals for a period of 50 sec. The final count rate in the liver was also measured after the plateau value had been reached.

A typical activity/time curve obtained over the liver area is shown in Fig. 3. Initially the curve rises rapidly as the colloid enters the liver during the injection period. This is followed by a more gradual

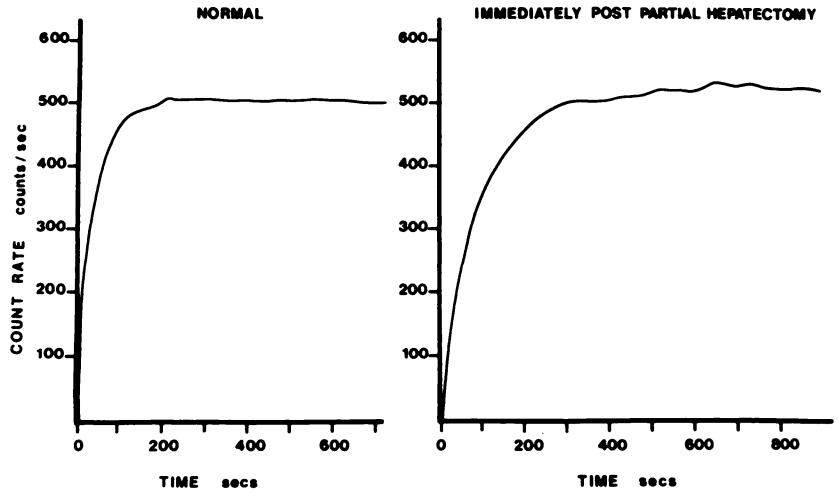

FIG. 2. Colloid uptake in liver following tail-vein injection in normal rat and immediately after partial hepatectomy.

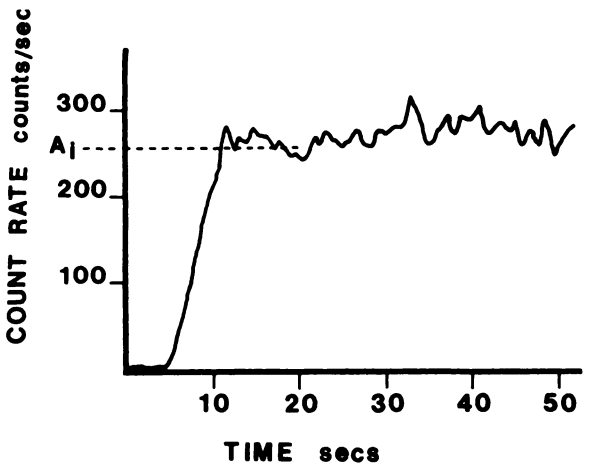

FIG. 3. Colloid uptake in liver following portal-vein injection.

rise due to activity not taken up by the liver on the first passage arriving back at the liver from the general circulation.

The activity initially taken up by the liver was assessed from the count rate 5 sec after the end of the injection (A_1). The extraction efficiency is then given by

$$E = \frac{A_1 x}{A_p - (1 - x)A_1},$$

where $x = K_1/(K_1 + K_2)$ and A_p is the plateau count rate in the liver. This procedure was carried out for normal rats and for hepatectomized rats at a range of times after partial hepatectomy.

Calculations. The liver uptake constant, $(K_1 + K_2)/V$, was calculated using the on-line computer. Each point on the liver uptake curve between 30 sec and 2½ min was subtracted from a plateau that was taken as the mean value of the curve between 12 and 15 min. The value of $(K_1 + K_2)/V$ was obtained by a least-squares linear regression on the logarithm of the subtracted curve.

The relative plateau count rates of the liver and extrahepatic sites at the end of the investigation, $L(\infty)$ and $E(\infty)$, were estimated in two ways:

1. In the necropsy studies the liver was removed and its activity and that of the remainder of the rat were estimated separately, final blood activity being ignored since it was less than 1% of that injected.

2. In the in vivo studies, regions of interest were chosen over the liver and over the whole of the remainder of the rat, and the counts in each region were measured using the computer. Both methods gave the ratio K_1/K_2. Thus, by obtaining $(K_1 + K_2)/V$ and K_1/K_2, and estimating V from body weight (*5*), K_1 was calculated. The blood volume, V, after partial hepatectomy was assumed to be 10% less than the normal value due to blood loss during the operation (*1*). The effective perfusion values were then divided by the appropriate extraction efficiency to obtain the true perfusion.

RESULTS

The results are summarized in Table 1 and Fig. 4.

For each animal the total liver perfusion rate (K_1/E) and perfusion rate per unit liver mass (specific liver perfusion rate) were calculated. The rats were grouped according to the time of perfusion measurement after partial hepatectomy. For each group the mean and standard error of the mean were calculated for both total and specific blood flows.

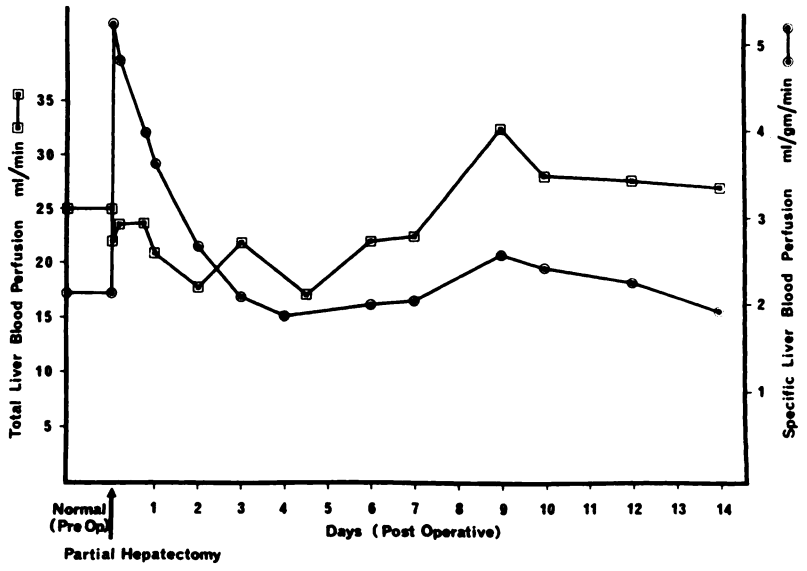

FIG. 4. Changes in total and specific liver blood perfusion following partial hepatectomy.

In normal rats the total perfusion rate was 25.1 ml/min. Immediately following partial hepatectomy this fell to 22.5 ml/min. Thereafter, the perfusion gradually increased, reaching a normal value at about 8 days postoperatively and progressing to higher values in the early part of the second week. The specific liver perfusion rate in normal animals was 2.15 ml/min-g. Following partial hepatectomy there was an immediate increase to 5.25 ml/min-g. Specific perfusion then fell to normal by 3 days. Values continued to fall to a minimum at 4–5 days, before oscillating around the normal value for the remainder of the regenerative period.

DISCUSSION

There are several possible sources of error in this technique. Errors involved in the calculation of liver uptake rate by linear regression were evaluated by considering confidence limits on the calculated rates, the mean percentage error being 1.9%. The fraction of the total activity in the liver was measured to an accuracy of ± 2%. This error is primarily because some activity in the carcass lies outside reticuloendothelial sites. The main error in the measurement of total effective hepatic perfusion results from the estimation of blood volume (*5*). In the sequential studies the fractional liver activity was obtained to within ± 4% due to variation in the definition of the liver region from the image. An additional error in the calculation of specific hepatic perfusion resulted from the estimate of liver mass (*3*). Another suggested source of possible error is the variation of extraction efficiency for different samples of colloid (*8*). The mean extraction efficiency measured in eight normal rats, based on different colloid samples from a variety of batches, was 81% ± 3% (1 s.d.). Consideration of all these factors gives a total error that is small compared with the changes seen after partial hepatectomy. The standard errors of the mean for the results of each group of rats are shown in Table 1.

Figure 5 shows comparison of results obtained using this new in vivo technique with previously reported sacrificial studies. Benacerraf et al. (*1*) and Rabinovíci and Weiner (*2*) both used I-131 labeled albumin colloid but different methods. Also illustrated are the results of Guest et al. (*9*), who used a krypton-85 clearance technique. All the values of specific liver perfusion in normal rats were comparable considering the variations in technique.

Following 67% hepatectomy, Benacerraf et al. (*1*) found a gradual increase in specific liver perfusion, with peak values at 16 hr after operation. Guest's results (*9*) showed an immediate rise followed by a more gradual increase to a peak value at 4 hr. In contrast, in our studies peak values were obtained *immediately* after hepatectomy. This immediate response was also shown by Rabinovici and Weiner (*2*). In all studies there was a fall to normal or possibly subnormal values within 3–5 days. The oscillation about normal seen thereafter suggests a possible feedback control in the restoration of hepatic perfusion.

One advantage of our technique over previously reported studies using colloid (*1*, *2*) is that serial blood sampling is not necessary. This makes the technique simpler and enables more accurate measurement of time/activity variations. In addition, use of the mathematical model to separate liver perfusion from total reticuloendothelial perfusion gives improved accuracy. Our method also has the advantage of being simpler to perform than inert-gas clearance techniques. By combining the technique with in vivo measurements of liver mass, sequential assessment of hepatic perfusion is possible. Thus this new technique offers the opportunity to per-

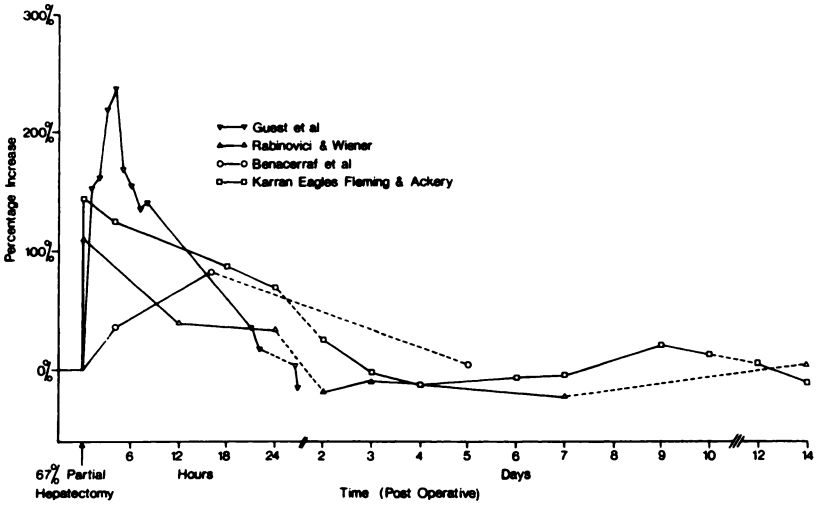

FIG. 5. Comparison of results using different techniques for assessing percentage changes in specific liver blood perfusion following partial hepatectomy.

form studies in a variety of pathophysiologic conditions, such as porta caval diversion and transposition and arterial ligation, in addition to partial hepatectomy.

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