A linear method for determining liver sinusoidal and extravascular volumes[1]

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GORESKY, CARL A. *A linear method for determining liver sinusoidal and extravascular volumes.* Am. J. Physiol. 204(4): 626–640. 1963.—Multiple indicator dilution studies of the hepatic circulation in the dog were carried out using labeled red cells, albumin, inulin, sucrose, sodium, urea, water, and T-1824. The materials were completely recovered in the outflow. Concentrations were expressed as fractions of the injected mass. The outflow pattern of each of the substances was displaced relative to the red cell curve, showing a lower peak concentration and longer transit time. The displacement was largest for water and urea, least for albumin and T-1824, intermediate for inulin, sucrose, and sodium. The results were analyzed using a flow-limited linear two-compartment model system. The analysis yielded estimates of sinusoidal blood volume and of the extravascular volumes of distribution of the diffusible labels. Water and urea volumes agreed with the liver weights and this agreement was taken as validation of the method of analysis. Volumes calculated for the other presumably extracellular substances demonstrated that the rapidly available extracellular space diminishes with increase in the molecular weight of the substance.

IN THE PAST the direct analysis of tissue has been used to define the composition of tissue in terms of accessible volumes of distribution for various substances and these volumes have been used in turn to define compartments within the tissue (e.g., vascular volume, extracellular space, and cellular space). The multiple indicator dilution technique may similarly be used to define the composition of an organ if a suitable method can be derived for calculating the capillary blood volume and the extravascular volumes of distribution for diffusible substances. The present study develops a method for calculating these parameters and uses this method to explore the composition of the dog's liver.

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An indicator substance introduced into the blood flowing into an organ becomes dispersed in the effluent blood and the concentrations of the substance in the effluent blood form an indicator dilution curve. If the substance is confined to the vascular compartment (e.g., labeled red cells), then flow through the circulatory labyrinth results in a dispersion of passage times, and the volume of the vascular compartment from injection site to sampling site is simply the product of flow through the system and of the mean transit time (i.e., the mean of this time dispersion) (1). When the indicator substance passes beyond the confines of the vascular compartment, the times of transit are increased by the times spent by the substance in the extravascular space. It appears obvious, then, that these increments in time must be related to the rate at which the diffusible indicator substance leaves and re-enters the capillary, to the size of the extravascular compartment related to each capillary, and to the total number and length of the capillaries across which exchange takes place. The rate at which the diffusible substance leaves and re-enters the capillary will be related in turn to the diffusion coefficient for the substance in the capillary wall and extravascular space and to the rate of presentation of material for exchange. When diffusion takes place slowly in relation to the blood flow rate, the rate of transcapillary passage will become limited chiefly by diffusion. In contrast, where diffusion takes place rapidly in comparison to the blood flow rate, the rate of transfer becomes limited chiefly by the rate of perfusion, i.e., by the rate of presentation of material. This will occur when the linear rate of flow is small and when the distances for diffusion in the extravascular compartment are small. In the present study molecular exchange across the walls of the hepatic sinusoid is examined and the relation of flow (i.e., of the rate at which the bulk transport of blood presents material for exchange) to the rate of transcapillary passage is critically assessed.

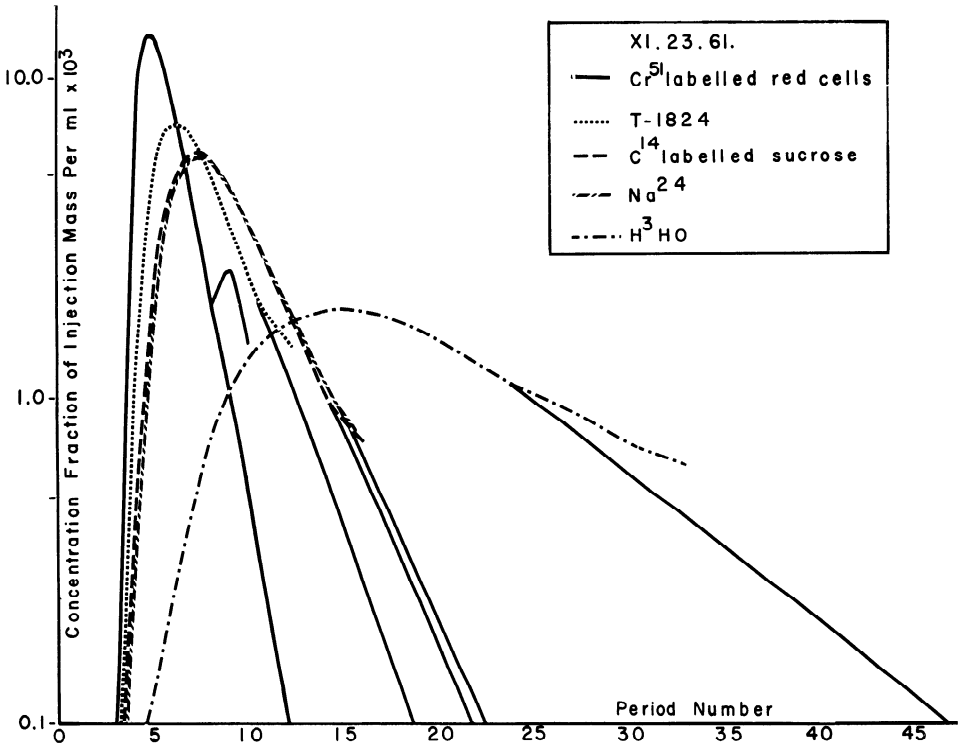

FIG. 1. Hepatic venous blood concentration time patterns, with extrapolated corrections for recirculation. *Ordinate:* concentration expressed as fractional recovery of injection mass per milliliter of blood, on a logarithmic scale. *Abscissa:* number of the sampling interval (one period is 1.67 sec). Concentrations are plotted at the midpoint of each interval.

METHODS AND PROCEDURES

General experimental procedures. The characteristics of the hepatic sinusoidal circulation were explored using the rapid single-injection multiple indicator dilution technique (2). In each experiment a group of indicator substances was mixed, and a known amount of mixture was placed in a single syringe. This mixture was rapidly injected into the portal vein, and 40 or more hepatic venous samples were collected in the following minute. The recoveries of test substances in each sample were determined.

Materials and injection solution. The following special materials were used: $Na_2Cr^{51}O_7$ solution, 18.7 c/mM, $Na_3(P^{32}O_4)_2$ solution, 10.5 c/mM, Na^{24} Cl, 2.13 mc/mM (Frosst, Montreal); T-1824 (Anachemia, Montreal); C^{14} sucrose, 32.9 mc/mM, C^{14} urea, 5 mc/mM (Merck, Montreal); inulin-COOH C^{14}, 118 mc/mM, tritium-enriched water, 2 mc/ml (New England Nuclear, Boston); inulin, recrystallized from ethanol (U.S. Standard Products, Mount Prospect, Ill.); I^{131} albumin, 13.6 mc/mM, prepared by Takeda (3). Red cells from 10 ml blood, labeled by incubation with 100 μc $Na_2Cr_2^{51}O_7$ at room temperature, or with 100 μc $Na_2(P^{32}O_4)_2$ at 37 C for 90 min, were included in the injection mixture of each experiment as an indicator which was without doubt confined to the vascular space. P^{32}-labeled red cells were used where I^{131} albumin was used; otherwise Cr^{51}-labeled cells were used. The injection mixtures were made up as follows: to blank dog plasma was added T-1824, 5 mg/ml. In some experiments one or more other indicator substances were also added: C^{14} sucrose, 10 μc/ml; C^{14} inulin, 5 μc/ml; inulin, 60 mg/ml; Na^{22}, 3 μc/ml; Na^{24}, 3.3 μc/ml; C^{14} urea, 10 μc/ml; tritium enriched water, 50 μc/ml; I^{131} albumin, 1.5 μc/ml. To this solution was added an equal volume of packed labeled red cells, washed three times in isotonic saline. This group of substances was selected because of an apparent absence of metabolic interaction with hepatic parenchymal cells (the recoveries in the outflow were essentially complete). Because of this lack of interaction, the study substance could be used to "probe" the transfer characteristics of the hepatic sinusoid. The volume of mixture used in each experiment was between 1.2 ml and 1.8 ml. The time required for injection was less than 0.5 sec.

Operative procedure and collection of samples. Mongrel dogs, weighing between 12 and 29 kg were used as experimental subjects. After pentobarbital anesthesia, blood is drawn for labeling red cells, an intravenous infusion is started, and an upper midabdominal incision is made. Catheters are placed in portal and hepatic veins and tied in place. Sutures passed through the wall of the relatively high pressure portal vein caused profuse blood loss in the heparinized animal and so the following procedure was adopted. The portal vein catheter is made from PE 190 polyethylene tubing tapered at the tip to an orifice approximately 0.015 in. in diameter. The catheter is fitted over a sharp-pointed flexible metal obturator and a collar, made from adhesive tape, is placed a half inch behind the tip. The peritoneum over the portal vein is incised and the vein is cleaned. The catheter tip and obturator are thrust through the wall of the vein, and advanced in midstream until the collar is wedged tight against the vein wall. The obturator is withdrawn,

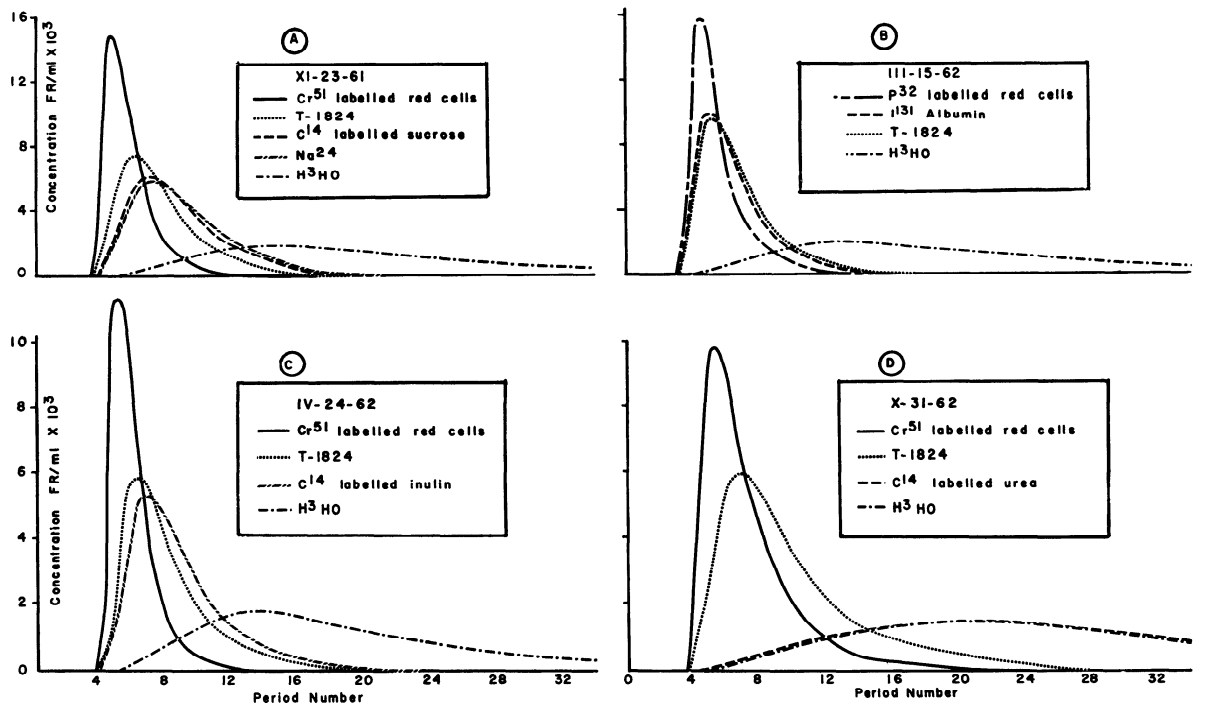

FIG. 2. Hepatic venous blood concentration time patterns. *Ordinates:* concentration expressed as fractional recovery of injection mass per milliliter of blood. *Abscissae:* number of the sampling interval (one period is 1.67 sec, in 2*A*; 1.63 sec, in 2*B*; 1.64 sec, in 2*C*; 1.72 sec, in 2*D*).

and a slow infusion of saline is begun. No subsequent blood loss occurs from the site of catheterization.

The second catheter is made of Teflon spaghetti tubing, has a tape collar, and three subterminal side holes. A single no. 00000 silk suture on a fused needle is passed through the wall of the left hepatic vein where it appears in the fissure between the left upper and lower hepatic lobes (4), a small slit is made adjacent, the catheter is passed through the slit, and is tied in place using the collar (5). No evidence of outflow obstruction occurred except on one occasion when autopsy demonstrated that the silk suture had constricted the vein.

Hepatic venous samples were pumped into a serial collection rack (J. Armant, Baltimore) using a Sigmamotor finger pump. The pumping rate was 75 ml/min. The volume of the sampling catheter was 3.2 ml. Trial experiments, performed with the abdomen open, resulted in bumpy indicator dilution curves. The abdomen is therefore closed prior to and during the experiment to allow the liver temperature to return to normal. The injection mixture is introduced through the portal vein catheter. The animal is fully heparinized to prevent blood clotting.

When tritium-enriched water was included in the injection mixture aliquots of liver tissue, secured at the end of the experiment by rapid excision of a portion of the edge of a liver lobe, were used to determine wet and dry weights of liver tissue. In every experiment the liver was excised and weighed.

Analysis of samples. Standards were prepared from the injection mixture by addition, in serial dilution, of blood obtained from the hepatic vein before the collection of samples. One milliliter of sample or of diluted standard was pipetted into 1 ml of saline and centrifuged. The sample was assayed in a well-type scintillation crystal gamma-ray spectrometer for gamma rays of appropriate energy (for Cr^{51}, I^{131}, Na^{22}, or Na^{24}). Where two gamma emitters were present the sample was counted in two channels and the counts were corrected, using appropriate standards and a decay factor, so that the activity due to each substance was evident. The supernatant was assayed for T-1824 color in a 1-cm microcuvette at 620 mμ in a Unicam SP-500 spectrophotometer. Inulin was determined using a modification of the method of Higashi and Peters (6). Then 0.25-ml supernatant or whole blood were planchetted into cupped stainless steel planchets, and the blood was lysed with distilled water. The samples were thoroughly dried, and radioactivity due to C^{14} was determined by a thin-window flow detector with an automatic sample changer and recorder. At least 1,000 counts were recorded from each sample analyzed; background was about 14 counts/min; where whole blood was used Cr^{51} contributed no significant increment to the activity. Where Na^{24} was used the samples were allowed to stand for more than 2 months before counting. P^{32} counts were corrected for I^{131} activity by using appropriate standards and a decay factor. Tritium activity was determined in water isolated from the supernatant using a microsublimation method (7). Then 0.25 ml water was added to 10 ml scintillator made up as follows: 77 ml dioxane, 23 ml ethanol, 13.3 g naphthalene, 25 mg 1,4-

bis-2-(5-phenyloxazolyl)-benzene (POPOP), and 1 g 2,5-diphenyloxazole (DPO). Samples were counted in a Packard Tri-Carb liquid scintillation counter at 5 C.

Criteria to be fulfilled. The use of the indicator dilution method to derive values for flow and volume implies the fulfillment of two criteria:

a) That the indicator be uniformly distributed across the whole at the inflow. The input catheter is placed in midstream approximately one inch proximal to the branching of the portal vein. The material is introduced as rapidly as possible through a catheter with a tapered tip, so that the linear velocity becomes high, and turbulent flow would be created. This should have created adequate mixing in the inflow.

b) That the samples obtained should be representative of the whole outflow. The left hepatic reservoir sampled drains approximately 44% of the liver by weight (4). The remainder of the outflow drains through other channels. If the liver is considered to be a homogeneous organ, and if uniform mixing in the inflow occurs, then the samples from the left reservoir may be assumed to be representative of the whole.

RESULTS

Instantaneous injection of a group of substances into the portal venous stream resulted in the appearance of a group of indicator dilution curves in the hepatic venous stream. The concentration of each substance rose rapidly to a peak, then fell off in an exponential manner until recirculation occurred, and thereafter remained fairly constant. The primary curves were corrected for recirculation by linear extrapolation of the downslope on a semilogarithmic plot, after the classical manner of Hamilton (8). In order to provide a basis for comparison among the group, the total amount of material injected was defined as 1 unit, and the concentration of material in the outflow was expressed as a fraction of the injected mass per milliliter of blood. Figure 1 illustrates the use of the linear extrapolation to correct the data for recirculation. The correction for the most rapidly traveling indicator is quite certain, whereas that for the most slowly traveling indicator is much less certain. Figures 2 *A–D* illustrate the primary curves, concentration being displayed on rectangular coordinates against time. The red cell label is the earliest to appear in the hepatic venous outflow, the concentration of injected cells rises to the highest and earliest peak, and decays most rapidly. The outflow pattern of each of the other indicator substances was modified relative to the red cell pattern, showing a diminution in peak concentration and delayed transit time. Labeled water and urea, which might be expected to enter the total organ water, showed a greater peak depression and prolongation of transit than that group of substances which might be expected to be excluded from intracellular water (inulin, sucrose, and sodium). Water and urea were dispersed in approximately the same manner. In contrast, inulin, sucrose, and sodium showed differences in their distribution. Inulin showed the least peak depression, sodium the most. Sucrose was intermediate. T-1824 showed even less peak depression than inulin, but was still markedly different from red cells. I^{131}-labeled albumin showed a peak very slightly higher than T-1824. The slight depression of the T-1824 peak relative to the I^{131}-labeled albumin was probably a consequence of the large amount of dye used. The concentration of dye exceeded the binding capacity of the albumin present in the injection solution (9), and also possibly exceeded the binding capacity of the albumin in the immediate vascular mixing volume.

TABLE 1. *Recoveries, relative flows, and average mean transit times*

Substance	No. of Exp.	Recoveries* Mean ± SD, %	Flow / T-1824 Flow Mean ± SD, %	Average $\bar{t}_m$† Mean ± SD, sec
T-1824	38	100.0	100.0	12.37±3.98
Cr^{51}-labeled red blood cells	32	99.0±2.9	100.9±3.1	8.36±2.66
I^{131}-labeled albumin	6	99.6±1.5	100.4±1.5	10.09±2.73
Inulin	9	101.3±2.5	99.4±2.5	12.55±2.54
C^{14} sucrose	8	99.6±3.2	100.0±2.8	15.11±4.82
Na^{22}, Na^{24}	6	98.7±1.4	101.7±1.7	14.42±2.15
C^{14} urea	3	98.4±1.5	101.4±2.4	42.22±12.96
H^3HO	23	99.5±2.2	100.6±1.8	36.38±10.43
P^{32}-labeled red blood cells	6	96.0±1.8	105.5±4.5	6.52±1.64

* Recoveries relative to T-1824. † $\bar{t}_m$ is mean transit time.

From each group of curves, several parameters used for the analysis of the data were obtained directly.

a) *Recoveries.* Some standard is needed to relate the recoveries of each substance used. The assumption was made that the recovery of Cr^{51}-labeled red cells was complete becuase there is no evidence to suggest that Cr^{51}-labeled red cells leave the circulation of or are retained in the liver. The recovery of T-1824 was found to be 101.1% ± 2.9% (mean ± SD) that of the Cr^{51}-labeled red cells. Recoveries were subsequently related to T-1824 (Table 1) since a dilution curve for this substance was available in each experiment. The recoveries of inulin, labeled sucrose, labeled sodium, I^{131}-labeled albumin, labeled urea, and tritium-enriched water were essentially the same as that of T-1824. P^{32}-labeled red cells showed some loss of activity during a single passage through the liver.

b) *Flows.* The average total blood flow for all experiments was 869 ml/min; expressed in terms of liver weight, this was 1.83 ± 0.55 ml/g min (mean ± SD). The calculation of blood flow from an indicator dilution curve depends on the assumption that all the material which has been put into the inflowing blood finally appears in the outflow. So long as there is no net retention of substance in the liver, the flow calculated for each indicator substance in an experiment should be the same. Table 1 illustrates that the only indicator showing flows appreciably different from T-1824 was the P^{32}-

labeled red cells. This was a consequence of P^{32} retention in the liver.

c) Mean transit times. These were calculated in the usual manner (1), and were corrected for catheter transit time by simple subtraction (10). Average values are illustrated in Table 1.

Analysis of Results

In order to interpret the results in terms of the flow and diffusion processes taking place in the hepatic sinusoids, it was found necessary to use a model. In this way information could be obtained which would have been otherwise inaccessible. The validity of the information depends upon the applicability to the biological system of the assumptions made in setting up the model. Two models have been used.

Lumped two-compartment system. This is essentially the model used by Lilienfeld et al. (11) to interpret the transcapillary migration of heavy water in the pulmonary circulation. The assumptions made are as follows.

1) Red cells are confined to the vascular compartment.

2) Another compartment is assumed to be present. Communication between the vascular and extravascular compartments is assumed to occur across such a short length of capillary wall that movement can be considered to occur at a single point just proximal to the point of sampling.

3) No axial streaming occurs in the larger vessels. Thus no red cell plasma separation occurs in the larger vessels.

4) Flow through the hepatic sinusoids is bolus flow (12): the red cells almost completely fill the lumen of the hepatic sinusoid, so that they push discrete segments or boluses of plasma along.

No separation of plasma and red cells would occur in such a system in the absence of passage of plasma label into an extravascular space. All labels for which the outflow concentration time dispersion is different from the red cell curve must have passed into an extravascular space. If we put

Q = proportion of injected label which is extravascular
t = time elapsed since injection
F = volume flow rate
C_e = expected concentration of label in the ouflow if there had been no extravascular diffusion (in this instance the concentration of labeled red cells will be used), and
C_o = concentration of label in the outflow when extravascular diffusion has occurred

then, by definition:

$$\frac{dQ}{dt} = F(C_e - C_o) \qquad (1)$$

Now, from the model, if V = the volume of the extravascular compartment and if there is a rate constant K for passage between the two compartments, which is the same in both directions, then

$$\frac{dQ}{dt} = KC_e - K\frac{Q}{V} \qquad (2)$$

A value for Q/V may be obtained from the crossover point of the red cell and the diffusible label concentration time curves, where $C_o = C_e$, hence $dQ/dt = 0$, and $C_e = Q/V = C_o$. Q is the difference between the red cell

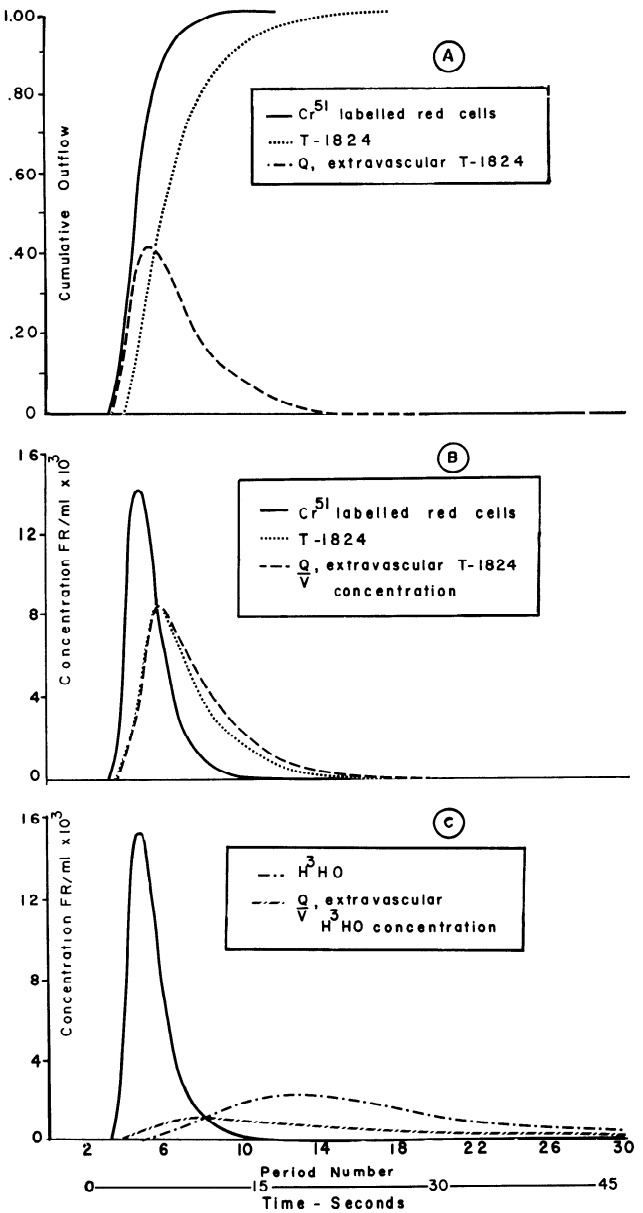

FIG. 3. *A:* cumulative hepatic venous outflow of the injected Cr^{51}-labeled red cells and T-1824. Q, the difference between these curves, represents the extravascular T-1824 in the lumped model. *B:* concentration time outflow patterns for Cr^{51}-labeled red cells and T-1824, and the computed compartmental concentration of T-1824. *C:* concentration time outflow patterns for Cr^{51}-labeled red cells and tritium-enriched water, and the computed compartmental concentration of tritium-enriched water in the same experiment.

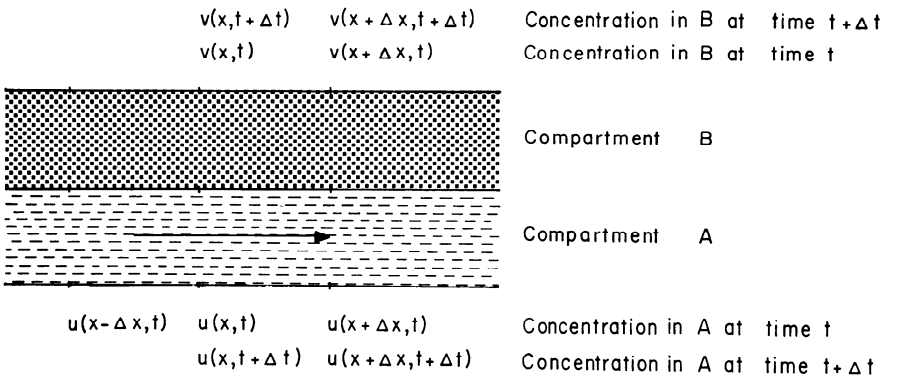

FIG. 4. A plane representation of the linear model.

and diffusible label cumulative outflows. Q is easily calculated from the data, hence V, the extravascular volume of distribution, and K, the rate constant for distribution, can be computed.

This analysis was applied to a group of experiments in which labeled red cells and the dye T-1824 were used. If the amount of material injected is defined as one unit, and if no material is lost in the system then the cumulative outflow will ultimately become one unit. Figure 3*A* illustrates the variation of Q, the cumulative difference between the red cell and diffusible label outflows, with time. Q rises to a maximum and then diminishes with time to zero. Unexpectedly, the compartmental concentration of the dye T-1824 was found to be approximately equal to that in the vascular compartment at the sampling point at any time (Fig. 3*B*). Comparison of *equations 1* and *2* shows that the rate constant K must be equal in magnitude to the flow rate F. This is another way of saying that the distribution of T-1824 appeared to be flow limited, i.e., equilibrium occurred so rapidly in comparison to flow that it was virtually complete at the rates at which label was being presented to the exchange site.

When the same analysis was applied to labeled red cell and tritium-enriched water dilution curves (Fig. 3*C*), three discordant results appeared. The calculated extravascular volume V for the water label was much larger than the size of the organ, the calculated compartmental concentration of the water label Q/V at the postulated exchange site could be shown to reach its peak much more rapidly in time than the concentration of water label appearing at the outflow, and the rate constant for the exchange of water showed a progressive increase with each sampling interval so that it far exceeded the flow rate. Logically, the flow-limited case ($K = F$) represents the maximum value of K, so that this is a physical impossibility. The apparently quite different results obtained for the two substances may be seen to be the result of the relationship of each curve to the red cell curve. At the crossover point Q, the total amount of label in the compartment, (and Q/V, its compartmental concentration), reaches its maximum. For T-1824 the concentration curve reaches a maximum at this time as well, and hence the Q/V curve is symmetrical to the concentration curve. The tritium-enriched water dilution curve reaches a maximum at a time which is much later than that at which it crosses the labeled red cell dilution curve. The peak compartmental concentration Q/V for the tritium-enriched water will thus occur much before the peak of the outflow concentration. This implies that isotope moves from low specific activity to high specific activity, an impossibility.

There is a perceptible lag between the outflow appearance of labeled red cells and diffusible substances. This lag increased with the mean transit time. The lumped model takes no account of this lag and indeed can provide no explanation for it.

This model was, then, not an adequate description of the biological system. It appeared that the defect of the model was the assumption that exchange occurred at a point locus. A more valid model of the behavior of diffusible substances in the liver was sought. Consideration of the first model suggested a model in which exchange is considered to occur along an extended vascular length corresponding perhaps to the length of the true capillary. This might be termed the exchange site.

Linear two-compartment system with flow-limited diffusion. Consider a two-compartment model (Fig. 4) with the following restrictions.

1) Flow is confined to one compartment (the vascular space A).

2) Diffusion in A or B along the length is effectively zero. Label will be transported from inflow to outflow only by the bulk transport of material in A. For all practical purposes, diffusion of material in the direction of flow is negligible compared to the rate of bulk transport of material during flow. Since there is no flow in the stationary compartment B, material which arrives there must come from A.

3) Diffusion is assumed to take place so rapidly in the direction perpendicular to the direction of flow that the concentration of label inside the vascular compartment and the concentration of label in the extravascular compartment may be considered equal, i.e., equilibrium of label is assumed to take place so rapidly in relation to flow that it is virtually complete at all points along the length. The sinusoid wall is not considered to be a phase boundary of any consequence for dissolved materials. This assumption was introduced as a consequence of the

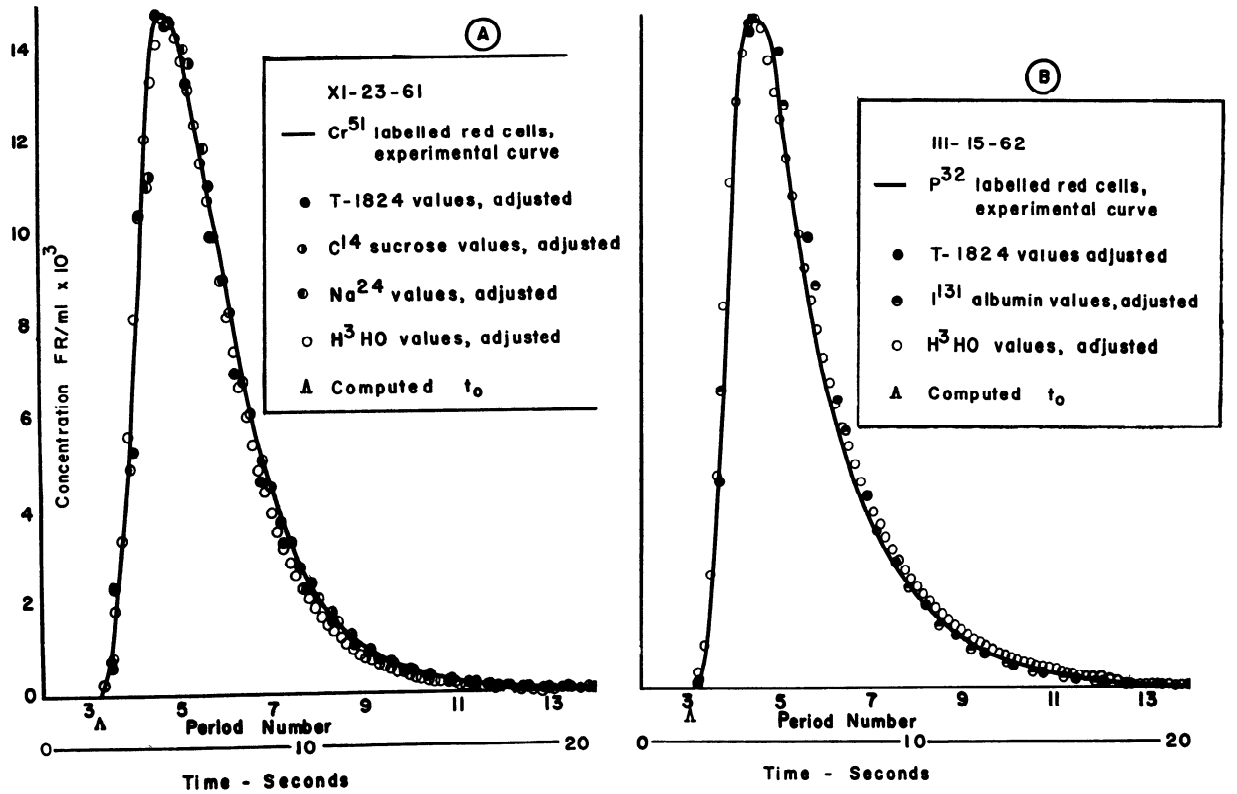

FIG. 5. *A:* theCr^{51}-labeled red cell curve and the adjusted values from the experiment illustrated in Fig. 2*A*. *B:* the P^{32}-labeled red cell curve and the adjusted values from the experiment illustrated in Fig. 2*B*.

application of the first model to the data for T-1824, but its chief justification for use rests upon a consideration of the rates of diffusion in elements of the size of a liver cell. At 37 C labeled water will enter an aqueous plate 30 μ wide at a rate such that the amount in the plate will reach 43 % of its equilibrium value in 10 msec and 96.5 % of its equilibrium value in 90 msec. The assumption of diffusion equilibration frees the model from any discrete consideration of the geometry of the system.

4) Flow through the sinusoids is again assumed to be bolus flow. In the area of label exchange the red cells are assumed to almost completely fill the lumen of the hepatic sinusoid so that discrete segments of plasma travel with the cells. Within the bolus of plasma each fluid element moves within a closed circuit (12); there is thus a mixing motion which increases the rate of presentation of vascular diffusible label to the extravascular space. The blood may be regarded, in a sense, as a well-stirred fluid. The hematocrit of the sinusoid blood is assumed to be the same as that of venous blood.

In the model, let

$u(x, t)$ = the concentration in compartment A at some point along the length, x, at time, t
$v(x, t)$ = the concentration in compartment B at some point along the length, x, at time, t
A = the volume per unit length for compartment A
B = the volume per unit length for compartment B
A and B = constant along the length

From the restrictions the amount added to compartments A and B at $(x, t + \Delta t)$ must come from compartment A at $(x - \Delta x, t)$, and so

$$u(x - \Delta x, t)A\Delta x = u(x, t + \Delta t)A\Delta x + v(x, t + \Delta t)B\Delta x - v(x, t)B\Delta x$$

$$B[v(x, t + \Delta t) - v(x, t)]\Delta x = -A[u(x, t + \Delta t) - u(x - \Delta x, t)]\Delta x$$

$$= -A[u(x, t + \Delta t) - u(x, t) + u(x, t) - u(x - \Delta x, t)]\Delta x$$

$$B\left[\frac{v(x, t + \Delta t) - v(x, t)}{\Delta t}\right] = -A\left[\frac{u(x, t + \Delta t) - u(x, t)}{\Delta t} + \frac{\{u(x, t) - u(x - \Delta x, t)\}\Delta x}{\Delta t \Delta x}\right]$$

Now let $\Delta x/\Delta t = W$, velocity of flow. Then in the limit

$$\Delta x, \Delta t \to 0, \qquad B\frac{\partial v}{\partial t} = -A\frac{\partial u}{\partial t} + W\frac{\partial u}{\partial x}$$

Now let $\gamma = B/A$ the ratio of volumes per unit length, then

$$\gamma\frac{\partial v}{\partial t} + \frac{\partial u}{\partial t} + W\frac{\partial u}{\partial x} = 0$$

Now the restrictions state that $u(x, t) = v(x, t)$, and hence

$$\frac{\partial v}{\partial t} = \frac{\partial u}{\partial t}$$

Therefore

$$\frac{1 + \gamma}{W} \cdot \frac{\partial u}{\partial t} + \frac{\partial u}{\partial x} = 0$$

This is the partial differential equation describing the passage of label along a single sinusoid.

The solution to this is a Fourier series which may be expressed in the complex plane. For a tube of length $0 \leq x \leq L$

$$u(x, t) = \sum_{n=-\infty}^{\infty} A_n \exp 2in \frac{\pi}{L} \left(x - \frac{Wt}{1 + \gamma} \right)$$

Now A_n, the complex Fourier coefficient must be defined. Suppose the initial concentration profile $u(x, 0) = \rho(x)$ so that

$$\rho(x) = \sum_{n=-\infty}^{\infty} A_n \exp 2in \frac{\pi}{L} x$$

whence

$$A_n = \frac{1}{L} \int_0^L \rho(x') \, dx' \exp - 2in \frac{\pi}{L} x'$$

whence

$$u(x, t) = \frac{1}{L} \int_0^L \rho(x') \, dx' \sum_{n=-\infty}^{\infty} \exp 2in \frac{\pi}{L} \left(x - \frac{Wt}{1 + \gamma} - x' \right)$$

$$= \frac{2\pi}{L} \int_0^L \rho(x') \delta \frac{2\pi}{L} \left(x - \frac{Wt}{1 + \gamma} - x' \right) dx'$$

$$= \rho \left(x - \frac{Wt}{1 + \gamma} \right)$$

The concentration behaves like a wave that travels at the rate $W/(1 + \gamma)$ instead of the expected W. When there is no accessible extravascular space

$$u(x, t) = \rho(x - Wt)$$

When there is an accessible extravascular space

$$u(x, t) = \rho \left(x - \frac{Wt}{1 + \gamma} \right)$$

The factor $1/(1 + \gamma)$ is the ratio of the cross-sectional area of the vascular space to the cross-sectional area of the whole space accessible to the diffusible label. Thus the red cells, which are confined to the vascular space,

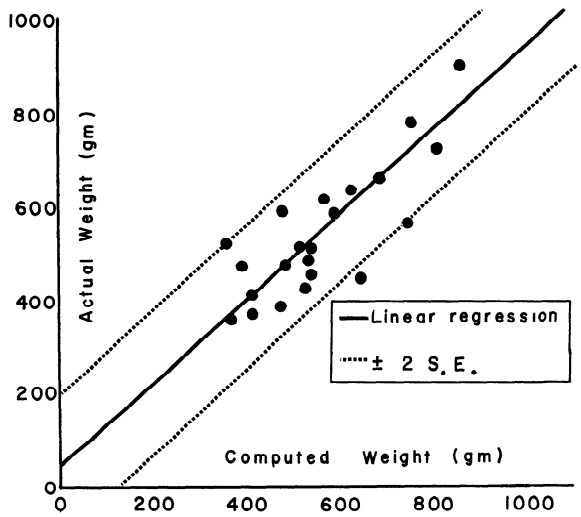

FIG. 6. Comparison of actual liver weights (*ordinate*) and computed liver weights (*abscissa*).

will flow along the sinusoid with a velocity W, whereas the diffusible label will behave as if it were flowing in a larger space (the sum of the vascular space and the space outside the sinusoids which is freely accessible to the label) and so will travel more slowly. The decrease in velocity of the concentration wave for the diffusible substance will result in a lag in the appearance of the wave at the outflow, and in a proportionate delay in all parts of a concentration wave. If corresponding parts of the wave are being considered, time t for a nondiffusible label corresponds to $(1 + \gamma)t$ for a diffusible label. The lag and the proportionate delay will increase as the size of the extravascular compartment increases.

The behavior of a large number of sinusoids of varying length must be considered. Let

$n(L)\,dL$ = the number of capillaries of length L to length $L + dL$
a = the mean cross-sectional area of a sinusoid
dQ = corresponding quantity of label appearing in the outflow/second

Then

$$dQ = \frac{aW}{1 + \gamma} u(L, t) n(L) \, dL. \quad \text{and therefore}$$

$$Q(t) = \frac{aW}{1 + \gamma} \int_{L_{\min}}^{L_{\max}} u(L, t) n(L) \, dL$$

$$= \frac{aW}{1 + \gamma} \int_{L_{\min}}^{L_{\max}} \rho \left(L - \frac{Wt}{1 + \gamma} \right) n(L) \, dL$$

Assume the system is originally empty, that the input is an impulse function, and that for each sinusoid $\rho(x) = q_0 \delta(x)$, where q_0 is a dimensionless factor weighting the proportion of the total input which enters each sinu-

soid. Then

$$Q(t) = \frac{aW}{1+\gamma}\int_{L_{min}}^{L_{max}} q_0\delta\left(L - \frac{Wt}{1+\gamma}\right) n(L)\,dL$$

$$= q_0 \frac{aW}{1+\gamma}\, n\,\frac{(Wt)}{1+\gamma}$$

The shape of $Q(t)$, the outflow rate from the system, gives the distribution of lengths of the sinusoids when there is no extravascular space accessible to label. For diffusible labels, $Q(t)$ is diminished by the factor $1/(1+\gamma)$. This is the result of the delay in each channel. Over the whole system this results in a proportionate dilution of labeled blood with unlabeled blood at corresponding times on the time concentration curve. There is thus a symmetry in time and concentration for the distortion of the indicator dilution outflow curve produced by passage of indicator into the extravascular space. The time for corresponding points on the curve is increased by the factor $(1+\gamma)$ and the corresponding concentrations are reduced by the factor $1/(1+\gamma)$.

Now if there is no loss of label, and if q is the total amount of label introduced into the system

$$q = \int_0^\infty Q(t)\,dt$$

$$= \frac{aW}{1+\gamma}\int_{L_{min}}^{L_{max}}\int_{t=0}^{\infty} n(L)\rho\left(L - \frac{Wt}{1+\gamma}\right) dt\,dL$$

and if

$$\frac{t}{1+\gamma} = \tau$$

then

$$q = aW\int_{L_{min}}^{L_{max}}\int_{\tau=0}^{\infty} n(L)\rho(L - W\tau)\,d\tau\,dL$$

and this is independent of γ.

The experimental results were analyzed to see if the hypothesis presented above was in accord with the data. The experimental curves for diffusible substances (fractional recovery of injection mass per milliliter of blood versus time) were adjusted in the following manner.

TABLE 2. *Volumes calculated using linear flow-limited model*

Volume	No. of Exp.	% Liver Weight, Mean ± SD
Sinusoidal blood volume	38	15.1±6.0
Extravascular volumes (in terms of equivalent plasma volumes)		
I^{131}-labeled albumin	6	6.5±3.2
T-1824	38	6.2±0.8
Inulin	9	7.8±1.3
Sucrose	8	9.5±2.1
Sodium	6	10.6±1.4

1) Peak or half peak concentrations were used to calculate the factor $1/(1+\gamma)$.

2) Concentrations were increased by this factor over the whole curve.

3) A zero time for exchange was calculated using the relationship that the velocity of propagation was diminished by the factor $1/(1+\gamma)$. If t_{RBC} is an aribtrary point in time on the red cell curve (for instance, the time of the peak), and t_{diff} is the corresponding point in time (here, the peak time) on the curve for diffusible label, and t_0 is the zero time, then

$$\frac{t_{RBC} - t_0}{t_{diff} - t_0} = \frac{1}{1+\gamma}$$

The calculated zero time for exchange effectively lumps all transit through feeder lines (portal and hepatic veins) at the beginning of the experiment. During this time no red cell-diffusible label separation is assumed to occur.

4) The time of each sample was diminished by the factor $1/(1+\gamma)$.

If the assumptions previously made are valid, it follows that:

1) All dilution curves for a group of substances not appreciably retained by the liver during a single circulation should form a single curve when adjusted in time by the factor $1/(1+\gamma)$, and when adjusted in concentration by the factor $(1+\gamma)$.

2) If there is little displacement between red cells and suspending plasma during passage through the large vessels (feeder lines), and if the displacement in time is due only to the exchange phenomenon, the single curve should superimpose on the red cell curve.

3) The difference between the mean time of transit for red cells and the zero time will represent the mean time of transit of red cells through the sinusoids. This value, multiplied by the flow, represents the sinusoidal blood volume.

4) γ (the ratio (extravascular space)/(intravascular space)) multiplied by the sinusoidal plasma volume will give an extravascular volume of distribution, in terms of equivalent plasma volume, for substances confined to the plasma compartment of blood.

5) From knowledge of the water content of the blood, the water content of the sinusoids may be computed. γ for water gives, then, the water content of the extravascular liver. The sum of these values should correspond to the water content of the whole organ. The weight of the organ may be computed from wet and dry weights of an aliquot. The computed weight should then correspond to the value measured for the extirpated organ.

The experimental data were treated in the manner described above. In each experiment, the adjusted curves for diffusible substances did indeed superimpose upon the labeled red cell dilution curve (see Fig. 5 *A* and *B*). The calculated t_0 was quite close to the point of appearance of labeled red cells, except in those experiments in which the hepatic blood flow was relatively low when there was a perceptible delay between the t_0 and the

TABLE 3. *Extravascular volumes related to average T-1824 extravascular volume*

Substance	Ratio of Volumes*	Corrected % Liver Weight†
I^{131}-labeled albumin	0.91±0.05	5.7±0.3
Inulin	1.24±0.07	7.7±0.4
Sucrose	1.42±0.20	8.8±1.2
Sodium	1.44±0.17	8.9±1.1

* Equivalent plasma volume/equivalent plasma volume of T-1824. †Ratio of volumes × 6.2%.

appearance of the red cells. The weight of the extirpated organ was compared to the weight calculated from the tritium-enriched water dilution curve. During removal of the liver no precautions were taken to preserve the blood within the organ, and it was found that the large veins were usually empty of blood. The calculated weight used therefore excludes the "feeder lines" volume. The average weight calculated from the tritium-enriched water curves was 100.4% of the actual weight (556 g) for this group of experiments. When the data were plotted as a scatter diagram (Fig. 6) the linear regression calculated by the method of least squares was: actual weight (g) = 0.901 (calculated weight (g)) + 46 g. The correlation coefficient was found to be 0.83. The standard error of the estimate was 74.7 g. There was a positive intercept on the y axis (actual weight of 46 g) when the calculated weight was zero. Tissue samples used to determine wet and dry weights were secured from the edge of liver lobes, an area lacking much of the connective tissue skeleton supporting large blood vessels and bile ducts in the center of the liver. The positive intercept may represent an average value for the weight of this tissue.

The average values for the calculated volumes from all experiments are given in Table 2. The values are expressed in terms of the percentage of actual liver weight. In each experiment the relation of each curve to the others was consistent. There appeared to be a greater spread in the relations between the average volumes than was present in any given experiment and the quantitative relations between the average volumes appeared much less consistent than the experimental data (e.g., the I^{131}-labeled albumin volume was always smaller than the T-1824 volume), and so the equivalent plasma volumes for the group of extravascular and presumably extracellular substances were related to the calculated volume for the one substance for which a dilution curve was available in each experiment, T-1824 (Table 3). The calculated dilution volume for the presumed extracellular substance decreases as the molecular weight increases. The ratio of the C^{14}-labeled urea dilution volume to the tritium-enriched water dilution volume averaged 0.97 for three experiments.

The superposition of the adjusted dilution curves upon the red cell dilution curve unequivocally establishes the relationship between the extravascular volume and the dispersion in time of the diffusible indicator. The agreement between calculated and actual liver weights justifies the assumption that mixing of indicators in the inflow was relatively complete, and validates the use of this method to calculate volumes of distribution which will correspond closely to the true volumes of distribution for substances in the liver. The analysis was carried out in a semigraphical manner, using the times of peak and half-peak values to determine an average t_0, since the most precise data are available in this part of the dilution curve, and the curve here is in general not greatly affected by the procedure for correction for recirculation. The calculated t_0 showed no progressive increase from values calculated from the half-peak upslope to those calculated from the half-peak downslope. This indicates that the t_0 for the longest paths was virtually the same as that for the shortest paths of transit. This validates another hidden assumption made in the application of the analysis.

The extravascular volumes may be calculated in a more direct manner. If $\bar{t}_{ref}$ is the mean transit time of the vascular volume reference substance, $\bar{t}_{diff}$ is the mean transit time of a diffusible substance, and t_0 is the calculated zero time, it may be shown that

$$\frac{\bar{t}_{diff} - t_0}{\bar{t}_{ref} - t_0} = 1 + \gamma$$

$$\frac{\bar{t}_{diff} - \bar{t}_{ref} + \bar{t}_{ref} - t_0}{\bar{t}_{ref} - t_0} = 1 + \gamma$$

whence

$$\frac{\bar{t}_{diff} - \bar{t}_{ref}}{\bar{t}_{ref} - t_0} = \gamma$$

Since $F(\bar{t}_{ref} - t_0)$ = sinusoidal blood volume, where F is the total blood flow rate, then $F(\bar{t}_{diff} - \bar{t}_{ref})$ = extravascular volume of distribution for diffusible substances. This value must be weighted appropriately for the manner of distribution of the diffusible label in blood (e.g., multiplied by the proportion of blood which is plasma if it is excluded from red cells). The volume calculated in this way has been called a transit time volume (13). The volumes calculated from the transit time might be expected to be affected somewhat more by correction for recirculation than those calculated using the semigraphical method. However the values were found to be in good agreement (Fig. 7). The linear regression relating the two volumes (from 93 curves for diffusible labels) was: transit time volume = 0.994 (peak time volume) + 1.48 ml. The correlation coefficient was 0.976. The standard error was 53.9 ml. There was, then, little perceptible statistical difference between the two values.

The relation of the values for extravascular volumes of distribution calculated from the lumped model to those derived from the linear model may now be examined. It was demonstrated that the distribution of diffusible label appeared to be flow limited in the lumped model only when the peak of the outflow curve for this label appeared on or close to the downslope of the vascular

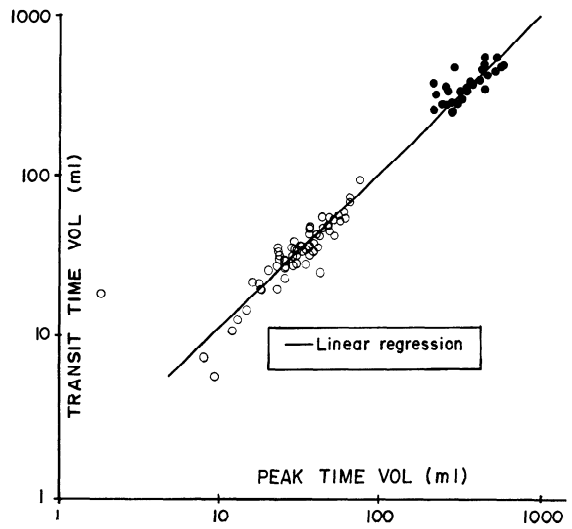

FIG. 7. Comparison of transit time volumes (*ordinate*) and volumes calculated using the semigraphical method (*abscissa*).

reference dilution curve. The basic assumption underlying the linear model is that the distribution of label is always flow limited. This suggests that since the peak of the T-1824 dilution curve falls on the downslope of the vascular volume reference curve, the volumes calculated by each method should be virtually the same for T-1824, whereas the volumes calculated for the extravascular distribution of water may be radically different. The average value for the T-1824 extravascular volumes computed using the lumped method was 28.8 ml equivalent plasma, and that computed using the linear method was 29.5 ml equivalent plasma. When the values were plotted on a scatter diagram (Fig. 8), the linear regression relating the two values was: value from the lumped analysis = 0.88 (value from linear analysis) + 3.0 ml. The correlation coefficient was 0.89. In contrast, the values calculated for the extravascular volume of distribution for water using the lumped method were invariably ridiculous, being much larger than the total liver water volume. The relatively good agreement between transit time volumes for the pulmonary extravascular water space and those calculated by Lilienfeld using the lumped method (13) has an explanation similar to that for T-1824. The peak of the water curve fell close to the downslope of the vascular volume reference curve.

DISCUSSION

Linear rates of transit and rates of diffusion equilibration. The linear method of analysis provides a means for estimating the blood volume in the exchange area (the sinusoidal blood volume) and the mean time spent by blood in this area. In this group of experiments the average liver weight was 513 g and the average sinusoidal blood volume was 77.5 ml, whereas the average volume of the headers was 42 ml. The mean sinusoidal transit time for red cells averaged 5.35 sec. If the length of the hepatic sinusoid is 0.5 mm (14), the mean linear rate of passage of red cells in the exchange area is approximately 93 μ/sec. The mean cross-sectional area of the vascular bed of the average dog liver is therefore 77.5 $cm^3/0.05$ cm = 1550 cm^2. The exchange of diffusible substances retards the rate of passage of a concentration wave along the sinusoid. The mean linear rate of passage for an albumin concentration wave is 53 μ/sec; for water, 15 μ/sec.

Rates of diffusion equilibration may be used comparatively to illustrate the relation between diffusion equilibration and flow displacement in the liver. Consider an aqueous plate 30 μ thick (the approximate diameter of a hepatic polygonal cell), exposed on both sides to a well-stirred aqueous fluid at 37 C. Diffusion equilibration for water is complete in 200 msec. In this time the red cell would travel 18 μ, the water concentration wave, 3 μ. Diffusion equilibration for albumin (the slowest of the extracellular group of diffusible substances) will be essentially complete in 4 sec. In this time the red cell would travel 372 μ; the albumin concentration wave, 222 μ. The distribution of albumin label into the extravascular space will not be flow limited if it lies between cells. In contrast, consider the 2-μ plate, exposed on one side (i.e., the instance where the space available for extracellular substances corresponds to the space of Disse, the anatomical site usually postulated for the formation of hepatic lymph). Diffusion equilibration for albumin is essentially complete in 60 msec, a time less than that required for water to equilibrate in the 30-μ plate. In this time the red cell will travel 5.6 μ; the albumin concentration wave, 3.2 μ. It is evident that it is the slow linear rate of blood flow in comparison to the rate of diffusion equilibration which provides a rational basis for the assumed flow-limited distribution of substances into an extravascular space.

When the linear method of analysis is applied to substances present within the red cell as well as in plasma, then, for equilibration of the material in the red cell with the extravascular space to occur, flow must transport the red cell only a very small distance during the half-time of exchange. Assuming the rate constant for the equilibration of labeled water within the dog red cell is the same as that for the human red cell, half equilibration will occur in 4.2 msecs (15). In this time the red cell will travel 0.42 μ. The water within the red cell will, then, participate in the flow-limited exchange. In contrast, if a diffusible substance is present in red cells for which the half-time of exchange is slow compared to the linear flow rate, then this substance will be carried along in part by the red cells, the extravascular exchange will not be completely flow limited, and equilibration of the vascular compartment with the extravascular compartment will be incomplete. This diffusible substance present within the red cells which does not equilibrate completely will appear more quickly in the outflow than one which does. The amount of incompletely equilibrated material which appears more quickly in the out-

flow should be quantitatively related to the size of the slowly exchanging red cell sink, that is, to the hematocrit.

The virtually instantaneous equilibration may be viewed in another way by considering the rapidly exchanging extravascular space to be an extension of the plasma volume, a space readily accessible to plasma, but not to the cellular elements of blood. The presence of this plasma space accounts for the low "tissue hematocrit" of liver. Table 4 compares the values for the amount of blood and the amount of "extra plasma" in liver, obtained from the indicator dilution curves, with those values obtained by others from analysis of tissue. The agreement is considered to be good.

Taylor (19) has examined the dispersion of soluble matter introduced into solvent flowing slowly through a tube and has shown analytically that the distribution of concentration produced in this way is centered on a point which moves with the mean speed of flow and is symmetrical about it in spite of asymmetry of flow, and that the longitudinal dispersion is a function of a virtual coefficient of diffusivity. The analysis applies to flows which are slow in relation to diffusion. In the sinusoids bolus flow creates radial convection which will preclude this as a mechanism of longitudinal separation. In the feeding vessels numerical calculations show that the effects would not be appreciable in the time of flow.

Red cell plasma separation. The linear method of analysis depends in part upon the assumption that there is no significant separation of red cells and plasma during flow through the larger vessels. If there were significant separation due to axial streaming of the cells a peripheral plasma layer would appear in the larger vessels. This peripheral sheath of plasma would have a lower mean velocity of flow than the central core. Diffusion exchange of plasma between the central core and the peripheral sheath in the smaller feeder vessels would tend to produce some retardation of the plasma concentration wave. The calculated t_0 would then occur somewhat earlier.

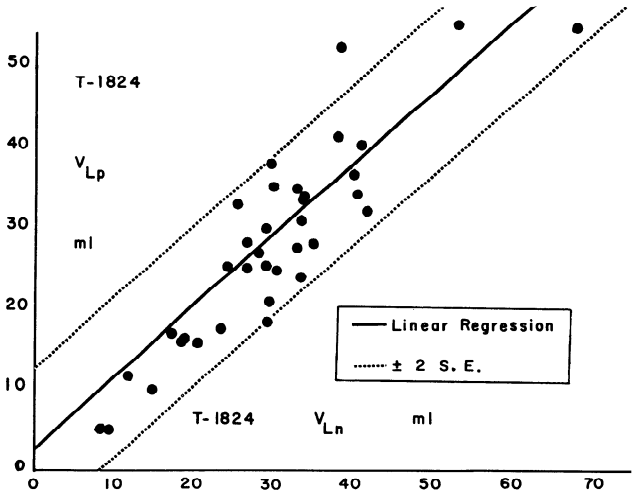

FIG. 8. Comparison of T-1824 volumes computed from the lumped model (L_p) and from the linear model (L_n).

For a diffusible substance like water which enters a very large space (the cell water), the predominant retardation would occur in sinusoids where there is bolus flow. The calculated t_0 would therefore be little affected. The average ratio of the t_0's calculated from red cell and T-1824 curves to the t_0's calculated from the red cell and tritium-enriched water curves was 1.012 ± 0.185 (SD). There is therefore little evidence of axial streaming.

When the ratio of particle to vessel size is small, axial streaming in a given suspension has been shown to depend upon the size and deformability of a particle and upon the rate of shear imposed on it (20). If there were significant axial streaming of red cells contributing to plasma red cell separation in transit through an organ, the greatest separation would be expected to occur in an organ with a high linear velocity of flow (e.g., the lung). The average value for the ratio of red cell to plasma transit times in the pulmonary circuit, taken from four series of experiments (21–24), is 0.95. If there is no rapidly exchanging extravascular space for albumin present in the lung, this is evidence of hydrodynamic separation of suspended particles and the suspending medium.

Could such separation occur in some way other than axial streaming? Goldsmith and Mason (25) have studied the flow of large bubbles suspended in wetting liquids undergoing Poiseuille flow through tubes when the tube radius was smaller than that of the bubble. It was shown that the bubbles were deformed into cylinders and moved in the tube surrounded by a film of suspending liquid. When the viscosity of the drops was insignificant compared to that of the medium, the liquid film was stationary. As the viscosity of the drop was increased, velocity profiles were set up in the film. In the limit, as the bubble viscosity is very high compared to that of the suspending liquid, plug flow occurs; the mean velocity of the film is then half that of the velocity of the bubble. If we assume the "red cell viscosity" to be very high compared to that of plasma, and if further we postulate bolus flow in the capillaries it is evident that while the plasma between the red cells moves with the same velocity as the red cells, that occupying the film between cell and wall moves with half the velocity.

Based on this model and a hematocrit of 45, the value for the film thickness giving a red cell plasma transit time ratio of 0.95 in the pulmonary capillaries averages 0.2 μ. This is a very thin layer of plasma and it is quite possible that such a layer accounts for the red cell plasma separation in the pulmonary capillaries. The average red cell:T-1824 transit time ratio for the liver is 0.65. The linear analysis appears to account for this larger red cell plasma separation in terms of extravascular exchange. If a small part of the difference of transit times between red cells and plasma in the hepatic sinusoid is due to a peripheral retarded intravascular plasma film, then the phenomenon could be treated quantitatively by weighting the reference curve (i.e., the red cell curve) for the proportion of substance present in the intravascular retarded plasma film and for the increased transit time of the film. A nonphagocytosed particulate

TABLE 4. *Extra plasma space in liver*

Species	Blood Space, % Liver Weight	Extra Plasma Space, % Liver Weight	Reference
Dog	15.2	6.2	Present study (indicator dilution Cr^{51}, T-1824)
Dog	15.0	6.7	T. H. Allen, E. B. Reeve (16) (extraction of T-1824, P^{32})
Rat	19.4	7.3	N. B. Everett et al. (17) (liquid N_2, extraction of Fe^{59} and I^{131})
Rat	11.6	6.0	R. W. Brauer et al. (18) (tissue analysis for Fe^{59} and I^{131})

plasma label would provide the necessary information for this correction.

Flow-limited exchange of labeled material present in cells and plasma will take place despite the presence of a thin retarded intravascular plasma film, if it is present, and the proportion of labeled material present in the extravascular space at any one time will be governed by the equilibrium partition of material in extravascular space and intravascular space, rather than by a diffusion coefficient. Thus Chinard and Enns found no measurable difference between the relative rates of passage of deuterium and tritium oxides in the hind limb and pulmonary capillaries in the dog (26). Again, Chinard, Enns, and Nolan found that the ratios of recoveries of inert gases in the pulmonary outflow approached the ratios of their solubilities rather than the ratios of their diffusion coefficients (27).

Calculated extracellular volumes. The calculated extracellular volumes decrease as the size of the probing molecule increases along the series: sodium, sucrose, inulin, and albumin. This is illustrated in Table 5, in which the equivalent plasma volume extracellular spaces, compared to the sodium space, are tabulated. If there is an extravascular space, it would be logical to assume that these species entered the same space. Is it possible that there exists a steady-state partition coefficient which progressively excludes larger molecules? Ogston and Philips (28) allowed molecular species in a buffer layer to diffuse into a layer of hyaluronic acid gel until complete equilibration occurred. At equilibrium the concentration of larger molecules in gel water was lower than that in the buffer water. The concentration of smaller molecules was virtually the same in gel and buffer water. As the molecular size of the probing molecule increased, more of the gel water enfolded within the domains of the hyaluronic acid chains became inaccessible to this molecule. The same phenomenon may be occurring in the liver extravascular space. A steady-state exclusion for larger molecules could explain the experimental results.

Could lack of diffusion equilibration explain the results? Examination of diffusion equilibration profiles for albumin and inulin demonstrate that there is a time at which the relative amounts of material which have passed into the extravascular space approximate the values listed in Table 5. What factors negate this explanation? The sodium space by tissue analysis exceeds the I^{131} albumin space in the isolated rat liver by a factor of at least two at a time when diffusion equilibration has occurred (18). Secondly, careful consideration of the kinetics demonstrates that lack of diffusion equilibration would change the shape of the curve so that the analysis carried out would not result in superposition of the curves for diffusible labels upon the red cell curve. The hypothesis of steady-state exclusion which is greater for larger molecules appears to explain the data more completely. However, completely certain assessment must await the solution of the nonequilibrium linear model.

Another lumped two-compartment model. Macy (29) has essentially extended a two-compartment lumped model by considering the behavior in toto of a large number of these small units. In each small unit the rate of accumulation of material in tissue is assumed equivalent to the difference of the rates of delivery and efflux of material, i.e.,

$$V_t dC_t/dt = F(C_i - C_o)$$

where V_t is the tissue volume for the element, C_t is the concentration of material in this volume, C_o is the observed outflow concentration, and C_i is the inflow concentration. Instantaneous equilibration is assumed. Thus at the outflow $C_o = C_t$. If C_i is constant beginning at time zero, then

$$C_o = C_i(1 - \exp(-Ft/V_t))$$

The length of the unit is not specified and, since no transport lag is present in the expression, must be negligible. Since capillary lengths are necessarily disregarded, the distribution of capillary lengths in the organ could be considered to have no effect on the final form of an effluent dilution curve. The assumption of instantaneous equilibration implicitly lumps capillary blood volume and the extravascular volume of distribution into the single parameter, V_t. Deviations of outflow concentrations for a whole organ from this paradoxical model describing the behavior of a small unit are then considered to result from heterogeneity of blood tissue perfusion ratios. In the absence of a group of indicator dilution curves to direct their attention to the importance of lag and hence the length of each unit, Thompson et al. (30) were able to construct a frequency distribution of regional blood tissue perfusion ratios which satisfactorily accounted for the deviation of the effluent dilution curve of the single indicator, D_2O, from the idealized curve. The consideration of heterogeneity of blood tissue perfusion ratios was unnecessary for satisfactory quantitative interpretation of data presented in the present paper when a linear two-compartment model was considered.

Slope volumes. When the data have been corrected for distortion produced by the catheter sampling system, the relationship between the capillary blood volume and the extravascular volumes may be defined using the

terminal slopes of a semilogarithmic plot of the primary indicator dilution curves. Let K_1 be the terminal slope for the intravascular label (red cells), and K_2 be the terminal slope for the diffusible label. Then

$$dy/dt = -K_1 y$$
$$dy'/dt' = -K_2 y'$$

But for corresponding parts of the curve

$$dy' = dy/(1+\gamma)$$
$$dt' = dt(1+\gamma)$$
$$y' = y/(1+\gamma)$$

so that

$$dy'/dt' = dy/[dt(1+\gamma)^2] = -K_1 y/(1+\gamma)^2 = -K_2 y'$$

or

$$K_1/K_2 = 1+\gamma = (V_A + V_B)/V_A$$

where V_A and V_B are the capillary blood volume and extravascular spaces, respectively. Thus

$$V_A = V_B/(K_1/K_2 - 1)$$

Now V_B is the transit time volume. The capillary blood volume may therefore be computed using the terminal slopes and the transit time volume, and should coincide with that found during the linear analysis demonstrated above. V_B will have been weighted for the manner of distribution of label in blood (e.g., it will represent the equivalent plasma volume in the instance of I^{131}-labeled albumin), and so V_A will be similarly weighted.

Now consider Macey's model. If the input into the system, FC_i, is a unit impulse function at time zero, then

$$C_o = (1/V_t)\exp(-Ft/V_t)$$

By considering an experimental lumped model representing a vascular mixing volume Newman et al. (31) derived a slope method for calculating this volume. The relation used was that terminal slope equals flow/volume. Now, in any system, volume equals flow times mean transit time. The definition implies that the slope is the reciprocal of the mean transit time defining the volume. Newman then, in reality, considered the response of Macey's model to a unit impulse function and the volume computed is that defined by the mean transit time of this response, i.e., the time taken for the response of the system to diminish to 1/e of its value at zero time, $1/V_t$. Agreement between the capillary volume computed from the linear model and the value derived from the red cell curve using Newman's method would be fortuitous.

Ramsey et al. (32) extended Newman's method to the calculation of the total volume of distribution for substances distributed into extravascular pulmonary volumes. If V_{A*} is the slope volume of the vascular compartment, then the total volume of distribution for diffusible substances would be $V_{A*} + V_{B*}$. To relate the volumes appropriately, both should again be weighted for the manner of distribution of the diffusible substance in blood. The method of Newman defines the relation

$$K_1/K_2 = (V_{A*} + V_{B*})/V_{A*}$$

This is the same relationship as that derived using the linear model. Values for V_{A*} which have been computed in the past using Newman's method will fortuitously correspond to values which would have been obtained using the linear model when V_{B*} has been found equal to V_B, the transit time volume.

TABLE 5. *Extravascular space relative to sodium space*

Substance	Space for Substance / Mean Sodium Space Mean ± SD
Sodium	1.00±0.14
Sucrose	0.98±0.13
Inulin	0.86±0.05
Albumin	0.64±0.03

Fluid balance in the tissue. The inference that albumin movement into the extravascular space is virtually instantaneous serves to reinforce the statement of Brauer et al. (18) that in the absence of protein-restraining hepatic endothelia, the fluid balance within the tissue should be extraordinarily labile, and should be restrained only by such mechanical factors as tissue tension, which in turn should reflect largely the mechanical rigidity of connective tissue scaffolding within, and of the capsule surrounding the hepatic parenchyma. Thus Brauer et al. (18) have shown that hepatic vein pressures of only 1 cm of water above the hilar level of the isolated rat liver results in engorgement of the organ, in expansion of the albumin and sodium spaces, and in the appearance of transudate at the liver surface.

General applications. The thesis presented is a general one. Flow-limited distribution of rapidly diffusible labels probably occurs in most richly perfused tissues. The permeability of the capillary walls varies, however. For instance, skin and muscle capillaries have been assumed to permit the transcapillary passage of albumin to occur very slowly. The distribution of this substance into the extracellular space of these tissues would then be predominantly diffusion limited. The distribution of labeled water would, in contrast, approach the flow-limited case. The linear method of analysis outlined above is applicable in organs other than liver to those indicator dilution curves resulting from the injection of a vascular volume reference label and a rapidly diffusible flow-limited label. The analysis will yield estimates of capillary blood volume, and of the extravascular volume of distribution of the rapidly diffusible label.

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