Measurement of Hepatic Blood Flow By Clearance Methods

A Review and a Theoretical Basis for a New Noncatheterization Method

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METHODS FOR MEASUREMENT OF REGIONAL OR ORGAN BLOOD FLOW have interested investigators for many years. The large number of devices used in experimental animals for the direct measurement of flow in tributary vessels can be classified as venous drainage, mean flow, or pulsatile flow recorders.[1] These require virtual isolation of the inflow or output circulation in order to measure total regional flow. Thermal conductivity of tissue is useful for estimation of perfusion of localized areas of tissue but not total organ flow,[2] while the "fractionation of the cardiac output" by isotope distribution requires extensive tissue sampling.[3] The foregoing methods are thus not applicable to the intact human. The development of atraumatic, valid, and accurate clearance methods, which have in common the principle of removal of a substance from the blood by an organ as the basis for measurement of blood flow to that organ, has been a significant advance in clinical medicine. It is the purpose of this paper to review those clearance methods which have been applied to the measurement of total blood flow to the liver and splanchnic bed, with emphasis on the principles on which they are based, their accuracy, and their ease of application to the human. After review of those presently available, the theoretical basis of a new method, which has been an outgrowth of earlier methods, will be presented.

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I. CURRENT METHODS OF MEASUREMENT OF BLOOD FLOW

The Fick Principle: Clearance:Extraction Ratio

As long ago as 1870, Fick[4] proposed a principle by which organ blood flow may be measured. Suppose a suitable test substance, *S*, maintained in the blood at a constant concentration, is removed by only one organ at a known rate, *R*. Assume both arterial inflow and venous outflow are accessible, so that, by sampling, the concentration of *S* in these blood vessels can be determined. Let *a* be the arterial concentration and *v* the venous concentration. In the steady state, the blood flow to the organ, *F*, in ml./minute, is stated by the Fick equation:

$$F = \frac{R}{a\text{-}v} \left(\text{i.e., } \frac{\text{amount removed per minute}}{\text{amount removed per ml. of blood}} \right). \quad \text{(Eq. 1)}$$

When the removal rate, arterial, and venous concentrations of *S* can each be measured directly, the method is known as the "direct" Fick principle; when one or more of these must be estimated by intermediate means, it is known as the "indirect" Fick principle.[5]

A well-known application of the direct Fick principle is the determination of systemic cardiac output, using right heart catheterization by the method of Cournand *et al.*[6] Here the test substance is oxygen. Since minute cardiac output is equivalent to total systemic blood flow per minute, *R* is the total body oxygen consumption in ml./minute, and can be measured directly by a spirometer or similar device. An arterial sample drawn from any accessible artery is suitable for measuring *a* (ml. O_2/ml. blood) because arterial blood is thoroughly mixed and of constant composition. Right heart catheterization permits completion of the Fick equation by direct means, samples of blood so obtained supplying the measure of the average oxygen content of systemic venous blood, *v*, from the site traversed, which in this case is the entire body.

If only the removal rate and arterial concentration are known, one can compute clearance, *Cl*. Clearance is the smallest volume of blood which must traverse the organ to yield the amount of *S* removed per unit time. It assumes complete extraction of *S* from each ml. of blood flowing through the organ. Thus

$$Cl = \frac{R}{a}. \quad \text{(Eq. 2)}$$

Extraction ratio,

$$E = \frac{a\text{-}v}{a}. \quad \text{(Eq. 3)}$$

If extraction is complete, v is zero. Therefore, the extraction ratio, E, is 1.0, and clearance equals flow:

$$E = \frac{a\text{-}o}{a} = 1.0 \text{ and } F = \frac{R}{a\text{-}o} = \frac{R}{a} = Cl. \qquad \text{(Eq. 4)}$$

If extraction is incomplete (i.e., less than 1.0), then

$$E \times F = \frac{R}{a\text{-}v} \times \frac{a\text{-}v}{a} = \frac{R}{a} = Cl \qquad \text{(Eq. 5)}$$

and clearance is less than flow.

In the familiar case of renal clearance of PAH, the extraction ratio is normally 0.90 or more, so that $Cl_{PAH} = \frac{UV}{P}$ may be loosely considered to be equivalent to renal blood flow, since it is in fact 90 per cent or more of that flow.[7] (In this instance, UV, the total amount collected in the urine [urine concentration, U, times urine volume, V], is a direct measure of R; P, the peripheral concentration, is equally well measured in artery or vein, for, since there is no extraction of PAH except by the kidney, elsewhere A equals V). In renal disease, however, the extraction is less than 90 per cent. In order to determine renal blood flow by the direct Fick principle, $\frac{UV}{a\text{-}v}$, measurement of renal vein concentration must be determined. Samples can be obtained by renal vein catheterization.[8]

Measurement of Hepatic Blood Flow: The Indirect Fick Principle

It is relatively simple to measure the rate of excretion of S by the kidney because the urine is formed at a rapid rate and can be collected easily *in toto*. The removal rate of substances extracted from the blood by the liver and excreted into the bile is not so readily measured, however, because bile flow is relatively slow and because biliary excretion is relatively inaccessible to collection. Nevertheless, Bradley and co-workers[9] have shown that with sulfobromophthalein (BSP) as the test substance the Fick principle can be applied to the liver. To accomplish this, the removal rate of the dye by the liver is estimated indirectly, and its concentration in hepatic venous blood obtained by hepatic vein catheterization.

Assuming excellent mixing, a constant volume of distribution, and the absence of extrahepatic removal of the dye, a given peripheral blood level, P, will remain constant if BSP is infused at *exactly* its rate of removal by the liver. (When there is no extra-

hepatic extraction, P represents both arterial and venous peripheral concentrations). In this circumstance, the Fick equation becomes:

$$\text{When } I = R,\ F = \frac{I}{P\text{-}H} \qquad \text{(Eq. 6)}$$

where H is hepatic vein concentration.

Without prior knowledge of the exact removal rate of BSP, however, it is unlikely that concentration equilibrium will be reached. Most often, a correction of the infusion rate will be necessary to estimate R accurately. If the infusion rate exceeds the removal rate, the peripheral concentration of BSP will gradually rise as the excess accumulates in the volume of distribution of the dye. The mg./minute excess can be estimated as the peripheral increment ΔP (mg./ml./min.) times volume, V, in ml. Since we are interested in a measure of R, this excess must be subtracted from the infusion rate. Thus

$$\text{When } I > R, \text{ then } R = I - \Delta P \cdot V. \qquad \text{(Eq. 7)}$$

The converse is true when I is less than R. Here correction for the deficit must be made:

$$\text{When } I < R, \text{ then } R = I + \Delta P \cdot V. \qquad \text{(Eq. 8)}$$

For these corrections, V is assumed to be the plasma volume.

In practice, the hepatic vein is catheterized in retrograde fashion by use of a cardiac catheter.[10] After introduction of the catheter into the right atrium in the usual manner, it is passed into the inferior vena cava and thence into one of the branches of the hepatic vein. With experience, one of these veins may be entered successfully as often as in 90 per cent of attempts to do so.[11] An amount of BSP sufficient to give a level of 1 mg./100 ml. or so throughout the plasma volume is injected as a "priming" dose. This is followed by a constant infusion of the dye at an exactly measured rate: in normals, the most satisfactory rate is between 0.06-0.10 mg./kg./-min., whereas in liver disease, a slower rate is better. After 20-30 minutes is allowed for equilibration, the periphery is sampled simultaneously with the hepatic vein every 10 minutes for three or four times. A semilogarithmic plot is made of the concentrations obtained (Fig. 1). Average values for each period are taken as the midpoints between two successive samples and ΔP calculated as the change in concentration for the first minute from the midpoint. If

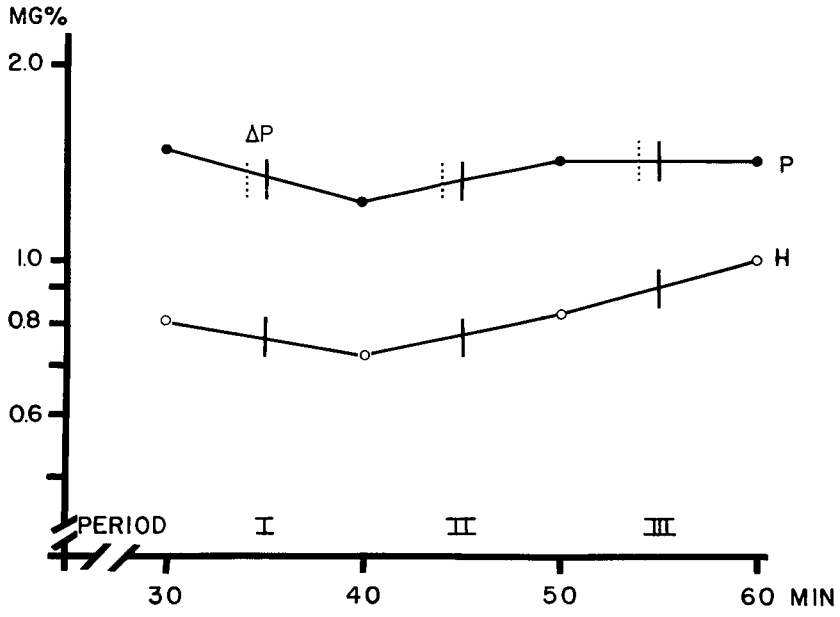

Fig. 1. **Method for estimation of hepatic blood flow from BSP clearance during constant infusion.[9] *P*, peripheral vein concentrations; *H*, hepatic vein concentrations. Values for *P* and *H* for Equation 9 (see text) are midpoints of each period *(vertical solid lines)*. ΔP is the difference in concentration of *P* between 1 minute before *(vertical broken line)* and at midpoint. Three possible corrections of infusion rate, *I*, to removal rate, *R*, are illustrated: Period I where *P* is falling, $R = I + \Delta PV$; Period II where *P* is rising, $R = I - \Delta PV$; Period III where *P* is steady, $R = I$. Extraction ratio, $P - H/P$, is approximately equal in I and II, but is decreasing in III.**

the BSP concentration is stated in mg./100 ml. and determined in plasma or serum, the completed Bradley equation for the estimation of hepatic blood flow becomes:

$$\text{EHBF} = \frac{\text{I} \pm \Delta\text{P} \cdot \text{V}}{.01\ (\text{P-H})} \times \frac{1}{1\text{-crit}} \qquad \text{(Eq. 9)}$$

where .01 is the correction for concentration of BSP to mg./ml./, *P* and *H* are the midpoint values for the period, and the second half of the equation a correction for plasma flow to whole blood flow. An average of two or three periods is recommended for most purposes. If the peripheral concentration changes too rapidly in either direction (ΔP more than 0.05 mg./100 ml./min.), the estimate of *R* is not valid and the test should be discarded.[9]

As has been indicated, certain assumptions are made in apply-

ing BSP to hepatic blood flow measurement. The first is that there is no extrahepatic loss of BSP. Actually, a small amount is lost in the urine, but if this is measured an appropriate correction can be made.[12] However, Cohn *et al.*[13] showed in hepatectomized, nephrectomized animals the continued disappearance of injected BSP with time, which may be taken to indicate metabolic destruction or progressive penetration into a larger volume of distribution. Further, an apparently inconsequential *a-v* difference across extrasplanchnic regions may represent very real extraction of BSP, in terms of mg./min., if the blood flow to the region is large. It has been shown by radioactive tracers, for example, that uptake of BSP is significant in skeletal muscle, where *a-v* differences are small but flow is high.[14]

Another assumption of the BSP method, or for that matter, of any method employing catheterization, is that the catheter sample represents true mixed hepatic venous blood. Bradley *et al.*[15] have shown that BSP concentration in samples of blood drawn from different hepatic veins in the same subject may vary as much as 20 per cent. This variation has been ascribed to different flows in different areas of the liver, and minute-by-minute variation in segmental liver flow is well known.[16] It is for this reason that average results from two or three periods were recommended originally. Recently, Sapirstein and Reininger[17] have presented evidence indicating that catheter depth influences the concentration of material in the venous sample. The deeper the catheter and the closer to wedging it is, the greater the likelihood of falsely low values. Different depths of catheterization may account for widely variant results between subjects and between lobes in the same subject. It is difficult to overcome this problem. As one places the catheter less deep in the hepatic vein, reflux aspiration of inferior vena cava blood becomes more likely.

Unless the peripheral concentration of BSP remains within narrow limits (1-2 mg./100 ml.), the accuracy of the method is questioned[18, 19] because of the possibility of abnormal extraction ratios. If the extraction ratio is less than 10-15 per cent, despite an optimal level in the periphery, the accuracy of the method is also questioned[15]; unfortunately, ratios of this order are not uncommon in hepatic disease. It is assumed that BSP distributes in the plasma volume, but in fact, it probably distributes in a larger volume.[20] An estimate of this volume is required by Equation 9. If

one uses T-1824 to estimate plasma volume, as suggested by Bradley, another variable is introduced, and there is evidence that BSP and T-1824 do not distribute in the same volume.[20]

The Bradley method assumes there is no extraction of BSP across the splanchnic bed, and that portal vein and hepatic artery levels are equal and accurately represented by the peripheral level. Many investigators prefer to avoid the question of portal vein-hepatic artery equivalence by considering the method a measure of estimated splanchnic blood flow. Only the inflow arterial level and not the portal contribution need thus be considered, since the hepatic vein is the final outflow in any event. There is indication of enterohepatic recirculation of BSP so that with time portal vein levels are possibly higher than arterial levels.[21] Even though the discrepancy is not marked, it will interfere with flow measurements whether "splanchnic" or "hepatic." For the reasons given above, then, the term *EHBF* for "estimated hepatic blood flow" has been used and appears to be a conservative approach to the problems encountered with use of the BSP method.

Alternative Methods Employing the Fick Principle and Catheterization

Sulfobromophthalein is not the only compound suitable as a test substance for liver-flow measurement by the above method. Rose Bengal dye has been used in animals and is claimed to have the advantage over BSP of restriction to the plasma volume, no urinary loss, and less extrasplanchnic uptake.[20] Lipscomb and Crandall[22] and Myers[23] have explored the use of endogenous urea as the test substance in animals and in humans, respectively. Urea has the advantage of being produced solely by the liver, and since the blood level of urea is quite constant over short periods of time, its urinary excretion is an accurate measure of liver production (output) and is easily collected. In this case, the Fick equation is adapted for production, rather than removal, of S:

$$F = \frac{\text{Output}}{\text{v-a}}. \qquad \text{(Eq. 10)}$$

The major objection to the method is the difficulty in accurate measurement of urea, the v-a difference being smaller, in most cases, than the error of chemical analysis.[23]

Ammonia, which is rapidly cleared by the liver for conversion to

urea, has been studied as a test substance by Sapirstein and Simpson,[24] but its extrahepatic extraction by a variety of tissues was found to be too great for validity.

Another application of the Fick principle has been described by Tybjaerg-Hansen, Tygstrup, and Winkler.[25] Using galactose as the test substance, they found that one can calculate the removal rate from the peripheral disappearance curve after a single injection, thus avoiding a constant infusion. The average difference between the parallel slopes of the peripheral and hepatic venous disappearance curves, the latter obtained by catheterization, is fitted into the denominator of the Fick equation. Work in our laboratory has extended the observations of these authors, so that it appears possible to employ galactose in the measurement of hepatic blood flow from the peripheral disappearance curve alone, without resort to catheterization.[26] Since this will be discussed in detail below, further consideration of the method of Tybjaerg-Hansen, Tygstrup, and Winkler will be deferred at this point.

It should be noted that each of the alternative methods employ hepatic vein catheterization as does the BSP method, and are thus subject to the same possibility of catheter induced sampling error as the Bradley method. A final objection to the catheterization technique is the complex equipment and teamwork needed to accomplish it. Although it may confidently be stated to be nonhazardous, it is hardly an office or bedside procedure and is thereby limited in its usefulness to research organizations with the necessary personnel and equipment.

Determination of Hepatic Blood Flow by Disappearance of Tagged Particulate Material

A fresh approach to the measurement of hepatic blood flow was supplied by Dobson and Jones[27] with the introduction of colloidal chromic phosphate clearance. Suppose a substance in the blood is completely removed by only one organ on one passage through that organ so that the extraction ratio is 1.0 and the venous concentration is zero. Thus, clearance equals flow (Equation 4). If the substance is injected by a single rapid injection rather than by constant infusion, the disappearance from the blood will be exponential, the disappearance curve being that of a first-order reaction, and described by the equation:

$$C_t = C_o e^{-kt}. \qquad \text{(Eq. 11)}$$

The constant, k, represents the fractional disappearance, that fraction of the amount remaining which disappears per unit time. Because extraction is complete, a volume of blood containing that particular amount must have traversed the organ per unit time. Since the material is distributed equally throughout the volume of distribution, the fraction of the total material being removed is contained in an identical fraction of the total volume and it is this fractional volume which flows through the organ:

$$F = kV. \qquad \text{(Eq. 12)}$$

If the extraction ratio is not known, Equation 12 describes a flow which can be no more than actual flow, but may be less, for if the ratio is less than 1.0, then the observed constant (k):

$$k = EK \qquad \text{(Eq. 13)}$$

where K is the true fractional flow constant. In this instance

$$F = KV \qquad \text{(Eq. 14)}$$

and

$$Cl = kV = EKV. \qquad \text{(Eq. 15)}$$

In other words, when E is less than 1.0, kV will measure clearance rather than flow, and the results will be smaller than actual flow (*Cf.* Equation 5).

Dobson and Jones showed that colloidal chromic phosphate is removed by the reticuloendothelial system, primarily in the spleen and liver, and to a lesser extent in the bone marrow. Since the spleen and liver are connected in series and are the major sites of removal, the disappearance of the material from the blood is essentially a measure of splanchnic, including hepatic clearance. Because extraction efficiency is in the vicinity of 90 per cent ($E \cong 0.90$), this clearance is a reasonable approximation of the splanchnic blood flow.[28]

To perform the measurement, 5 ml. of radioactive colloidal chromic phosphate, containing 2-4 μc. of P^{32}, is injected intravenously and the disappearance of radioactivity from the peripheral blood measured by venous sampling.[28] The disappearance constant, called the "colloidal disappearance constant," is determined from a semilogarithmic plot of the curve as

$$k = \frac{\log_e 2}{T\frac{1}{2}} = \frac{0.693}{T\frac{1}{2}}. \qquad \text{(Eq. 16)}$$

and the volume of distribution, V, is calculated by T-1824 dilution (a measure of total plasma volume). Recently, Dobson *et al.*[29] have indicated that the correct volume to use in Equation 12 is not the total volume of distribution but only the extrasplanchnic blood volume because the disappearance of the colloid being measured is that which occurs as the particles leave the extrasplanchnic volume. Since measurement of total blood volume with T-1824 includes the splanchnic volume, they recommend estimation of the extrasplanchnic volume by extrapolation of the disappearance curve itself.

Vetter *et al.*[30] have applied the same principle to a method which employs radioactive colloidal gold Au^{198}. This preparation avoids venipuncture since the disappearance curve can be traced over the thigh by external scintillation counting. Results obtained in humans with this method correspond reasonably well with those obtained by the BSP method in the same subjects.[31]

Halpern *et al.*[32] propose heat-denatured, human serum albumin tagged with I^{131} as the test substance for the disappearance method. Once denatured, the albumin is phagocytosed by the reticuloendothelial system as are the above materials. The product appears to have one advantage not shared by the others: once removed from the circulation, it is broken down and the radioactivity released and excreted along the usual pathways for I^{131}. The hazard of radiation injury to the reticuloendothelial system thus seems lessened by the material used by Halpern's group.

Determination of splanchnic blood flow by the disappearance of tagged particulate material has been studied in both animals and humans with reasonably satisfactory results.[28-32] The objections to this approach include: (1) extrasplanchnic removal of the material, particularly in the bone marrow, but possibly elsewhere[30]; (2) nonuniform particle size resulting in uneven clearance[27]; (3) incomplete extraction on one passage through the liver so that flow is only approximated; (4) possible toxicity of the material and its contained radioactivity; and (5) the need for equipment for counting radioactivity.

Results Obtained with Current Methods

It should be noted that all clearance methods require active work on the part of the liver, be it extraction or production of the test substance by parenchymal or reticuloendothelial cell. These are

thus "effective flow" measurements, and not necessarily true perfusion measurements. Furthermore, because of the variation in hepatic blood flow from moment to moment, these methods should be thought of as estimating only average flow over a period of time, and other techniques used if detailed study of the fluctuation in flow is desired.[16] Tables 1 and 2 summarize many reports of results obtained by use of the various methods already discussed. Values in "normal" humans in the resting state, in response to a variety of physiological and pharmacological situations, and in diseases are listed. No attempt has been made to include all of the large literature on the subject; rather, representative results have been selected. Animal studies have not been included, but in general, parallel findings in humans.

TABLE 1. Representative Values for Estimated Hepatic or Splanchnic Blood Flow in Normal Humans

Method	Authors and Ref.	Year	Mean EHBF ml./1.73 M^2/min.[a]	ml./min.
BSP	Bearn[42]	1951	1444	--
BSP	Bearn[43]	1951	1868	--
BSP	Bondy[45]	1952	1296	--
BSP	Bradley[9]	1945	1497[b]	--
BSP	Bradley[15]	1952		1530
BSP	Brandt[50]	1955	1955	--
BSP	Culbertson[44]	1951	1713[b]	--
BSP	Kessler[49]	1954	1332	--
BSP	Mendeloff[48]	1954	--	1627
BSP	Munnel[39]	1947	1548[b]	--
BSP	Myers[23]	1947	1384	--
BSP	Myers[40]	1948	1470	--
BSP	Myers[41]	1950	1405	--
BSP	Reynolds[46]	1953	1609	--
BSP	Shackman[47]	1953	--	1258
BSP	Sherlock[18]	1950	1446	--
BSP	Vetter[31]	1956	--	1083
Urea	Myers[23]	1947	1730	--
Galactose	Tybjaerg-Hansen[25]	1954	--	1673
Colloidal phosphate	Dobson[28]	1953	--	1500-1800
Colloidal gold	Vetter[30]	1954	--	1217
Colloidal gold	Vetter[31]	1956	--	921
Denatured HSA	Halpern[32]	1956	--	1060

In general, subjects studied in fasting state and supine position.

[a]Recalculated from authors' mean values given in ml./M^2/min. to facilitate comparison with values in ml./min.

[b]Given in authors' paper as ml./1.73 M^2/min.

TABLE 2. Representative Values for Estimated Hepatic or Splanchnic Blood Flow in Physiologic and Pathologic Conditions

Method	Author and Ref.	Year	Condition studied	Mean EHBF ml./1.73 M^2/min.[a]	Mean EHBF ml./min.
			A. PHYSIOLOGIC CHANGES		
BSP	Brandt[50]	1955	Postprandial	2716	..
BSP	Bearn[42]	1951	I.V. adrenalin	2787	..
BSP	Bearn[42]	1951	I.V. noradrenalin	952	..
BSP	Mendeloff[48]	1954	I.V. alcohol	..	2346
BSP	Culbertson[44]	1951	Upright posture	1070[b]	..
BSP	Bearn[43]	1951	Fainting	952	..
BSP	Reynolds[46]	1953	Hexamethonium admin.	1193	..
BSP	Shackman[47]	1953	Anesthesia	..	884
BSP	Munnel[39]	1947	Pregnancy	1554[b]	..
			B. LIVER DISEASE		
BSP	Bradley[15]	1952	Cirrhosis	..	1090
BSP	Brandt[50]	1955	Cirrhosis	1263	..
BSP	Kessler[49]	1954	Fatty liver	1142	..
BSP	Vetter[31]	1956	Liver disease		973
Colloidal gold	Vetter[31]	1956	Liver disease	..	779
Colloidal gold	Vetter[30]	1954	Cirrhosis		527
Denatured HSA	Halpern[32]	1956	Cirrhosis	..	733
Denatured HSA	Halpern[32]	1956	Cirrhosis with ascites	..	460
			C. OTHER DISEASE		
BSP	Munnel[39]	1947	Toxemia of pregnancy	2056[b]	..
BSP	Bearn[52]	1951	Diabetes ± insulin	1418	..
BSP	Myers[41]	1950	Hyperthyroidism	1520	..
BSP	Culbertson[44]	1951	Hypertension	1357[b]	..
BSP	Wilkins[51]	1951	Hypertension, postsympathectomy	1472[b]	
BSP	Myers[40]	1948	Cardiac disease	925	..

For control values, see respective author, Table 1.

[a]Recalculated from authors' mean values given in ml./M^2/min. to facilitate comparison with values in ml./min.

[b]Given in authors' paper as ml./1.73 M^2/min.

II. THEORETICAL CONSIDERATIONS OF A PROPOSED NONCATHETERIZATION METHOD

We should now like to present the theoretical basis for a noncatheterization method recently developed in our laboratory. Specific data relative to this method are in preparation for publication and will be presented at a later date.[26]

Consider the behavior of a hypothetical substance brought to the liver via the blood stream, which is removed from the blood by an active metabolic process having a maximum, limiting rate. The amount delivered to the liver per minute is the product of the rate

of blood flow and the concentration of this substance in the blood. When presented with the test substance in excess of the capacity, the maximum amount is removed and the excess is returned to the general circulation. When presented with an amount less than capacity, that amount is removed completely and none is returned to the circulation. Assume that the following hypothetical conditions are met by the system: the substance is removed only by the liver, it distributes instantaneously throughout a water volume of distribution somewhat greater than the extracellular fluid compartment, and mixing is so rapid and complete that any removal of the substance by the liver is immediately reflected in decreased concentration in the entire extrahepatic volume of distribution. (The liver, in this situation, is considered an organ of extraction, but not distribution, so that the volume of distribution is extrahepatic). Figure 2*a* is a model for the situation in the initial period after intravenous introduction of a large amount of the substance as a single, rapid injection. For the sake of simplifying explanation, specific quantities for volumes, hepatic blood flow, and maximum removal rate are assumed and given. Upon injection of the substance, the immediate concentration is calculated as

$$C_o = \frac{I}{V} \qquad \text{(Eq. 17)}$$

where V is the extrahepatic volume of distribution. In the case illustrated by Fig. 2*a*, C_o equals 35,000 mg./17,500 ml., or 2.0 mg./ml. (200 mg./100 ml.). The substance is now delivered to the liver via the portal vein-hepatic artery inflow, which have identical concentrations because there is no extrahepatic removal. In the first minute, 3000 mg. is delivered (2 mg./ml. $\times$ 1500 ml./min. flow) from which the liver extracts 700 mg., its maximum, and returns 2300 mg. to the general system via the hepatic vein, whose concentration is now 2300/1500 or 1.53 mg./ml. At this point, V contains 700 mg. less than initially, and the concentration, C, correspondingly falls. The next minute, and each minute thereafter, so long as the amount delivered exceeds the removal maximum ($F \cdot C_t > R$), 700 mg./min. is removed from V. During this phase, the decrease in concentration is rectilinear, and concentration at any time, t, may be expressed as:

$$\text{When } F \cdot C_t > R, \text{ then } C_t = \frac{I - R \cdot t}{V} \qquad \text{(Eq. 18)}$$

where R is removal maximum in mg./min.

Eventually, a point is reached where the amount delivered equals R ($F \cdot C_t = R$). From here on, the liver removes all of the substance brought to it, and the hepatic vein concentration falls to, and remains, zero (Fig. 2*b*). Now the situation is that described for the disappearance techniques when the extraction ratio, E, is 1.0. The decrease in concentration is exponential and C_t is expressed by Equation 11.

If one calculates C_t minute by minute, assuming the conditions and quantities given above, the disappearance curves for both peripheral vein (or artery) and hepatic vein concentrations shown in Fig. 3 are obtained. In a rectilinear plot (Fig. 3, *left*) the curves are linear until the hepatic vein concentration, H, reaches zero; thereafter, the peripheral curve follows a curvilinear line. In the semilogarithmic plot (Fig. 3, *right*), the peripheral curve is linear after H reaches zero. From the plots, the following equations permit calculation of volume of distribution, liver removal maximum, and hepatic blood flow.

By extrapolation of the rectilinear first phase, C_o is obtained as that concentration at which the curve crosses the ordinate. By rearrangement, Equation 17 becomes:

$$V = \frac{I}{C_o}. \qquad \text{(Eq. 19)}$$

The slope of the rectilinear phase can be calculated from the plot as

$$K_1 = \frac{C_{t_1} - C_{t_2}}{t_2 - t_1}. \qquad \text{(Eq. 20)}$$

Since this describes the disappearance in this phase in mg./ml./min., multiplying by total number of milliliters (V) gives total mg./min. removal (R):

$$R = K_1 V. \qquad \text{(Eq. 21)}$$

Let the time at which the peripheral curve begins its exponential phase be x, and the peripheral concentration at this time be C_x. By knowing C_x exactly, it is not necessary to catheterize the hepatic vein in order to use the Fick equation. At the exact moment H becomes zero, the peripheral concentration is C_x. This is equivalent to the first phase P-H difference, since the curves are parallel in the first phase and $C_x = P$-H when H first equals zero. The Fick equation, $F = R/P$-H at time, x, can be restated as

$$F = \frac{R}{C_x}, \text{ since } H = 0. \qquad \text{(Eq. 22)}$$

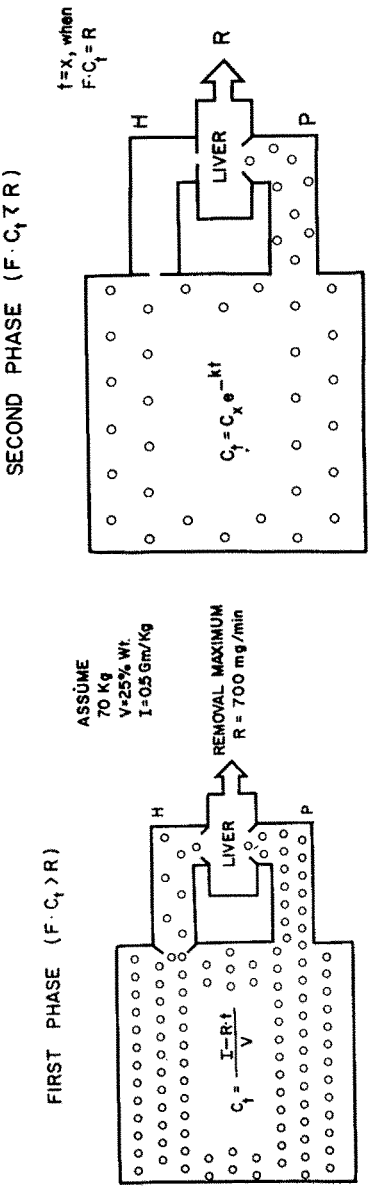

Fig. 2. Behavior of hypothetical substance for which liver has removal maximum. *A*, model for first phase of disappearance after a single intravenous injection. Amount delivered to liver via portal vein and hepatic artery (*P*) exceeds removal maximum. Excess is returned via hepatic vein (*H*). C_t (concentration in volume of distribution at time, t) is calculated by Equation. See text for explanation. *B*, model for second phase after injection. Concentration in V has fallen, so that $F \cdot C_t = R$, at time x, and $F \cdot C_t < R$, thereafter. Removal by liver is complete and hepatic vein concentration is zero. C_t is calculated by the equation. See text for explanation.

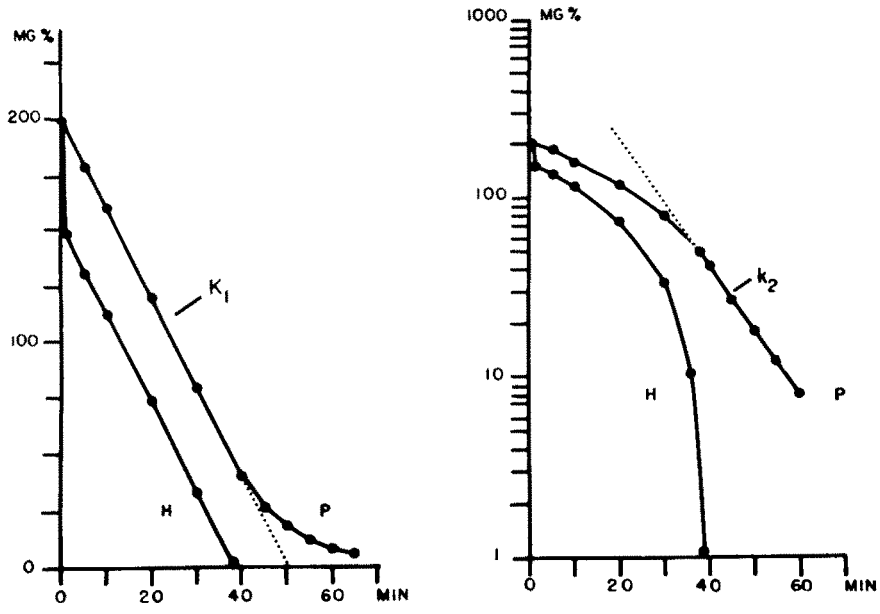

Fig. 3. **Disappearance of hypothetical substance assuming conditions given in text and in Fig. 2*a*. *Left*, rectilinear plot of peripheral *(P)* and hepatic vein *(H)* concentrations. K_1 derived from first phase of peripheral curve (extrapolated to abscissa). *Right*, semilogarithmic plot of peripheral and hepatic vein concentrations. k_2 derived from second phase of peripheral curve (extrapolated towards ordinate). See text for pertinent equations.**

As previously noted, however, from C_x on, the peripheral curve is exponential so that the fractional disappearance constant for the second phase, k_2, can be calculated by Equation 16, $T½$ being obtained from the semilogarithmic plot of this phase. The hepatic blood flow is then calculated by Equation 12 without reference to C_x. However, if one wishes to calculate C_x to fit into Equation 22, then

$$C_x = \frac{R}{F} = \frac{K_1 V}{k_2 V} = \frac{K_1}{k_2}. \qquad \text{(Eq. 23)}$$

Possible Use of Galactose as Test Substance for Noncatheterization Method

Tygstrup and Winkler[33] presented good evidence that after single injection galactose disappears initially in a rectilinear manner rather than exponentially as is commonly held.[34] From results of our studies, we agree.[26] But, where they state that the disap-

pearance curve "flatten[s] out" below 50-30 mg./100 ml. and "undergoes a change," without further analysis of the kinetics beyond the point of change, it is our opinion that the galactose curve describes an exponential phase resembling the theoretic model we have outlined. Tybjaerg-Hansen, Tygstrup, and Winkler,[25] as noted previously, proposed that galactose be used to measure hepatic blood flow by a single-injection technique, using the Fick principle. They recommend finding the numerator, R, as in Equation 21, and the denominator, P-H, as the average difference between peripheral and hepatic vein disappearance curves, the latter to be obtained by hepatic vein catheterization. Because in our unpublished studies,[26] rectilinear and semilogarithmic plots of galactose disappearance curves in normals after the rapid, single intravenous injection of 0.5 gm./kg. closely resemble the theoretic plots shown in Fig. 3, we propose that *hepatic vein catheterization is not necessary,* and that calculations of the type developed in the previous section may be applied to the peripheral disappearance curve alone.

Galactose is removed from the blood primarily by the liver, wherein several known enzymatic processes convert it to glucose.[35] Metabolism elsewhere, for example in the brain and red cells, does not appear to occur at a rate fast enough to interefere with its proposed use as a test substance.[26] However, renal loss in normals may be significant. Our studies[26] and those of others[36] indicate urine excretion parallels blood concentration. This can be taken to mean constant renal clearance of galactose, to account for which the theoretic model must now be modified (Fig. 4). An average renal clearance is calculated during the rectilinear first phase, when blood levels are highest and renal losses greatest. Let U_{cl} be the average renal clearance of galactose, U_m the minute excretion, and U_t, the total urine galactose collected from injection to time, t. Then

$$U_m = C_t \cdot U_{cl} \qquad \text{(Eq. 24)}$$

and, from the plot of the rectilinear phase

$$U_{cl} = \frac{U_t/t}{\frac{(C_o + C_t)}{2}}. \qquad \text{(Eq. 25)}$$

During the first or rectilinear phase, C_t will be determined by the amount injected less the amount removed by the liver (whose

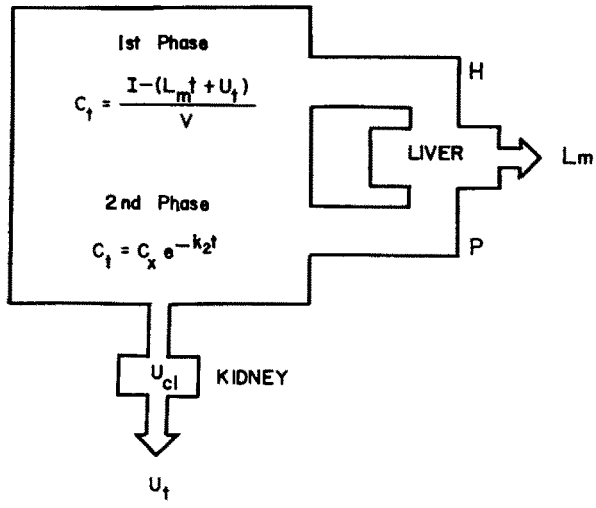

Fig. 4. **Model for liver removal maximum (L_m) plus urinary loss. U_{cl} is the average renal clearance of the substance, U_t the total amount lost in the urine from injection to time, t. Compare with Figs. 2*a* and 2*b*. See text for explanation.**

minute removal maximum we shall now call L_m, as have others[12, 37]) *plus* the amount lost in the urine. Thus

$$C_t = \frac{I - R \cdot t}{V} = \frac{I - (L_m t + U_t)}{V}. \qquad \text{(Eq. 26)}$$

The liver removal maximum can now be derived:

$$L_m = \frac{I - U_t - C_t V}{t}. \qquad \text{(Eq. 27)}$$

During the second or exponential phase, the fractional disappearance constant, k_2, represents the fractional volume due both to hepatic blood flow and renal clearance:

$$F = k_2 V = HBF + U_{cl}. \qquad \text{(Eq. 28)}$$

Thus

$$EHBF = k_2 V - U_{cl}. \qquad \text{(Eq. 29)}$$

Due to changing renal excretion (Equation 24), the peripheral disappearance curve will not be strictly rectilinear during the first phase. From our many model calculations, however, it can be shown that the error of assuming rectilinearity for the purpose of calculation will be small.[26]

It is, of course, appreciated that the behavior of galactose after injection can at best only approximate the theoretic conditions we have stipulated in constructing our models. Nevertheless, we be-

lieve the approximation is satisfactory enough to permit use of the peripheral disappearance curve of galactose alone, without resort to hepatic vein catheterization, for the estimation of liver removal maximum (Equation 27) and hepatic blood flow (Equation 29). Specific data pertaining to degree and sources of error and results in subjects with and without liver disease are in preparation for publication.[26]

The proposed method using galactose as the test substance appears to offer the advantage of simplicity, avoids the need for catheterization or equipment for counting radioactivity, estimates hepatic blood flow as a result of over-all, rather than lobular, hepatic extraction, and supplies a second parameter of hepatic function at the same time it estimates blood flow.

SUMMARY

The estimation of hepatic and splanchnic blood flow in humans by clearance methods has been reviewed (Part I). Methods based on the Fick principle and hepatic vein catheterization employ BSP, Rose Bengal, urea, or galactose as the test substance. Methods based on the disappearance of particulate material after injection use P^{32}-labeled colloidal chromic phosphate, Au^{198}-labeled colloidal gold, or I^{131}-labeled denatured human serum albumin as the test substance.

The hypothetical behavior of a substance after intravenous injection for which the liver has a removal rate maximum and which is lost otherwise only into the urine is developed (Part II). Equations are derived from these considerations by which to estimate the liver removal maximum and hepatic blood flow from the peripheral disappearance curve and urine collection alone, without resort to hepatic vein catheterization. The actual behavior of galactose after single intravenous injection suggests it may be a suitable test substance for the application of the proposed noncatheterization method to humans.

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