INVESTIGATION OF HEPATIC FUNCTION BY CLEARANCE TECHNIQUES[1]

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THE purpose of this investigation is to adapt the methods and concepts of renal clearance (1) to the study of hepatic function. The clearance of a given compound is usually defined as the virtual volume of blood (or plasma) completely cleared of that compound within a standard period of time. Clearance may be more realistically defined as the smallest volume of plasma at a given concentration which could have yielded the amount of material removed from the plasma. Mathematically, clearance may be stated as follows:

$$C \times P = Qe,$$

where C = clearance, P = plasma concentration, and Qe = the amount of material cleared either by excretion or metabolic conversion.

In studying renal function, the amount of material excreted, Qe, is measured in the urine. However, in the study of hepatic function, a comparable collection of bile is useless because of the latent period between removal from plasma and appearance in bile (2). Furthermore, many compounds are cleared by storage or metabolic conversion. A technique for the measurement of hepatic clearance must then depend on some scheme to determine Qe indirectly.

Our measurements of Qe are obtained by utilizing an intravenous infusion at a constant rate. During the early part of the infusion, the plasma concentration rises rapidly and eventually reaches a stable equilibrium level. At this level the rate of excretion or removal is equal to the rate of infusion, R, in mg/minute. Earle and Berliner (3) have used this method successfully in measuring renal clearance where it was possible to compare R directly with Qe.

If clearance is independent of plasma level in the range of values studied, P will be a simple linear function of R, and extrapolation of the line can pass either through the ordinate, the abscissa or the origin. If the line passes through the ordinate, the corresponding value of P will represent the threshold. If the line passes through the abscissa, the corresponding value of R will represent the maximum rate at which the liver can *completely* clear the blood during a single circulatory passage. If the line passes through the origin there is neither a threshold nor a level of complete clearance.

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In clinical studies, an empirical approximation of clearance is obtained by so-called tolerance and retention tests. In a previous paper (4) we discussed the relationship of these methods to clearance, and derived the following relationship:

$$\frac{C}{V} = \frac{(P - P')}{tPm}$$

where V = the volume of the fluid space containing the injected material, t = time elapsed between samples, P and P' the concentration of the initial and final plasma samples, and Pm = the mean plasma concentration during the time interval t. For practical clinical purposes, Pm may be taken as the geometric mean of P and

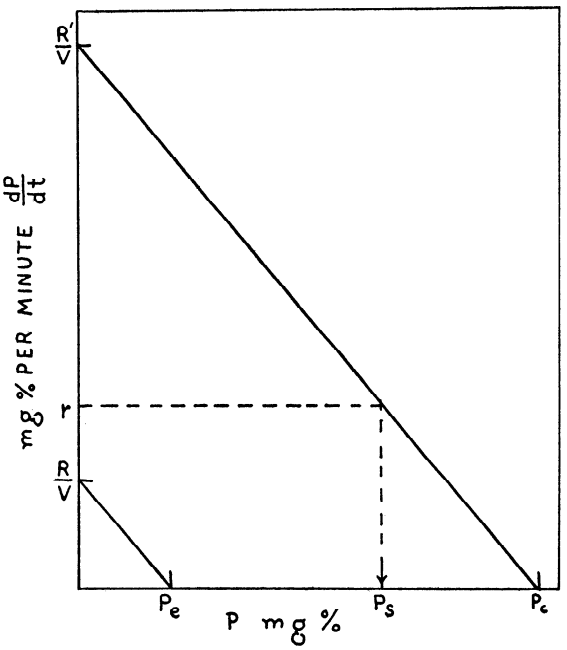


Fig. 1. PROPORTIONAL INCREMENT METHOD

P', which equals $\sqrt{P \times P'}$, but for the precision required in a theoretical analysis, it is necessary to consider the true mean. The relationship is then expressed as:

$$\frac{C}{V} \int_0^t P' \, dt = P - P',$$

which can finally be resolved as:

$$\frac{C}{V} = \frac{2.3 \, (\log P - \log P')}{t}$$

Although this last equation has been derived to apply to falling plasma levels after a single injection, calculations of C/V from the falling plasma levels after a period of constant infusion are more accurate, since possible errors due to mixing

time are eliminated. Furthermore, auxiliary removal systems (e.g. the reticulo-endothelial system), when present would be saturated and less likely to influence significantly the rate of fall in plasma levels.

The fractional clearance, C/V, may also be calculated from the rising plasma

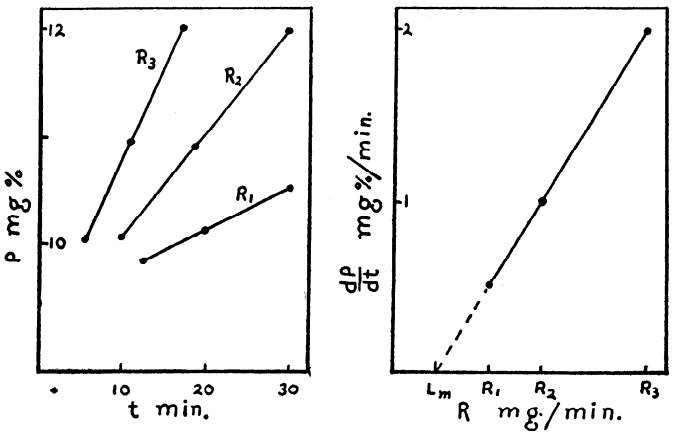


Fig. 2. SERIES INCREMENT METHOD

levels measured at the beginning of a constant infusion as indicated by the following derivation:

$$\frac{dP}{dt} = \frac{R - CP}{V}$$

$$R = CPe$$

$$\frac{dP}{dt} = \frac{C(Pe - P)}{V}$$

$$\frac{C}{V} = \frac{2.3\ [\log Pe - \log (Pe - P)]}{t}$$

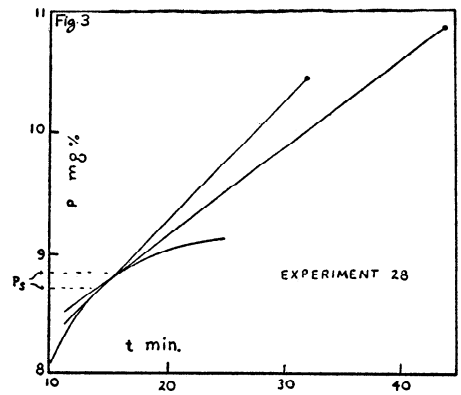
where Pe = the plasma level at equilibrium. If some auxiliary clearance system, the activity of which is not a function of the plasma concentration, is present, the fractional clearances determined from rising plasma levels will differ significantly from the falling level fractional clearances. In the derivations above, it has been assumed that the injected material (bromsulfalein in this instance) is confined to the plasma. If 2 or more fluid compartments are entered to any significant degree at different rates, a graph of log P plotted as a function of time would give a curve that was convex downward rather than a straight line. Fractional clearances calculated from points on the early portion of this curve would then differ from those calculated from later points.

In a quantitative study of the function of any organ, a distinction must be made between capacity and intensity. The intensity of an organ's function is defined as the rate at which it does some form of physiological work. The *maximum* rate at which the organ can function defines capacity. If the function of some organ changes in intensity while the capacity is unchanged, we may assume that the change is part

of an integrated response by the entire organism. If the capacity of the organ changes with or without a change in intensity, we can assume that the organ in question has been directly affected. Changes in capacity and intensity may be closely associated, but we can only expect changes in capacity to show any consistent correlation with anatomically demonstrable changes.

There are 4 methods of measuring maximum excretory capacity of the liver without catheterization of the hepatic vein. All 4 methods are basically similar; they measure the increment of material retained in the plasma/unit time while it is being injected at a rate well above the capacity of the liver to remove it. These are as follows:

1) *M-H-S Direct Increment Method.* In this method devised by Mason, Hawley and Smith (5) a constant intravenous infusion of BSP is maintained at a rate higher



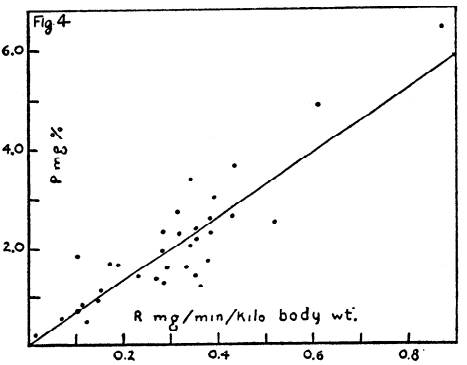


Fig. 3. GRAPHIC METHOD
Fig. 4. PLASMA LEVELS AS A FUNCTION OF INFUSION RATES

than the liver's capacity to remove it. Their calculation is summarized in the following equation:

$$Lm = (\text{Infusion rate} - \text{renal excretion rate}) - \frac{V(P - P')}{t},$$

where Lm = maximum hepatic excretory capacity, V = plasma volume calculated on the basis of weight, P and P' are 2 plasma levels, and t = the number of minutes elapsed between the 2 plasma levels. We did not employ this method, because of inaccuracy of plasma volumes calculated on a weight basis in rabbits.

2) *Proportional Increment Method.* After determining the values of C and C/V on a rabbit, the rate of change, r, in plasma concentration was determined by taking 3 or more blood samples after 30 or more minutes of constant infusion at a relatively high rate, R'. The plasma level at which the clearance mechanisms are saturated, Ps, is calculated from the available data as follows:

$$Ps = \frac{R'}{C} - \frac{C}{Vr}$$

$$CPs = Lm$$

The relationships of these factors are indicated graphically in figure 1.

3) Series Increment Method. In this method, 2 or 3 different relatively concentrated solutions are given in series at a constant rate. The corresponding rates of plasma level increase are a function of the rate of infusion, and *Lm* may be obtained by calculation or extrapolation as shown in figure 2.

4) Graphic Method. This method is useful when technical errors make it impossible to be objectively certain about the rate of increase in the plasma level after saturation. This situation is depicted in figure 3, where it can be seen that each plasma level after saturation gives an independent determination of *Ps* when a tangent to the theoretical time-plasma level curve is drawn from each point.

TABLE 1. BSP CLEARANCES OF FED AND FASTED ANIMALS

ANIMAL NO.	CLEARANCE (FED)	CLEARANCE (FASTED)	DIFFERENCE
	cc/min/kg.	*cc/min/kg.*	
1	13.7	8.5	5.2
2	14.2	9.8	4.4
3	14.2	12.1	2.1
4	21.2	10.1	11.1
5	12.7		
6	16.9	7.3	9.6
7	14.6	12.8	1.8
8	16.7	9.1	7.6
9	16.4	10.1	6.3
10	21.3	9.5	11.8
Mean	16.19	9.92	6.66
Standard Deviation	3.01 (18.6% of mean)	1.71 (17.3% of mean)	1.22

MATERIALS AND METHODS

Ten mature male Dutch rabbits were used in this investigation. No anesthesia was required since the animals were restrained in the supine position on an animal board. One ear vein was used for the constant infusion, while samples were withdrawn from the opposite ear vein with syringes moistened with heparin solution to prevent clotting or hemolysis. The time required to withdraw these blood samples varied from 0.5 to 1.5 minutes. The time that blood first appeared in the syringe and the time of withdrawal was recorded to the nearest 0.5 minute and the average of these times taken as the time of the sample. Each blood sample was immediately transferred to a small test tube for mixing with oxalate crystals. The plasma was separated from the cells by centrifuging at 2500 rpm. for 45 minutes in mercury-calibrated Wintrobe tubes, so that the volume of plasma taken for color analysis could be accurately determined. The plasma (0.15–0.30 cc.) was then pipetted into cuvettes to which known volumes of 0.1 N NaOH (1.0–1.33 cc.) had been added. These were then read in a spectrophotometer (Coleman Junior) at 575 mμ for determinations of BSP content.

The infusion solutions were made from standard ampules of BSP (Hyson, Wescott and Dunning, 50 mg/cc.), diluted with pyrogen-free physiological saline.[2] The rectal temperature of each animal was recorded before and after each experiment; over 35 experiments were performed, and in no instance was there a rise in temperature or noticeable change in thickness of the white cell layer in the Wintrobe tubes.

Constant infusion rates were obtained by driving the plunger of a calibrated 50-cc. syringe with a screw (N.S. ½ inch–20), turned manually at the rate of one rpm. Determinations of *C*, *C/V* or *Lm* require from 10 to 15 venepunctures. Scarring and small scab formations over the veins due to shaving and multiple venepunctures made it impossible to use the rabbits at closer than 10- to 14-day intervals.

[2] Supplied through the courtesy of Don Baxter, Inc., Pasadena, California.

RESULTS

Exploration of Equilibrium Plasma Levels as a Function of Infusion Rate. In order to obtain sufficient data on the relationship of clearance to plasma level, the results obtained with 10 rabbits were pooled by plotting P as a function of R in terms of mg/minute/kg. body weight. These results are presented in figure 4. Although there is some scattering of points about the mean line, the data justify the conclusion that the line passes through the origin. If the line actually passes to one side of the origin, this deviation is too small to be detected by our methods and too small to invalidate conclusions based on the assumption that the line does pass through the origin. The slope of the line measures clearance, and numerical values for the measure of scatter about this line are given with the data on clearances.

BSP Clearances of Fed and Fasted Animals. In table 1, the BSP clearances of the 10 animals are presented. It can be seen that there is considerable variation between determinations on individual rabbits and between separate determinations on the same rabbit. The standard deviation is 3.01 cc/minute/kg. or 18.6 per cent

TABLE 2. FRACTIONAL CLEARANCES OF BSP

	C/V, FRACTION OF PLASMA VOLUME CLEARED/MINUTE	
ANIMAL NO.	FED	FASTED
1	.361	.212
2		.227
3		.248
4	.278	.259
6	.295	.179
7		.214
8	.557	.301
9	.238	.207
10	.332	.322
Mean	.344	.219
Standard Deviation	.113 (32.8% of mean)	.052 (23.7% of mean)

of the mean. These determinations were repeated on animals deprived of food for 17 hours. As shown in table 1, the variability is not significantly diminished, but in each instance the measured clearance is definitely lower in the fasting state. The mean decrease is 6.66 cc/minute/kg. The probability of obtaining this consistent decrease by chance is less than 0.01.

BSP Fluid Compartment. The volume of the fluid compartment containing BSP was estimated by dividing C by C/V. In 9 fasting animals the mean was 41.2 cc/kg., with a standard deviation of 7.38 cc/kg. (18% of the mean). This is of the same order of magnitude as the plasma volumes which can be calculated from reported ranges of blood volume and hematocrit (6). Slightly higher and more variable values were obtained with fed animals, but the difference was not significant.

Fractional Clearance, (C/V), of BSP. As indicated in the discussion of theory and equations, early (before 5 minutes) and late (after 5 minutes), falling plasma levels were used to calculate C/V, and their mean values were compared. Each of these mean values differed from the combined mean by less than one per cent. We may therefore conclude that BSP remains in a single fluid compartment, presumably

plasma. A comparison of fractional clearances obtained from rising plasma levels with those obtained from falling levels reveals a difference of less than 4 per cent from the combined mean. The probability of obtaining this difference by chance is greater than 0.05 and therefore the difference is not significant.

Table 2 presents the fractional clearances of fed and fasted animals together with the statistical properties of the data. The probability of obtaining the observed difference by chance is greater than 0.01, but less than 0.02.

Maximum Excretory Capacity. Table 3 presents the measurements of *Lm.* There is no significant difference in the capacity between fed and fasted animals and the standard deviation of the pooled data is 0.104 mg/kg/minute or 10.4 per cent of the mean.

There is evidence that BSP is removed to some small degree by the kidneys, but in our studies with rabbits, we have found that the renal excretion of BSP, even with unusually high blood levels, is so small as to be barely detectable.

TABLE 3. MAXIMUM EXCRETORY CAPACITIES OF BSP

ANIMAL NO.	Lm (FED)	Lm (FASTED)	DIFFERENCE
	mg/min/kg.	*mg/min/kg.*	
4		.924	
1	.939	.945	.006
6	1.10	1.03	.07
8	1.09	.986	.10
9	1.07	.890	.18
10	1.01	.970	.04
7		1.097	
Mean	1.042	0.977	.076

Combined mean = 1.004 mg/min/kg.
Standard deviation = 0.104 (10.4% mean).
Probability of difference by chance = 0.3.

DISCUSSION

Certain facts must be established for each compound used in measuring hepatic clearance. These are: *1*) the liver is the sole route of departure from the plasma, or if there are other routes, these are negligible; *2*) before removal by the liver the compound remains confined to the plasma, or, if other fluid compartments are entered, equilibrium is rapidly established; *3*) clearance is independent of plasma concentration.

The data on fractional clearance indicate that BSP is confined to a single fluid compartment prior to removal from the plasma. If certain cells or spaces other than those involved in excretion are entered, penetration must be either insignificantly small, or if this volume is of significant size there is almost instantaneous equilibration with the plasma. In any event, in the study of normal rabbits the BSP compartment may be considered as representing the plasma volume.

Injection of BSP into eviscerated animals (7) reveals that some BSP may be removed by other tissues (approximately 6%). It is generally believed that the reticulo-endothelial system accounts for this removal (8). It is clear, however, that the liver is certainly the major excretory route, and the available clinical and experi-

mental evidence indicates that variations in rate of BSP removal are correlated with variations in the status of the liver (9). There is no evidence that variations in other tissues produce corresponding variations in BSP removal, but it will be necessary to investigate this possibility before it is possible to interpret pathological BSP clearance data without qualification.

The constancy of *Lm* between rabbits and in the same rabbit, whether fed or fasted, is remarkable. The lability of rabbits is notorious, particularly as regards their circulatory responses. The digestive tract in the rabbit is a relatively large one, and no doubt the blood flow to this organ and consequently to the liver is greatly augmented after feeding. The increase in clearance and fractional clearance in fed animals probably is a consequence of this increased blood flow but might be related to the nutritional state of the cell. If the response is caused by increased blood flow, the response might be related either to the filling of additional capillary beds within the liver or to a response of the individual cells. Since *Lm* does not change after feeding, the possibility that additional capillary beds are opened and more functional units reached must be ruled out. The constancy of *Lm* also rules out the possible role of the nutritional state of the cell in BSP clearance. This does not mean that prolonged fasting and malnutrition might not change this clearance, but refers to the more rapid changes in glycogen storage occurring between feedings. Therefore, the immediate clearance response of the liver is caused by increased blood flow, and this increase is regulated by mechanisms residing in responsive arteriolar beds outside the liver; arterioles within the liver have no appreciable effect in this particular response.

SUMMARY

Equations have been derived which analyze the kinetics of clearance and the distribution of compounds injected intravenously. These have been applied to the study of the hepatic clearance of bromsulfalein in fed and fasted normal rabbits. Bromsulfalein is confined to the plasma until excreted by the liver. Below saturation levels hepatic clearance is independent of plasma concentration. Determinations of clearance and maximum excretory capacity indicate that fasting diminishes blood flow to the liver. Hepatic clearance varies with hepatic blood flow, but maximum excretory capacity, *Lm*, does not vary with blood flow. Changes in blood flow to the liver in response to feeding or fasting are controlled by arteriolar beds outside the liver and are not appreciably affected by arterioles within the liver.

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