APPLICATION OF THE SATURATION PRINCIPLE TO THE ESTIMATION OF FUNCTIONAL HEPATIC MASS IN NORMAL DOGS

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Studies of renal function have demonstrated that the maximum tubular secretion or reabsorption rates obtained for certain solutes at plasma concentrations saturating the mechanisms concerned may be considered a measure of the active tubular mass (Tm) in respect to the substance employed (1). Similarly, measurement of the maximum excretion of a substance largely or exclusively secreted into the bile should give a corresponding estimate of the functional hepatic mass ('Lm') for the solute employed. The following report deals with studies on the maximum excretion of bromsulphalein by dogs under conditions of apparent saturation of the mechanisms concerned and is an extension of a previous communication (2).

PROCEDURE

The principle of the procedure for determination of hepatic excretory mass is a simple one previously suggested for application to studies of renal function (3). It avoids the necessity of making a quantitative collection of bile and may be applicable to a number of substances which are excreted or metabolized largely or exclusively by the liver.

If the substance to be excreted is intravenously infused at a rate so that a constant plasma concentration of sufficient magnitude to saturate the excretory mechanisms is obtained, then, providing the plasma concentration remains constant, the rate of infusion is equal to the rate of excretion. In actual experiments a correction may be made for observed rise or fall in plasma levels, provided that the nature of distribution of the substance in the body is known. In addition, correction must be made for extrahepatic disposal of the substance if such occurs. From the corrected infusion rate the maximum mass of substance excreted per unit time is readily calculated. The estimation of bromsulphalein excretion in man by infusion at a rate producing a constant blood level has been previously described in an abstract by Ingelfinger (4) and in more detail by Bradley *et al.* (5) in connection with the determination of hepatic blood flow.

In applying this principle to the excretion of bromsulphalein, no assumptions are made regarding the mechanisms of hepatic excretion of this dye other than that these are 'saturated' during the test at the plasma concentrations achieved (see below). Extrahepatic loss via the urine is readily measured and in any event in the case of dogs, it is an almost negligible fraction of the dye infused over a range of plasma levels considerably above the apparent saturation limit.

The correction applied to the infusion rate for a rise or fall of plasma concentra-

tion is made upon the basis that any dye entering the circulation when the plasma level is already above the saturation limit is distributed in the plasma only (5). The details of the means of further establishing this will be reported in a subsequent communication. Tentatively, it would appear that in dogs the correction may be made with sufficient accuracy by estimating the plasma volume from the hematocrit reading and a blood volume calculated as 9 per cent of the body weight, as is the case in the experiments reported below. The error involved in estimating the correction by such means decreases as the slope of the curve relating plasma concentration to time approaches zero.

EXPERIMENTAL

Dogs were used in the experiments. No anesthetic was employed other than novocaine occasionally used to infiltrate sites of subsequent venepuncture. It was found, however, that the animal had to be sufficiently trained to submit to the procedures quietly. The actual measurement of maximum hepatic excretion of bromsulphalein was made as follows.

A needle was inserted into a saphenous vein. This was connected by a short piece of tubing to a 50 cc. syringe containing the bromsulphalein solution to be injected at a constant rate. The syringe was seated in the slot of an apparatus equipped with a threaded rod which was connected by a worm gear to an electric motor and so arranged that the threaded rod drove the plunger of the syringe at any rate desired. Alternately the gear mechanism was operated by hand.

Infusion of the bromsulphalein (Hynson, Westcott and Dunning, sterile solution, 50 mgm. dye/cc., buffered to pH 7.4 with disodium phosphate and diluted with sterile saline to whatever strength desired) was started at a rate estimated to represent the maximum rate of excretion. For normal dogs of an average size (10 to 15 kgm.) this is about 0.45 mgm/kgm. body wt/min. As soon as the infusion was started a priming dose of the dye amounting to from 15 to 30 mgm/kgm. body wt. was injected, employing either some other accessible vein or the already inserted needle connected to the infusion apparatus. In some instances the priming dose was given first, with constant infusion following immediately. After allowing a period of 10 to 15 minutes to elapse to establish mixing and saturation, blood specimens of 5 cc. each with or without anti-coagulant added were taken at intervals of 5, 10 or 15 minutes from the femoral vein of the leg opposite the site of infusion. The constant injection was continued for periods of 30 minutes to an hour or more from the time of the withdrawal of the first blood specimen. Shorter infusions may be found to be sufficient, although to date it has been noted that in some dogs the infusion must be maintained for from 20 to 40 minutes before the plasma concentration of the dye ceases to exhibit considerable fluctuation. In most instances the animals were catheterized so that urine was collected at intervals and renal loss of the dye exactly measured.

Bromsulphalein in serum, plasma or urine was determined by the photoelectric procedure of Gaebler (6), which is quite precise even in the presence of moderate degrees of lipemia, hemolysis and icterus. The exact concentration of the infused

dye solution was also determined by analysis, rather than by calculation from the degree of dilution of the stock solution. The hematocrit reading of a portion of the first blood specimen drawn was determined by the Wintrobe procedure, employing isotonic oxalate anticoagulant. Of the several formulations available for the calculation of the surface area of dogs (7), that of Meeh and Rubner was employed.

TABLE 1. 'Lm' (BROMSULPHALEIN); NORMAL DOGS

DOGS	WEIGHT	AVERAGE PLASMA BROMSULPHALEIN CONCENTRATION DURING TEST	Lm	
			Mgm/kgm/min.	Mgm/min./sq.M. surface area
	kgm.	*mgm. %*		
1	10.90	5.5–27.0	0.49 (av. of 5 det'ns.)	9.9 (av. of 5 det'ns.)
2	14.50	4	0.42	9.1
3	10.46	4	0.43	8.5
4	8.20	10	0.42	7.5
5	9.50	10	0.41	7.8
6	20.40	5	0.41	9.9
7	12.30	4	0.41	8.6
8	13.40	8	0.44	9.4
9	19.50	8	0.39	9.5
10	15.40	9	0.33	7.5
Average.....			0.44	9.1

TABLE 2. REPRODUCIBILITY OF 'Lm' (BROMSULPHALEIN)

(Dog No. 1)

DATE	AVERAGE PLASMA BROMSULPHALEIN CONCENTRATION DURING TEST	Lm		REMARKS
		Mgm/kgm/min.	Mgm/min/sq.M. surface area	
	mgm. %			
3/18	5.5	0.48	10.0	
3/25	17.0	0.47	9.9	
4/1	27.0	0.52	10.3	
4/4	25.0	0.48	9.6	
4/10	9.0	0.37	7.2	48 hours after CCl_4; 1 cc/kgm.
4/22	13.5	0.49	9.7	
8/21	7.0	0.42	9.3	

RESULTS

A typical experiment and the calculations involved are shown in figure 1. Table 1 summarizes the results of 14 tests on 10 normal dogs. It is seen that the maximum hepatic excretory rate for bromsulphalein ('Lm', bromsulphalein) is of the order of 9.1 mgm. per minute. Table 2 summarizes the results of repeated tests upon the same dog and includes the various plasma concentrations

of dye attained in each experiment. In addition, the results of three tests after the oral administration of a small dose of carbon tetrachloride are given.

DISCUSSION

The 'Lm' (bromsulphalein) of normal dogs. A considerably larger series of animals must be studied in order to assess properly the spread of values to be anticipated in normal animals; however, these preliminary results indicate that the range will not be a large one. Similar results have been obtained by others employing the procedure described (8).

It may be noted that the 'Lm' for the dog on whom many tests were performed (table 2) is distinctly greater than that of most of the remaining animals reported. This may be due to physiological variability, as upon subsequent laparotomy this animal exhibited an unusually large liver. Sections from a lobe removed revealed entirely normal architecture. In addition, this animal was the most satisfactorily trained of the group studied and had received a diet containing large supplements of horse meat for some time, and these factors may be concerned. It was consistently observed that the excretion rate became slower and highly variable when the animals manifested hard struggling or excitement. The plasma levels of the dye, instead of rising or falling smoothly as the infusion progressed, exhibited marked fluctuations. With moderate restlessness the excretory rate may fall somewhat, which may account for the rather low values obtained for *dogs 4, 5,* and *10* (table 1). In the calculation of the 'Lm' it is only the initial and final plasma concentrations for the time period chosen which are employed in making the corrections for retention or extra excretion of dye. However, obtaining intermediate values permits an estimate as to whether or not conditions influencing excretion of the dye (probably hepatic blood flow) have been reasonably constant during the test.

In a few experiments in which light anesthesia was induced with pentobarbital, most of the excretory rates obtained were much lower than those observed in unanesthetized animals.

These values for 'Lm, bromsulfalein' obtained for dogs are considerably greater than the maximum excretory rate of approximately 3 mgm/sq. M/min. reported by Inglefinger (4) employing human subjects. We have applied the procedure described here to a series of normal patients and have found 'Lm' to be about 14. The reason for this discrepancy is not clear at the present time.

The serum concentration of bromsulphalein required for saturation. It is seen in table 2 that the maximum excretory rate remained essentially constant over a wide range of serum concentrations of dye, lending support to the assumption of saturation of the excretory mechanisms. The maximum level at which saturation is achieved has not been carefully ascertained as yet. Preliminary data make it appear that the saturation level is below 4 mgm. per cent plasma concentration of the dye. It is the priming dose which is largely responsible for the rapid attainment of saturation, and the analysis of certain data not reported here further indicates that in the conventional bromsulphalein test of liver function the rate of the initial rapid disappearance of most of the dye from the blood

stream has no relation to functional hepatic excretory mass but is more nearly a function of the mass of reticulo-endothelial tissue (5, 9–11). Serum concentrations well above the saturation limit have always been obtained in dogs with priming doses of dye of 15 to 20 mgm/kgm. body weight. We have not observed a diminution in maximum excretory rate with rising plasma dye concentrations such as has been reported to occur in case of perfusion of isolated fish livers (12).

Sensitivity of the test. Data upon the correlation between reduction of 'Lm' as measured by the procedure described and that produced by partial hepatectomy and experimental liver damage caused by hepatotoxic agents will be reported subsequently. In the single experiment of this nature reported above (table 2),

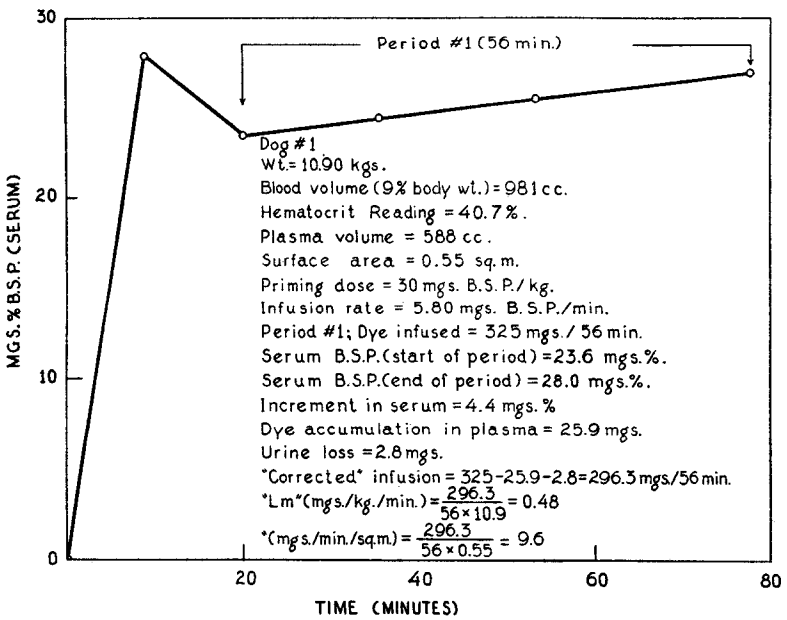

FIG. 1. LIVER MAXIMUM, bromsulphalein (= 'LM', bromsulphalein); dog

a small but distinct reduction in 'Lm' was observed two days after the administration of carbon tetrachloride. The animal had not been ill and the quantity of carbon tetrachloride employed produced no symptoms at any time other than the immediate ataxia and depression during absorption of the drug. Fourteen days later the excretory rate was essentially normal again.

Toxicity of bromsulphalein. No evidence of toxicity resulting from infusions of the amounts of dye required has been observed with the exception of some local irritation of the vein employed for infusion. This in some instances has been followed by thrombosis of a small segment. This may be minimized by increasing the dilution of the solution infused. Temperature, pulse, respiration and blood pressure do not significantly alter during the infusion or afterwards, providing the infusion and priming solutions are *freshly* prepared.

Extrahepatic disposal of bromsulphalein. A rather large fraction of the initial priming dose of the dye is quickly removed from the vascular compartment probably by cells of the reticulo-endothelial system, until this means of disposal is saturated. Subsequently no further uptake of the dye occurs by this means or else the rate of transfer of the dye from this system to the liver cells attains a constant maximum rate (unpublished observations). At maintained plasma levels of 5 to 15 mgm. per cent, excretion via the urine was found to be only a small quantity, ranging from 1 to 4 per cent of the amount which had been infused during the period. At very high plasma levels the fraction escaping into the urine appears to increase considerably. At the plasma levels employed in the experiments reported here, no dye appears in extravascular, extracellular fluid. Thus hypertonic glucose administered intraperitoneally at the beginning of an experiment and withdrawn later was found to be essentially free of the dye. There is no escape via the salivary route nor does the dye appear in the cerebrospinal fluid. These findings agree with those previously reported by Bradley *et al.* (5) as obtaining in man.

SUMMARY

A technique for determination of the maximum rate of hepatic excretion of bromsulphalein ('Lm' bromsulphalein) has been described. It is believed that this rate obtained under conditions of saturation of the mechanisms involved should be proportional to the functional hepatic mass in respect to the substance excreted. The values of 'Lm' for a small series of normal dogs ranged from 7.5 to 10.3 mgm. min/sq. M. surface area. The value was found to be rather constant and reproducible in the same animal.

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