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Chapter 8

Clinical and Experimental Aspects of
Sulphobromophthalein and
Related Compounds

By NORMAN B. JAVITT, M.D., Ph.D.

CONSIDERING THAT MOST of the pharmaceuticals used today have been in existence less than 15 years, it is remarkable that sulphobromophthalein* (BSP), introduced by Edwin C. White and Sanford Rosenthal in 1925,¹ is still employed as a diagnostic agent.

From its inception, studies of BSP have yielded several surprising and puzzling results. Synthesis of this compound was, in part, an outgrowth of screening compounds with bactericidal properties that might be excreted in urine.² BSP was found to be almost completely recovered in bile, presumably without any metabolic transformation. The studies of Brauer and associates,³ however, led to the recognition that BSP is conjugated with glutathione. The assumption that BSP is bound exclusively to albumin in plasma has been shown untenable.³ Another recent finding is that the maximum excretion rate of BSP (T_m) partly depends on the flow rate of bile.³³ These unexpected findings should serve as an admonition to investigators attempting to decipher the physiologic basis of the sensitivity of the BSP test in detecting hepatic disease.

The purpose of this review is to: (1) evaluate the factors that may act on BSP on its way from the glass ampul to the ampulla of Vater; (2) relate clinical abnormalities in BSP metabolism and excretion to these determinants; (3) discuss other compounds used to evaluate hepatic excretory function particularly as they relate to BSP.

PROTEIN BINDING OF BSP

BSP is usually administered as a 5 per cent aqueous solution by rapid injection into a forearm vein. Anaphylactic-type reactions have been reported³⁴ and facilities for immediate treatment should be available. Probably, injected BSP binds almost instantaneously with plasma proteins. Assumptions derived from the studies of the binding of methyl orange (p-dimethylaminoazobenzene sulfonate) to the free epsilon amino group of the lysine moieties of albumin³⁵ led to the thought that BSP was bound similarly. However, studies by Baker and Bradley indicate heterogeneity in binding to albumin.⁴

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* The official U.S. Pharmacopeia name is sulphobromophthalein sodium. One pharmaceutical company uses the registered trade name, Bromsulphalein, from which the abbreviation, BSP, is probably derived. Other more chemically descriptive names are phenoltetra-bromphthalein disulfonate and 3,4,5,6,-tetra-bromo- α - α ,di(4-hydroxysulphophenyl)phthalide.

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In addition,³ analysis of sera containing BSP showed that from 23-38 per cent of BSP is bound to globulins, preponderantly α_1 -lipoprotein. The low concentration of α_1 -lipoprotein in plasma and its high molecular weight compared to albumin suggested that the α_1 -lipoproteins have a higher binding affinity.

HEPATIC UPTAKE OF BSP

The entrance of protein-bound BSP into the perisinusoidal space of Disse is followed by rapid accumulation of BSP by the liver cell. This is a unique function of the hepatocyte. Whether transport across the sinusoidal surface of the hepatocyte is an active or passive process is not known. It is clear, however, that in common with other exchange areas of the body, a very broad surface is presented to the entering plasma. Possibly, cellular proteins in contact with this hepatic sinusoidal surface may have a greater affinity for BSP than circulating plasma proteins. Since BSP in bile is not protein bound to an appreciable extent, dissociation from protein must occur somewhere in the domain of the cell.

Goresky¹² provided direct information concerning the characteristics of the uptake process. A bolus of BSP was injected directly into the portal vein of a dog and 40 or more samples collected from the hepatic vein within 60 seconds. Since the collections were completed prior to accumulation of significant concentrations of BSP in the liver, a measure of unidirectional BSP movement from plasma to liver cell was obtained.

The appearance time of BSP in the hepatic vein and the shape of the curve of changing BSP concentration when compared to other simultaneously injected compounds give insight into the events occurring in the exchanging area of the liver. The exchange area comprises the sinusoid, the space of Disse and the bordering hepatocytes and is rapidly stirred by plasma water flow. The volumes of distribution of erythrocytes, albumin, sucrose, sodium ion and other constituents dissolved or suspended in plasma are proportional to their permeability through natural barriers. Because the exchange area is rapidly stirred by incoming plasma water, virtually all of the tracer compounds are swept back into the sinusoid and recovered in the hepatic vein during a single passage.

Increasing the concentration of BSP in a single bolus results in an increase in the amount of BSP taken up by the liver cell. Uptake rates of 5.95 mg./Kg. of body weight have been obtained. The percentage of BSP removed in a single passage through the exchange area diminishes with increasing doses, indicating that there is probably an upper limit to the absolute rate of BSP uptake. That BSP uptake is a rate-limited process suggests that it is carrier-mediated, possibly but not necessarily active in nature. The design of these studies provides a precise way of determining what other compounds may share the same carrier-mediated process.

HEPATIC STORAGE

The uptake rate of BSP exceeds the maximum excretion rate of 0.19 mg./kg. body weight reported by Wheeler, Meltzer and Bradley.¹⁴ As a consequence of this difference between uptake and excretion, BSP accumulates in the liver.

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By administering BSP as a constant infusion above the rate of maximum excretion and assuming that the molecules are present only in the liver, plasma and bile, it is possible to estimate the amount in liver and the rate of excretion in bile by determining the change in plasma levels occurring at known different infusion rates.⁴⁴ Although storage or accumulation in the liver probably has a limit, the capacity is greater than the amount which can be approached experimentally. In a steady state, the quantity of BSP stored in the liver is directly proportional to the plasma concentration.

A storage phenomenon also occurs using indocyanine green, phenoldibromphthalein disulfonate and sodium taurocholate. Thus the accumulation of compounds by the liver is not dependent on metabolic transformations of the molecules. The estimation of BSP storage and excretion is complicated, however, by conjugation of the molecule which changes its physical and chemical properties. The appearance of BSP conjugates in plasma 3 minutes following a single injection⁴³ and studies using $BS^{33}P^{34}$ indicate a rapid movement from hepatocyte to plasma probably as a consequence of the gradient that occurs as the conjugates accumulate in the liver and bile. BSP conjugates bind to serum proteins with lesser affinity than does unconjugated BSP.⁴ Although during BSP infusion a constant total plasma BSP concentration was assumed to reflect an equilibrium between infusion and hepatic removal (uptake + excretion) of BSP, probably a more complex equilibrium exists between infusion and hepatic removal of BSP and subsequent distribution of conjugated BSP in plasma, extravascular tissues and bile. Entry of BSP conjugates from the liver into plasma has been shown to occur following sodium dehydrocholate administration.⁴⁶ This phenomenon might be recognized by an increase in total BSP concentration in the hepatic vein, perhaps transiently exceeding the simultaneous arterial concentration. A similar transient phenomena could occur with other compounds stored in the liver but should not affect estimates of hepatic blood flow done in a steady state over a long period of time.

BSP CONJUGATION

BSP is representative of many compounds which are conjugated with glutathione via a thioether linkage.¹⁹ Glutathione conjugation occurs particularly with halogenated compounds and several S-glutathione transferase enzymes may exist.⁴⁴

The factors involved in the conjugation of BSP were suggested from studies of the excretion of the three isomers of phenoldibromphthalein derived from dibromphthalic anhydride.²² After isolation of the appropriate dibromphthalic anhydride it can be reacted with phenol to form phenoldibromphthalein, and with sulfuric acid to form the disulfonate (Fig. 1). BSP is prepared similarly following bromination of phthalic anhydride under more vigorous conditions to obtain tetrabromphthalic anhydride.

The three phenoldibromphthaleins were found to be excreted in rat bile as ether-type glucuronides.^{22,23} However, only two, phenol 3,4-dibromphthalein and phenol 4,5-dibromphthalein were found to form glutathione conjugates. Since sulfonation was found to hinder conjugation of BSP as a glucuronide,²¹ (Fig. 2), it was predictable that sulfonation of phenol 3,6-dibromphthalein

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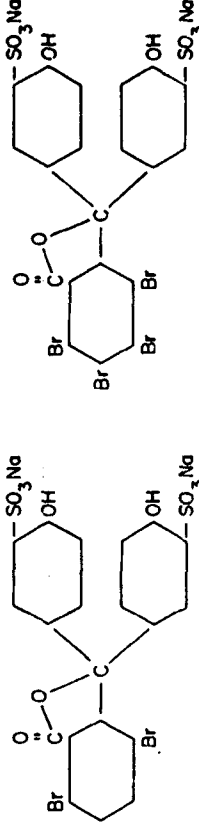


FIG. 1—Structural formulas of phenol 3,6-dibromophthalein disulfonate and phenol-tetrabromophthalein disulfonate (BSP).

would yield a disulfonate that would be excreted unchanged in bile.²⁰ These findings have been confirmed.^{9,26} The small amounts of glutathione conjugates occasionally detected following administration of phenol 3,6-dibromophthalein disulfonate can be attributed to small amounts of other isomers. Since the two phenoldibromophthaleins that form glutathione conjugates have in common a bromide at the 4 position, during BSP conjugation this position probably is substituted by the sulfhydryl of glutathione to form the thioether linkage.

The phenomena of hepatic uptake, storage and excretion are demonstrable with both compounds. Therefore, specific effects on conjugation should be detectable by demonstrating alterations in BSP excretion but not phenoldibromophthalein disulfonate excretion.^{9,26}

BSP EXCRETION

With constant infusion of sufficient BSP into an animal with a bile duct fistula, the excretion of BSP in bile becomes constant. When the infusion rate exceeds the excretion rate and BSP accumulates in liver and plasma, maximum transport into bile (T_m) is assumed. By analogy with renal mechanisms,²⁹ maximum transport rate was not thought to be affected significantly by flow rate. The decrease in the flow rate of bile attributable to depletion of the bile salt pool was neglected as a factor that might influence BSP excretion.

However, O'Maille, Richards and Short³² and others^{12,36} showed that during an infusion of sodium taurocholate or sodium dehydrocholate the excretion rate of BSP increases two to threefold. The increment in BSP excretion has been attributed to acceleration of the active transport process induced by these compounds.³² Alternatively, owing to the concentration gradient of BSP between the biliary tree and surrounding tissue, possibly back diffusion occurs from the biliary tree. This is directly related to biliary BSP concentration and, hence, inversely related to the bile flow rate. Regardless of mechanism, the relationship of BSP excretion to bile flow has important implications in the excretion of conjugated bilirubin.

CLINICAL ASPECTS OF BSP METABOLISM AND EXCRETION

The results of the BSP test are so frequently abnormal in the presence of diffuse liver disease severe enough to cause jaundice that the test has little purpose.³³ These findings have frequently been misinterpreted to mean that bilirubin either interferes with the accuracy of BSP estimation in plasma or

	CONJUGATION GSH	BROMINATION		SULFONATION		CONJUGATION GLUCURONIC A.	WATER SOLUBLE	% DOSE RECOVERED IN BILE	RATE OF DISAPPEARANCE FROM PLASMA
		4	5	6	3				
PHENOL-TETRA- BROMOPHTHALEIN	YES	4	5	6	3	YES	NO	—	
PHENOL-TETRA- BROMOPHTHALEIN MONOSULFONATE	YES	4	5	6	3	YES	NO	100 %	
PHENOL-TETRA- BROMOPHTHALEIN DISULFONATE (BSP)	YES	4	5	6	3	NO	YES	80 + %	
PHENOLDIBROM- PHTHALEIN	NO	2	5	6	3	GLYH, HESTAR RAT (B) YES	NO	—	
PHENOLDIBROM- PHTHALEIN DISULFONATE	NO	2	5	6	3	NO	YES	80 + %	
PHENOL-TETRA- BROMOPHTHALEIN TETRASULFONATE	IN VITRO IN VIVO YES NO	4	5	6	3	NO	YES	10 % 2 HRS	

FIG. 2.—Relationship of bromination and sulfonation of the phenolphthalein skeleton to conjugation with glutathione and glucuronic acid. The structural formula of phenolphthalein is shown as a condensation between 1 mol of phthalic anhydride and 2 moles of phenol. In the series of compounds studied, bromination is always of the phthalic moiety and sulfonation is always adjacent (ortho) to the hydroxy groups of the phenols. The phenoldibromophthalein shown is the 3,6 dibrom isomer.

blocks the uptake of BSP by the liver. Bilirubin has very little light absorption at the wave length used for estimation of BSP and this effect on BSP estimation can be minimized by subtracting the optical density of a plasma sample obtained just prior to the injection of BSP. Hemolysis is a much greater problem for accurate BSP estimation. Although in animals bilirubin injected intravenously can decrease the rate of BSP removal from plasma,¹⁷ in man the standard BSP test can be normal with serum bilirubin levels as high as 20 mg./100 ml.¹⁸ With defective conjugation, and also in hemolytic jaundice, BSP can be used as a valid test of liver function.

Correlation of individual liver diseases with specific disturbances in the processes involved in the transfer of BSP from plasma to bile is difficult. In attempting to make a correlation, perhaps it is best to argue from the defects existing in man that have been characterized carefully.

In the Dubin-Johnson syndrome there is a marked deficit in the transport of BSP from the liver cell into bile.⁴⁴ Since there is conjugated hyperbilirubinemia without an increase in serum bile salt levels,² the defect appears selective for some but not all anions. In many patients the initial disappearance rate of BSP from plasma is normal, particularly if corrections are made for the increased amounts of BSP conjugates which appear in serum. The per cent of BSP retained in plasma at 45 minutes is often normal.⁶ Therefore, in man, a severe deficit in excretion of BSP and its conjugates does not alter the initial disappearance rate of BSP from plasma.

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Conversely, a decrease in the initial disappearance rate of BSP from plasma¹⁰ should correlate with disturbances in either hepatic uptake or storage. Factors that may influence the rate of BSP uptake are: (1) shunting of blood from the sinusoidal circulation; (2) changes in BSP binding to albumin and α -1 lipoproteins or in the relative proportion of BSP bound to each protein; (3) changes in the volume to surface area ratio in the perisinusoidal space of Disse; (4) changes in the rate of flow through the space of Disse; (5) alterations of the surface properties of the liver cell. Considering the variety of possible abnormalities and the inability to evaluate some of them, it is not surprising that precise explanations for BSP retention in most liver diseases have not been obtained.

Since BSP in liver exchanges with that in plasma,¹¹ a change in the physical and chemical properties of BSP by conjugation can alter the relationship between plasma and liver BSP. Possibly conjugation can influence the net rate of BSP removal from plasma. However, no known clinical entities correspond to a specific defect in BSP conjugation. The effect of natural and synthetic estrogens^{11,12} and phenobarbital¹³ are not related to conjugation. Moreover, in a few studies in man, increased plasma retention of phenoldibromophthalein disulfonate (Dibromsulfonephthaleine, Serb Pharmaceuticals, Paris, France) was found during the course of viral hepatitis.⁶ Analysis of the material excreted in human bile again indicated that no conjugation had occurred.²⁴

For clinical purposes, the determination of BSP conjugates in plasma is of particular help in evaluating the relative involvement of the transfer processes. Thus, the Dubin-Johnson syndrome can be distinguished from other bile secretory defects, including benign recurrent cholestasis⁴⁵ by the upward inflection in the BSP disappearance curve attributable to conjugation.³¹

Attempts to estimate accurately hepatic blood flow in liver disease using BSP have been limited by the low arterio-venous difference in BSP concentration. The higher extraction ratio for indocyanine green (ICG) has made this compound more useful.²⁹ Detection of ICG by an ear densitometer provides a continuous monitoring of the plasma level of ICG. This technique may yield more information than blood sampling and a more uniform method for repeated studies during recovery from acute liver disease.³⁰

Although rose Bengal- I^{131} has proved useful for scanning purposes, its disappearance from plasma has not been a sensitive indicator of liver disease in man.⁴¹ Recently attempts have been made to label BSP with I^{131} . In a strict sense the compound is no longer BSP⁴² but it seems probable that physiologically it will behave similarly. I^{131} BSP has been used to obtain adequate liver scans.⁴⁰ Provided that all the radioactivity remains with the BSP, scanning of other areas and monitoring of blood levels may provide new information.

It is surprising that probing continues into a diagnostic area that is more than 40 years old. Some of the impetus may be derived from the belief that disturbed excretory function is the Achilles heel leading to the downfall of the rest of the organ.

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