Bromsulphthalein metabolism in acute alcoholic liver disease

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Routine biochemical tests of hepatic function are useful in detecting subclinical acute alcoholic liver disease, but they do not correlate well with abnormalities of hepatic histology, and they are too nonspecific to use in unravelling the pathophysiology of the condition. The most frequently observed biochemical abnormalities are raised levels of serum glutamic oxaloacetic transaminase (SGOT), serum alkaline phosphatase (SAP), and bromsulphthalein (BSP) retention, suggesting either parenchymal cell injury (Leevy, 1962; Green, Mistilis, and Schiff, 1963), abnormal canalicular function (Ballard, Bernstein, and Farrar, 1961; Sataline and Matre, 1963), or both. The histological changes observed in patients with acute alcoholic liver disease vary greatly. In a minority of patients the liver appears normal under light microscopy, whilst in the majority there is either fatty infiltration alone, or acute alcoholic hepatitis in which parenchymal cell necrosis and inflammation accompany the fatty change. Such histological changes, however, are not always associated with abnormal physical signs or altered liver function tests, and conversely clinical and biochemical evidence of liver damage may coexist with a liver biopsy which appears normal under the light microscope (Leevy, 1962; Green, 1965).

Just as the electron microscope has been used to reveal the early structural changes of acute liver disease not apparent on light microscopy, so might more refined biochemical methods be used to study specific alterations in hepatic function. In this way it might ultimately be possible to learn not only about the nature and consequences of acute alcoholic liver injury but also about normal metabolic pathways. In the present investigation BSP metabolism has been studied in patients suffering from acute alcoholic liver disease and the results obtained have been correlated with other clinical, laboratory, and histological data. Bromsulphthalein follows a metabolic pathway involving uptake by the liver, conjugation and biliary excretion, and abnormalities

of the BSP retention test are the most sensitive routine index of early alcoholic liver disease (Leevy, 1962; Green, 1965). Unfortunately, this test does not differentiate between parenchymal injury and biliary obstruction. However, a clinical technique is available (Wheeler, Meltzer, and Bradley, 1960) which makes it possible to measure independently in man the storage (S_{BSP}) of the dye by the liver and its maximum rate of excretion (Tm_{BSP}). This method of investigating BSP metabolism has been used in the study reported here. The values obtained for S_{BSP} and Tm_{BSP} have been examined in relation to details of clinical assessment, routine liver function tests, and liver biopsy histology, and the results and their interpretation are the subject of the remainder of the present paper.

PATIENTS AND METHODS

Two groups of men were investigated. In the first group were 27 patients who were referred to the University of Melbourne Department of Medicine Alcoholism Clinic for treatment of chronic alcoholism, their ages ranging from 27 to 59 years (mean 46.1 years). All were admitted to hospital as inpatients where they received a normal diet with oral or intramuscular vitamin B supplements. Thirteen also received chlordiazepoxide¹ to suppress or prevent withdrawal symptoms. No other tranquillizers or sedatives were given. In the second group were three convalescent patients, aged 24, 42, and 47 years, who had neither a history of alcoholism nor evidence of hepatobiliary or cardiac disease.

In each alcoholic patient liver biopsy, routine liver function tests, and measurements of S_{BSP} and Tm_{BSP} were performed within a period of 48 hours. These initial investigations were carried out between two and 19 days after the cessation of drinking. In six of these alcoholic patients measurement of S_{BSP} and Tm_{BSP} were carried out a second time. In one patient this was 21 months later, after a second bout of heavy drinking, the patient being abstinent in between. In the other five patients the second series of investigations was made

¹Librium (Roche Products Pty Ltd).

after periods of abstinence ranging from nine to 150 days.

MEASUREMENT OF S_{BSP} AND T_{MBSP} The hepatic storage capacity (S_{BSP}) and biliary transport maximum (T_{MBSP}) for BSP were measured using the two-phase constant infusion technique of Wheeler *et al* (1960).

All patients were examined following an overnight fast. Using a Harvard peristaltic constant infusion pump, a solution of BSP in normal saline was infused into an antecubital vein at a rate which was adjusted during the first phase to give a rising plasma concentration of the dye. After 30 minutes' equilibration a series of five venous or arterial samples was obtained from the opposite arm at five-minute intervals. Then, for the second phase, the infusion was slowed to about one third of the initial rate and after another 30 minute equilibration period, a further series of samples was obtained at five-minute intervals. At the end of the infusion the plasma volume was measured, using ^{131}I -labelled human serum albumin. The BSP concentrations in the plasma samples were estimated by the method of Seligson, Marino, and Dodson (1957).

Values for S_{BSP} and T_{MBSP} were then derived by solving the equation $I = Tm + P(PV + S)$ for the two phases of BSP infusion. I (infusion rate) was measured in milligrams per minute, P (rate of change of plasma concentration of dye in milligrams per 100 ml per minute) was calculated mathematically from the plasma concentrations of dye during the first and second phases of infusion, and PV (plasma volume) was expressed in 100 ml of plasma. The value for S_{BSP} was expressed as milligrams of dye stored per milligram per 100 ml of plasma concentration and T_{MBSP} was measured in milligrams per minute.

RESULTS

DRINKING HABITS OF ALCOHOLIC PATIENTS Nine of the alcoholic patients studied were 'spree' drinkers who alternated periods of excessive drinking with intervals of relative or complete abstinence. The remainder had drunk heavily and consistently for several years until the time of admission. The estimated intake of alcohol in these 27 patients ranged between 120 and 400 g per day (mean 250 g per day). The type of alcoholic beverage varied greatly. Seventeen men drank various combinations of beer, wine, spirits, and methylated spirits. The

remaining 10 favoured one beverage only; in nine this was beer, in the tenth whisky.

CLINICAL AND LABORATORY FINDINGS IN ALCOHOLIC PATIENTS A palpable, tender liver was detectable in 16 patients. Eight patients had reversible peripheral neuropathy, none had clinical evidence of cardiac disease.

No patient was clinically jaundiced when studied, the highest serum bilirubin value being 1.6 mg per 100 ml; SGOT and SAP estimations were either normal or showed modest rises, the highest SGOT being 95 Sigma-Frankel units per ml and the highest SAP 16 King-Armstrong units per 100 ml.

Six patients had histologically normal liver biopsies; six had fatty infiltration without cell necrosis; six had acute alcoholic hepatitis, that is, parenchymal cell necrosis and inflammatory response as well as fatty change; and three had patchy cell necrosis without fatty infiltration. Four of the 12 patients with acute alcoholic hepatitis also had minor degrees of portal tract fibrosis. No patient had histological evidence of cirrhosis.

VALUES FOR S_{BSP} AND T_{MBSP} The results obtained for S_{BSP} and T_{MBSP} in the three non-alcoholic patients agreed well with the mean values reported by Preisig, Williams, Sweeting, and Bradley (1966) for normal male volunteers (Table I).

The initial studies carried out in the 27 alcoholic patients revealed a reduction of S_{BSP} in 15, T_{MBSP} remaining normal in all subjects. This defect in S_{BSP} could not be correlated with age, drinking habits, SGOT or SAP elevations, or histological changes seen in the liver biopsies. It did not coincide with either the presence of peripheral neuropathy or medication with chlordiazepoxide. In Table II, the results have been tabulated according to the histological findings and arranged in order of the severity of reduction in S_{BSP} .

The storage defect was most severe in those patients who had drunk most recently. Thirteen out of the 15 patients who were studied within a week of alcohol withdrawal had abnormal S_{BSP} values, while all but two of the 12 patients studied during

TABLE I

BROMSULPHTHALEIN METABOLISM IN NON-ALCOHOLIC MEN WITHOUT LIVER DISEASE

Patient	Age (yr)	Weight (kg)	Diagnosis	S_{BSP} (mg per mg per 100 ml)	T_{MBSP} (mg per min)
A	24	71.7	Peptic ulcer	77	11.1
B	42	88.0	Peptic ulcer	58	10.0
C	47	66.2	Anxiety state	60	10.6

Values for 18 normal men (Preisig *et al*, 1966)

6.9 ± 17

9.6 ± 19

TABLE II
BROMSULPHTHALEIN METABOLISM IN 27 ALCOHOLIC MEN RELATED TO HEPATIC HISTOLOGY

Liver Biopsy	Patient No.	Age (yr)	Weight (kg)	Time Abstinent before Test (days)	S _{BSP} (mg per mg per 100 ml)	T _{mBSP} (mg per min)
Normal	2	42	60.4	6	11	9.3
	8	57	68.0	8	19	13.2
	15	33	62.5	7	34	6.3
	17	56	56.2	14	42	15.1
	22	42	58.6	11	49	7.8
Fatty liver	25	47	72.8	9	54	12.6
	4	57	63.4	3	14	9.6
	11	43	72.6	2	24	13.8
	16	32	62.2	4	41	10.3
	19	37	60.5	11	44	13.6
	20	35	61.9	8	46	12.9
Acute alcoholic hepatitis	27	55	77.0	9	67	11.8
	1	46	68.6	5	2	9.6
	3	49	66.3	6	13	13.1
	5 ¹	45	57.0	5	16	13.7
	6	45	121.0	3	17	5.7
	7 ¹	53	61.5	15	18	14.1
	9 ¹	52	52.9	6	20	8.2
	10a	42	60.4	2	21	13.3
	b	43	61.9	3	26	8.9
	12	31	75.0	6	26	6.8
	13	57	57.2	3	27	9.8
Patchy cell necrosis	18 ¹	53	63.5	11	42	13.5
	24	39	58.4	6	52	12.0
	26	45	62.4	10	64	16.4
	14	50	59.4	4	32	13.3
	21	42	58.6	15	48	12.0
	23	59	60.4	19	50	5.8

¹Denotes portal fibrosis

TABLE III
BROMSULPHTHALEIN METABOLISM IN 27 ALCOHOLIC MEN RELATED TO PERIOD OF ABSTINENCE BEFORE STUDY

Time Abstinent Before Test (days)	Number of Subjects	S _{BSP} (mg per mg per 100 ml)	T _{mBSP} (mg per min)
7 or less (4.5 ± 1.7)	15	23.3 ± 12.7	10.3 ± 2.8
8 — 21 (11.7 ± 3.4)	12	45.3 ± 14.8	12.4 ± 2.9
P value		<0.001	Not significant

their second or third week of abstinence were normal (Table III, Fig. 1).

In the five patients who were studied again after intervals of abstinence ranging from nine to 150 days, the initially abnormal S_{BSP} values had all returned to normal (Table IV).

DISCUSSION

The results of the present investigation, viewed in the light of present knowledge of BSP metabolism and alcoholic liver disease, allow certain speculations and conclusions, and suggest some further in-

FIG. 1. Bromsulphthalein storage capacity (S_{BSP}) and transfer maximum (T_{mBSP}) in 27 alcoholic patients. The rectangle encloses the area of the normal mean ± 2SD for T_{mBSP} and S_{BSP}. All values are plotted logarithmically. The solid line marks out equal percentile changes in T_{mBSP} and S_{BSP}: where S_{BSP} is reduced to a greater extent than T_{mBSP} the result lies above the line, and where T_{mBSP} is affected more severely it lies below (after Preisig et al, 1966). ● = tested less than one week, ○ = tested more than one week, after alcohol withdrawal.

TABLE IV
BROMSULPHTHALEIN METABOLISM IN FIVE ALCOHOLIC MEN RESTUDIED AFTER ABSTAINING

Patient No.	Weight (kg)	Time Abstinent before Test	S _{BSP} (mg per mg per 100 ml)	T _{mBSP} (mg per min)
4	63.4	3	14	9.6
	66.5	5	54	14.5
5	57.0	95	16	13.7
	57.0	26	119	10.4
12	75.0	6	26	6.8
	70.5	156	71	14.5
14	59.4	4	32	13.3
	59.4	13	45	12.4
15	62.5	7	34	6.3
	64.2	128	101	14.4
Mean for initial study			24.4 ± 8.9	9.94 ± 3.46
Mean for second study			68.0 ± 17.5	13.24 ± 1.85
			p < 0.001	Not significant

vestigations. Following its intravenous injection BSP is carried as an albumin complex in the plasma from which it is rapidly extracted and taken up by the hepatic parenchymal cells. Its removal from the plasma by the liver consists of two processes, uptake and storage in the liver which is proportional to the plasma concentration of the dye, and biliary excretion which has a maximal transport rate (Wheeler *et al*, 1960). During its passage from plasma to bile, BSP is enzymically conjugated with glutathione (Javitt, Wheeler, Baker, Ramos, and Bradley, 1960; Combes and Stakelum, 1961), but it is not known whether it is stored in the liver in the conjugated or unconjugated form. Baker and Bradley (1966) postulate that the dye may be conjugated at the hepatocyte border during its extraction from the BSP-albumin complex, so that it would be stored in conjugated form. However, Combes (1962) found that S_{BSP} was independent of conjugating ability, suggesting that it is stored in the unconjugated state.

Previous studies of BSP metabolism (Wheeler *et al*, 1960; Preisig *et al*, 1966) have shown that where there is loss of total functioning liver cell mass, as in cirrhosis and viral hepatitis, S_{BSP} and T_{mBSP} are proportionately reduced. The only conditions in which a selective reduction of S_{BSP} has previously been reported are extrahepatic portal hypertension, due to portal vein thrombosis (Thompson, Williams, and Sherlock, 1964) and advanced age (Thompson and Williams, 1965). In the present study, S_{BSP} was similarly reduced in 13 out of 15 patients who had been drinking within a week of investigation. The abnormality was unrelated to changes visible with the light microscope, and it was rapidly reversible with time. These results suggest that the reduction in S_{BSP} does not indicate any loss of overall cell function, but rather a selective cellular change. This could be caused by some intracellular injury too subtle to be recognized by the light microscope, or

by some disturbance of the splanchnic circulation.

Early structural changes in the liver cell, which have been identified by electron microscopy in acute alcoholic liver disease, include damage to the mitochondria and endoplasmic reticulum (Kiessling, Pilstrom, Strandberg, and Lindgren, 1965; Porta, Bergmen, and Stein, 1965). Rubin and Lieber (1967) have demonstrated that such changes can be induced by excessive alcohol ingestion combined with an otherwise normal diet, that they occur very early in alcoholic liver disease and precede fatty infiltration observable under the light microscope. Decreased oxidative powers of the mitochondria or disturbed enzyme activity in the endoplasmic reticulum of damaged cells could explain the reduced S_{BSP} observed in the alcoholic patients reported here. A selective disturbance of S_{BSP} might thus be the earliest biochemical indicator of liver damage, T_{mBSP} falling commensurately only when severe cell damage or cell necrosis ensued.

Alternatively, selective abnormalities of S_{BSP} could result from decreased delivery of portal blood to the liver cells. Hepatic perfusion is abnormal in extrahepatic portal hypertension and is also possibly reduced with advancing age. In both of these conditions a selective reduction of S_{BSP} has been found (Thompson *et al*, 1964; Thompson and Williams, 1965). Leevy, Zinke, Baber, and Chey (1958) and Chiandussi, Greco, Indovina, Cesano, Vaccarino, and Muratori (1963) have demonstrated reversible portal hypertension in patients with alcoholic fatty livers. Therefore it is possible that in acute alcoholic liver disease disturbances to vascular perfusion may impede the delivery of either dye or oxygen, or both, to the hepatocytes, and so lead to the reduction in S_{BSP} reported here.

The change in BSP metabolism described in these patients may be a specific effect of prolonged alcoholic excess, or it may be caused by other

factors such as vitamin or protein deficiencies, congeners or excessive iron in certain alcoholic beverages, coexistent viral hepatitis, or some combination of these. Some workers have thought that alcoholic fatty liver is but a manifestation of the alcoholic's poor food intake (Best, Hartroft, Lucas, and Ridout, 1949). Traditionally, certain alcoholic beverages have been thought to be more toxic than others. Recently, however, Rubin and Lieber (1967) have shown that ethyl alcohol itself can cause many of the morphological changes seen in acute alcoholic liver disease. Likewise, it seems possible that the functional abnormality found in the alcoholic patients studied here is predominantly, if not exclusively, due to alcohol excess. The disturbance of BSP metabolism was not related to any coexisting clinical deficiency state such as peripheral neuropathy, the type of beverage drunk, or the pattern of drinking. Well nourished, continuous heavy drinkers were affected as severely as 'spree' drinkers who neglected to eat during drinking bouts, and beer drinkers as badly as those who drank wine or spirits. The crucial factor for the disturbance in BSP metabolism appeared to be the period of withdrawal of ethyl alcohol.

Two possible extensions of the present study are apparent. First, the direct effect of ethyl alcohol on the liver could be assessed by a controlled investigation similar to that of Rubin and Lieber (1967). Bromsulphthalein metabolism, hepatic ultrastructure, and possibly splanchnic haemodynamics could be studied simultaneously while patients received large amounts of alcohol together with an otherwise normal diet. Secondly, by measuring conjugated and unconjugated BSP circulating in the plasma of alcoholic patients infused with BSP, it might be possible to determine whether the dye is normally stored in the liver in the conjugated or unconjugated form. Such studies are warranted because of the frequency and potential morbidity of liver damage in chronic alcoholism. They may also elucidate some of the pathways of normal hepatic metabolism.

SUMMARY

The relative storage capacity of the liver (S_{BSP}) and the biliary transport maximum (Tm_{BSP}) for bromsulphthalein were measured by a constant infusion method in 27 male patients who had recently been drinking excessive amounts of alcohol. Other biochemical tests of liver function and needle biopsies of the liver were also performed. Patients with histological evidence of cirrhosis were excluded.

In 15 of the 27 men a selective reduction of S_{BSP} was found while Tm_{BSP} remained within normal

limits. This reduction was not related to any hepatic histological changes observable by light microscopy. It was more severe in patients tested within a week of alcohol withdrawal than in those tested later. Five patients in whom S_{BSP} was initially reduced had normal values when studied again after intervals of abstinence ranging from nine to 150 days.

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