Journal of Clinical Investigation
Vol. 42, No. 3, 1963

STUDIES OF HEPATIC EXCRETORY FUNCTION. THE EFFECT OF 17α-ETHYL-19-NORTESTOSTERONE ON SULFOBROMOPHTHALEIN SODIUM (BSP) METABOLISM IN MAN *

By J. SCHERB,† M. KIRSCHNER,‡ AND I. ARIAS

(From the Department of Medicine, Albert Einstein College of Medicine and the Bronx Municipal Hospital Center, New York, N. Y.)

(Submitted for publication July 26, 1962; accepted November 24, 1962)

The administration of large doses of 17α-ethyl-19-nortestosterone to man is frequently associated with hyperbilirubinemia, with conjugated bilirubin in the serum and increased retention of sulfobromophthalein (BSP) in the serum 45 minutes after the intravenous administration of 5 mg BSP per kg body weight (1). Light microscopy studies of hematoxylin- and eosin-stained sections of liver reveal normal liver cells with occasional canalicular bile casts (2). Treatment of rats with 17α-ethyl-19-nortestosterone results in reduced capacity to excrete BSP and conjugated bilirubin in the bile (3). These observations suggest that the steroid may functionally interfere with the excretion of BSP and conjugated bilirubin by the liver cell into the bile. The mechanism by which BSP retention is produced in man has not been established.

The metabolism of BSP by the liver involves uptake and storage of BSP (4), intracellular formation of a glutathione conjugate (5–7), and subsequent excretion of free and conjugated BSP into the bile (5–7). The storage of BSP in parenchymal liver cells is directly proportional to the plasma concentration of BSP, whereas the transfer of BSP from the liver cell to bile is by a rate-limiting transfer mechanism (8, 9). Conjugation of BSP with glutathione is an important determinant of the maximal rate at which BSP is transferred from blood to bile (10, 11). A method for the simultaneous estimation of hepatic BSP storage and excretion and data regarding its derivation, validity, and reproducibility have been presented by Wheeler and associates (8, 9). Values for the relative hepatic storage of BSP (expressed as milligrams of BSP stored per milligram BSP per 100 ml plasma) and the transport maximum of BSP (expressed as milligrams of BSP excreted per minute) are derived from estimations of the concentration of BSP in samples of plasma serially obtained during separate intravenous BSP infusions at three different rates (9). In the present study, this technique (9) was used to study the effect of 17α-ethyl-19-nortestosterone on BSP metabolism in man.

MATERIALS AND METHODS

Eighteen patients who demonstrated neither clinical evidence of liver disease nor abnormal tests of liver function were selected for study. The following tests of liver function were performed in each patient by using standard procedures: estimation of the concentration of serum albumin, globulin, and bilirubin; electrophoresis of serum proteins; serum cephalin cholesterol and thymol flocculation; serum alkaline phosphatase, glutamic oxaloacetic transaminase, and glutamic pyruvic transaminase activities; and retention of BSP in serum 45 minutes after the intravenous administration of 5 mg BSP per kg body weight.

Ten patients served as controls and did not receive 17α-ethyl-19-nortestosterone. The age, sex, clinical diagnosis, and treatment of the eight patients who received the steroid are presented in Table I. One hundred mg of 17α-ethyl-19-nortestosterone was ingested per day by each patient except patient J.V., who preferred parenteral administration. Between days 7 and 10 of steroid administration, the previously mentioned liver function tests were repeated. If BSP retention in the serum exceeded 10% 45 minutes after the intravenous injection of 5 mg BSP per kg body weight, the BSP infusion study was performed. If BSP retention in serum did not exceed 10%, the steroid was administered for an additional 5 days, at which time BSP retention in each patient exceeded 10%, and the infusion study was performed.

The relative hepatic BSP storage (S) and transport maximum (Tm) were estimated (9) in ten control sub-

* Investigation supported by research grants from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service (A-2019, H-3838, M-2562, and A-2966), and the G. D. Searle Co. of Chicago, Ill.

† Fellow of the Kellogg Foundation. Present address: Recife, Brazil.

‡ Present address: National Institutes of Health, Bethesda, Md.

TABLE I

Clinical data

Patient	Sex	Age	Diagnosis	Treatment	Total dose of 17 α-ethyl-19-nortestosterone administered
					mg
J.V.	M	53	pulmonary TBC, arrested	INH* (300 *mg/day*) PAS† (12 *g/day*) Meprobamate (800 *mg/day*)	1,200
D.T.	M	28	pulmonary TBC, arrested	INH (300 *mg/day*) PAS (12 *g/day*)	1,100
J.F.	M	47	peripheral neuropathy	Thiamine HCl (100 *mg/day*)	1,100
M.L.	F	53	idiopathic osteoporosis		1,100
G.F.	F	74	cerebral arterial insufficiency		1,500
J.L.	M	43	pulmonary TBC, arrested	INH (300 *mg/day*) PAS (12 *g/day*)	1,200
A.M.	M	50	neuropathy	Thiamine HCl (100 *mg/day*) ASA‡ as necessary	1,400
W.J.	M	46	fractured femur		1,000

* INH = isonicotinic acid hydrazide.
† PAS = para-aminosalicylic acid.
‡ ASA = acetylsalicylic acid.

jects, three patients during steroid administration, three patients before and during steroid administration, and two patients both during and after steroid administration. A Harvard multispeed infusion pump was used to inject BSP at rates of 15, 5, and 10 mg per minute during the three infusion periods before the steroid was administered and at 8, 0, and 5 mg per minute after the steroid was given. The latter rates were selected to avoid potentially toxic reactions to large doses of BSP which could not be metabolized as readily as by normal liver (9).

The concentration of free and conjugated BSP was estimated in plasma obtained at the end of each hour of the BSP infusion before and during steroid administration in two patients (D.T. and J.F.) with the method described by Combes and Stakelum (6).

Aspiration liver biopsy was performed in two patients (D.T. and J.F.) within 24 hours after the cessation of steroid administration. The liver biopsy specimens were fixed for electron microscopic examination and the following procedures: hematoxylin and eosin, oil red orange and ATPase, acid phosphatase, alkaline phosphatase, and 5-nucleotidase activities.

The tests of liver function were repeated in each patient one week after steroid administration was discontinued.

RESULTS

The results of the BSP infusion studies are presented in Table II. In the control group, the mean relative hepatic storage (S) of BSP was 61 ± 18 (SD) mg of BSP stored per mg BSP per 100 ml plasma and the mean transport maximum (Tm) for BSP was 7.6 ± 1.7 mg BSP excreted per minute. Estimates of S and Tm were within the range of normal in each of the three patients who were studied prior to steroid administration. These three patients and five additional patients were studied during steroid administration, and they demonstrated normal relative hepatic storage of BSP; however, BSP Tm ranged from +.35 to 2.1 mg per minute. BSP Tm returned to normal limits one week after steroid was discontinued in the two patients (A.H. and W.J.) in whom this was studied. The average plasma BSP concentrations during each BSP infusion period before, during, and after administration of 17α-ethyl-19-nortestosterone are presented in Table III.

The ratios of conjugated to total BSP in plasma obtained at the end of each hour of the BSP infusion study in two patients (D.T. and J.F.) before and during steroid administration are presented in Table IV. Prior to steroid administration, the proportion of conjugated to total BSP in plasma increased only when the infusion rate exceeded Tm (i.e., in infusion periods I and III). After

TABLE II

Effect of 17 α-ethyl-19-nortestosterone on Sulfobromophthalein sodium (BSP) metabolism in man

	Relative hepatic storage of BSP			Hepatic Tm for BSP*		
	mg BSP stored/mg per 100 ml BSP in plasma			*mg BSP excreted/min*		
Control subjects	61 ± 18 (SD)			7.6 ± 1.7 (SD)		
Relationship to steroid administration:	Before	During	After 7 days	Before	During	After 7 days
Patients						
J.V.	35	41		8.0	+ 35	
D.T.	55	62		7.9	.75	
J.F.	68	57		8.4	.8	
M.L.		65			0	
G.F.		74			1.8	
J.L.		40			2.1	
A.M.		56	64		0.7	10.1
W.J.		49	40		1.0	8.9

* Tm = transport maximum.

steroid administration, the proportion of conjugated to total BSP increased in each infusion period. Although no BSP was infused during period II, the plasma BSP concentration remained relatively constant, and almost 90% of the BSP in the plasma was in a conjugated form.

Light microscopic examination of hematoxylin- and eosin-stained specimens of liver obtained in patients D.T. and J.F. after steroid administration revealed normal appearing liver cells except for a pale, pink, vacuolar, cytoplasmic inclusion without a membranous limit. Exposure of the tissue sections to ammonia fumes resulted in appearance of a purple color with an absorption maximum of 580 mμ. This is the absorption maximum for BSP (7). These biopsy specimens were obtained within 24 hours after the second BSP infusion study, and the vacuolar inclusions within liver cells probably represent BSP. Histochemical findings were normal except for ATPase staining of bile canaliculi which revealed variable distortion. Electron microscopy revealed normal cellular architecture. Several bile canaliculi appeared slightly dilated; however, these changes were variable and their significance could not be evaluated. Lysosomes, the Golgi apparatus, mitochondria, and other cytoplasmic organelles appeared unaltered.

Liver function tests performed in each patient between days 7 and 10 of steroid administration were normal except for the following. The serum bilirubin concentration was 1.4 mg per 100 ml (1.1 mg per 100 ml direct reacting) in patient J.V. The serum bilirubin concentration was 1.6 mg per 100 ml (1.2 mg per 100 ml direct reacting) in patient J.L. The serum glutamic oxaloacetic transaminase activity was 85μ in patient A.M. Liver

TABLE III

Average plasma BSP concentration (mg per 100 ml) during each BSP infusion period before, during, and after administration of 17α-ethyl-19-nortestosterone

Patients	Relationship to steroid administration								
	Before			During			After		
Infusion period:	I	II	III	I	II	III	I	II	III
J.V.	4.6	1.8	3.6	5.7	5.3	8.4			
D.T.	3.8	2.1	4.4	4.6	4.4	7.8			
J.F.	5.4	3.0	3.8	6.6	6.4	9.2			
M.L.				6.2	6.0	8.9			
G.F.				6.0	5.1	6.9			
J.L.				3.2	2.6	5.4			
A.M.				5.1	5.0	8.9	4.8	1.9	5.2
W.J.				4.9	4.5	7.7	3.9	2.4	4.0

function tests including conventional BSP test were normal in all patients one week after steroid administration was discontinued.

DISCUSSION

The observed reduction in hepatic Tm for BSP with normal relative hepatic storage of BSP in patients receiving 17α-ethyl-19-nortestosterone could result from a deficiency in hepatic glutathione, reduced activity of the enzyme which catalyzes the formation of BSP glutathione, or an abnormality in hepatic parenchymal cell excretory function, or all. Because BSP was retained in plasma primarily as a conjugate after steroid administration, it is unlikely that there is a limitation in either hepatic glutathione content or activity of the conjugating enzyme in man. In rats, steroid administration in comparable doses does not decrease either hepatic glutathione content or conjugating enzyme activity (12). These observations are most consistent with the conclusion that 17α-ethyl-19-nortestosterone interferes with the transport of BSP from the liver cell into the bile. This effect of the steroid may prove useful in the biochemical study of the normal mechanism and regulation of excretory transport by the liver cell.

Philp, Grodsky, and Carbone (10) and Combes (11) have demonstrated that the formation of conjugated BSP is a major determinant of the maximal rate at which BSP is excreted from the liver cell into the bile. The fact that BSP is retained in plasma primarily as a conjugate in patients treated with 17α-ethyl-19-nortestosterone supports this conclusion.

Heaney and Whedon (13) observed delayed removal of BSP from the plasma of normal subjects treated with 20 to 100 mg of 17α-ethyl-19-nortestosterone daily for 7 to 10 days following the intravenous injection of 5 mg BSP per kg body weight, and suggested that the steroid primarily affects the excretion of BSP by the liver. Leevy, Cherrick, and Davidson (14) studied the effect of the steroid on the disappearance of injected BSP from the plasma and suggested that the steroid affects hepatic uptake as well as excretion of BSP. The method of Wheeler, Meltzer, and Bradley (9) when combined with quantitation of free and conjugated BSP in plasma avoids some of the difficulties of single injection experiments, as BSP storage and Tm can be quantitatively estimated.

TABLE IV

Ratio of concentration of conjugated to total BSP in plasma obtained at the conclusion of each hour of the BSP infusion study before and during administration of 17α-ethyl-19-nortestosterone

	Before steroid administration			During steroid administration		
Infusion period	I	II	III	I	II	III
Infusion rate, *mg/min*	15	5	10	8	0	5
Patient D.T.	.15	.10	.25	.25	.85	.70
Patient J.F.	.12	.08	.25	.20	.90	.83

In a study of BSP metabolism in patients with hepatitis, cirrhosis, biliary obstruction, and jaundice after chlorpromazine administration, both BSP storage and Tm were reduced (9). The functional abnormality observed after administration of 17α-ethyl-19-nortestosterone in this study is similar to that seen in patients with chronic familial nonhemolytic jaundice with conjugated bilirubin in the serum with and without an unidentified pigment in the liver cells, in whom hepatic storage of BSP is normal, BSP Tm is reduced virtually to zero (9, 15), and BSP is retained in the plasma primarily as a conjugate (16). An inherited abnormality in the transfer of conjugated bilirubin and other substances from the liver cell into the bile has been postulated to explain this syndrome (15, 17).

Carbone, Grodsky, and Hjelte (18) demonstrated that after administration of 17-methyltestosterone to normal subjects, BSP is retained in plasma primarily as a conjugate. The effects observed with 17α-ethyl-19-nortestosterone in the present study may, therefore, be produced by other steroids of similar structure.

Alterations in hepatic cell membrane staining reactions and absent bile canalicular microvilli have been described in rats (19, 20) and humans (2) after administration of 17α-ethyl-19-nortestosterone. In this study, in two patients, the functional abnormality in BSP metabolism was observed without significant alterations in liver cell ultrastructure or histochemical staining reactions. This observation suggests that the previously described structural abnormalities may not be etiologically related to the functional defect.

SUMMARY AND CONCLUSIONS

The administration of 17α-ethyl-19-nortestosterone to subjects with neither clinical nor chemical evidence of liver disease resulted in reversible reduction in the hepatic transport maximum for sulfobromophthalein sodium (BSP). The relative hepatic storage of BSP was unimpaired and BSP was retained in the plasma primarily as a conjugate. Light microscopic examination of two liver biopsies was normal except for BSP within parenchymal cells and fragmented ATPase staining reaction of bile canaliculi. Electron microscopic examination revealed variable dilatation of bile canaliculi and normal intracellular organelles.

These studies suggest that 17α-ethyl-19-nortestosterone interferes with the transfer of BSP conjugates from the liver cell into the bile.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Henry Wheeler of the Department of Medicine, College of Physicians and Surgeons, for advice regarding the BSP infusion method; Dr. Boris Gueft of the Department of Pathology, Albert Einstein College of Medicine, for the light microscopic examination of liver and assistance in the identification of BSP within liver cells; Dr. A. B. Novikoff, Dr. S. Goldfischer, and Dr. E. Essner of the Department of Pathology, Albert Einstein College of Medicine, for the histochemical and electron microscopic studies; and Dr. Thomas Hayes of the G. D. Searle Co. for a generous gift of 17α-ethyl-19-nortestosterone.

REFERENCES

1. Arias, I. M. The effects of anabolic steroids on liver function. Ciba Symposium on "Protein Metabolism." Berlin, Springer-Verlag, 1962, p. 434.
2. Schaffner, F., H. Popper, and E. Chesrow. Cholestasis produced by administration of norethandrolone. Amer. J. Med. 1959, **26**, 249.
3. Arias, I. M., S. Goldfischer, A. B. Novikoff, and E. Essner. The effect of 17-ethyl-19-nortestosterone and icterogenin on the hepatic metabolism of bilirubin in normal and Gunn rats (abstract). J. clin. Invest. 1961, **40**, 1023.
4. Combes, B., H. O. Wheeler, A. W. Childs, and S. E. Bradley. The mechanisms of bromsulfalein removal from the blood. Trans. Ass. Amer. Phycns 1956, **69**, 276.
5. Grodsky, G. M., J. V. Carbone, and R. Fanska. Identification of metabolites of sulfobromophthalein. J. clin. Invest. 1959, **38**, 1981.
6. Combes, B., and G. S. Stakelum. Conjugation of sulfobromophthalein sodium with glutathione in thioether linkage by the rat. J. clin. Invest. 1960, **39**, 1214.
7. Javitt, N. B., H. O. Wheeler, K. J. Baker, O. L. Ramos, and S. E. Bradley. The intrahepatic conjugation of sulfobromophthalein and glutathione in the dog. J. clin. Invest. 1960, **39**, 1570.
8. Wheeler, H. O., R. M. Epstein, R. R. Robinson, and E. S. Snell. Hepatic storage and excretion of sulfobromophthalein sodium in the dog. J. clin. Invest. 1960, **39**, 236.
9. Wheeler, H. O., J. I. Meltzer, and S. E. Bradley. Biliary transport and hepatic storage of sulfobromophthalein sodium in the unanesthetized dog, in normal man, and in patients with hepatic disease. J. clin. Invest. 1960, **39**, 1131.
10. Philp, J., G. M. Grodsky, and J. V. Carbone. Mercaptide conjugation in the uptake and secretion of BSP. Amer. J. Physiol. 1961, **200**, 545.
11. Combes, B. The importance of conjugation with glutathione on sulfobromophthalein (BSP) transport from blood to bile (abstract). J. clin. Invest. 1962, **41**, 1351.
12. Scherb, J., and I. M. Arias. Unpublished observations.
13. Heaney, R. P., and G. D. Whedon. Impairment of hepatic bromsulphalein clearance by two 17-substituted testosterones. J. Lab. clin. Med. 1958, **52**, 169.
14. Leevy, C. M., G. R. Cherrick, and C. S. Davidson. Observations on norethandrolone induced abnormalities in plasma decay of sulfobromophthalein and indocyanine green. J. Lab. clin. Med. 1961, **56**, 193.
15. Arias, I. M. Studies of chronic familial nonhemolytic jaundice with conjugated bilirubin in the serum with and without an unidentified pigment in the liver cells. Amer. J. Med. 1961, **31**, 510.
16. Wegmann, R., M. Rangier, J. Eteve, A. Charbonnier, and J. Caroli. Mélanose hépato-splénique avec ictère chronique à bilirubine directe: maladie de Dubin-Johnson? Sem. Hôp. Paris 1960, **36**, 1761.
17. Dubin, I., and F. Johnson. Chronic idiopathic jaundice with unidentified pigment in liver cells: new clinico-pathologic entity with report of 12 cases. Medicine (Baltimore) 1954, **33**, 155.
18. Carbone, J. V., G. M. Grodsky, and V. Hjelte. Effect of hepatic dysfunction on circulating levels of sulfobromophthalein and its metabolites. J. clin. Invest. 1959, **38**, 1989.
19. Goldfischer, S., I. M. Arias, E. Essner, and A. B. Novikoff. Cytochemical and electron microscopic studies of rat liver with reduced capacity to transport conjugated bilirubin. J. exp. Med. 1962, **115**, 467.
20. Schaffner, F., H. Popper, and V. Perez. Changes in bile canaliculi produced by norethandrolone: electron microscopic study of human and rat liver. J. Lab. clin. Med. 1960, **56**, 623.