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Research Article

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ALTERATIONS IN SULFOBROMOPHTHALEIN SODIUM-REMOVAL MECHANISMS FROM BLOOD DURING NORMAL PREGNANCY *

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During the past few years, we have been impressed by the observation that our patients with pre-eclampsia and eclampsia usually demonstrate sulfobromophthalein sodium (BSP) retention in blood in the 45-minute BSP test. On the basis of these findings, we undertook a study of hepatic BSP-removal mechanisms during the course of normal pregnancy. Increased BSP retention in 45 minutes was not detected in normal pregnancy when compared to results obtained in a group of nonpregnant women of comparable age. When hepatic BSP-removal mechanisms were appraised quantitatively by a prolonged-infusion method (1, 2), however, significant changes in both hepatic uptake and excretion of BSP were observed. The following study presents in detail such data obtained from women in various stages of normal pregnancy and in the early postpartum period, and compares these findings with data obtained in a separate group of control nonpregnant women.

METHODS

The method used in the present studies was devised by Wheeler and his associates (1, 2) and is based on the observations that removal of BSP from plasma depends on the simultaneous operation of at least two separate hepatic mechanisms: *1*) uptake of BSP into a hepatic "storage compartment," and *2*) active secretion into bile (1-8). The quantity of BSP taken up into the "storage compartment" appears to be directly proportional to plasma concentration (1). Thus, uptake into storage during a period of changing plasma concentration can be represented in symbols as $S \times \Delta P/\Delta t$, where the "relative storage capacity" S, a proportional factor, is defined as the number of milligrams of BSP stored in the liver per milligram per 100 ml of plasma concentration, and $\Delta P/\Delta t$ signifies the rate of change of plasma concentration in milligrams per 100 ml per minute. Secretion of BSP into bile is a rate-limited process characterized by a maximal excretory rate or T_m (1, 2, 8), defined in terms of milligrams per minute. The maximal rate of BSP secretion into bile is achieved when plasma BSP concentration is maintained in excess of 2 to 3 mg per 100 ml during prolonged infusions of BSP (1, 8). At these plasma concentrations, the general equation for the hepatic removal rate of BSP (1), R_H, in milligrams per minute, is as follows: $R_H = S \times (\Delta P/\Delta t) + T_m$.

Values for R_H and $\Delta P/\Delta t$ obtained during two or more different rates of BSP infusion are substituted in the formula, providing a set of simultaneous equations that can be solved for both S and T_m. In practice, values for R_H and $\Delta P/\Delta t$ were obtained during three separate infusion rates of BSP, each given for an hour. As indicated by Wheeler, when the corresponding values for R_H and $\Delta P/\Delta t$ are plotted on the ordinate and abscissa, respectively, the relationship between R_H and $\Delta P/\Delta t$ appears to be approximately linear. Thus, the equation of the line that best fits the three points is calculated by the method of least squares (9). Since the general equation for hepatic removal rate of BSP is in the form of a straight line, $y = m \times + c$, S is equal to the slope of the least-squares line, and T_m is equal to the value of the y intercept.

Procedures

Single, prolonged-infusion studies were conducted before surgery on 10 control nonpregnant women in the reproductive age range admitted to the gynecology service, usually for therapy for stress incontinence or for simple hysterectomy; on 15 women in various stages of normal pregnancy; and on 11 women during the first week postpartum. The studies were usually carried out in the morning, approximately 1 hour after a fat-free breakfast. The procedure used was essentially the same as that described by Wheeler, Meltzer, and Bradley (2), with the exception that the BSP administered was di-

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TABLE I

Hepatic sulfobromophthalein sodium (BSP)-removal mechanisms in control nonpregnant women

Patient Age, years BSA, m^2	Plasma volume	Hemato-crit	Total blood volume	BSP						BSP retention 45 min	S†	T_m‡
				Infusion period	Infusion rate	Average P*	Distribution in plasma: Conjugated	Distribution in plasma: Free	Average ΔP			
	ml	%	*ml*		*mg/min*	*mg/100 ml*	%	%	*mg/100 ml/min*	%	*mg/mg/100 ml*	*mg/min*
O.S.				I	14.70	7.84			+.123			
24				II	5.01	3.77			−.037			
1.45	1,835	41.1	3,115	III	8.75	5.12			+.004	2.3	38.0	8.7
E.S.				I	12.95	8.28	5.7	94.3	+.126			
35				II	4.12	6.44	25.9	74.1	−.039			
1.52	2,150	40.0	3,585	III	7.10	6.74	26.3	73.7	+.004	2.8	27.8	6.8
J.N.				I	21.91	11.83	8.6	91.4	+.207			
35				II	7.51	11.53	28.8	71.2	−.005			
1.57	2,315	35.2	3,575	III	12.96	15.64	30.1	69.9	+.091	2.5	45.2	7.4
A.H.				I	17.20	5.02			+.087			
28				II	5.80	3.09			−.032			
1.58	3,050	37.9	4,910	III	10.40	3.94			+.032		57.4	8.0
R.W.				I	16.43	9.01			+.125			
38				II	5.85	6.34			−.064			
1.69	2,590	35.8	4,035	III	9.74	7.95			+.026	3.7	32.4	9.2
M.R.				I	14.40	9.48	7.2	92.8	+.127			
39				II	5.30	6.46	32.9	67.1	−.054			
1.72	2,405	35.8	3,745	III	8.35	6.39	25.0	75.0	−.002	3.7	26.3	8.2
B.B.				I	23.28	12.77	6.5	93.5	+.225			
32				II	7.84	12.09	24.9	75.1	+.015			
1.80	2,550	42.8	4,460	III	14.61	17.48	22.8	77.2	+.099	4.5	48.0	7.0
G.M.				I	22.90	9.23	5.3	94.7	+.133			
45				II	7.92	7.88	21.8	78.2	−.034			
1.80	2,770	40.4	4,650	III	14.65	10.14	18.9	81.1	+.039	3.5	61.8	10.8
J.A.				I	18.48	8.17			+.140			
34				II	6.60	7.52			−.038			
1.81	2,565	41.0	4,345	III	11.44	10.01			+.032	6.1	41.3	9.2
D.C.				I	18.16	7.85	5.3	94.7	+.102			
29				II	6.65	5.02	24.6	75.4	−.032			
1.86	2,815	39.0	4,615	III	11.08	5.34	23.6	76.4	+.006		55.5	9.8
Mean				I	18.04	8.95	6.4	93.6	+.140			
				II	6.32	7.01	26.5	73.5	−.032			
				III	10.91	8.88	24.5	75.5	+.033	3.6	43.4	8.5
											±SD 12.3	±SD 1.3

* P = plasma concentration of BSP.
† S = relative storage capacity, defined as number of milligrams BSP stored in liver per milligram per 100 ml plasma concentration.
‡ T_m = maximal excretory rate of BSP into bile.

luted in isotonic saline. Plasma volume was measured in each subject during the first hour of the study with I^{131}-labeled human serum albumin. A plasma sample was obtained 10 minutes after injection of approximately 5 μc of radioactive tracer for estimation of plasma volume. A 45-minute BSP test (10) was carried out in most control and pregnant subjects on the day before, and in postpartum women, just before the prolonged-infusion study.

In 14 additional studies, constant infusions of BSP were administered for 24 to 156 minutes to women in labor. At the time of delivery, samples of maternal and fetal cord blood were obtained simultaneously for estimation of plasma concentration of BSP. Placentas were obtained from 10 of these women and analyzed for BSP content.

The quantity of BSP excreted into urine during 65 to 97 minutes of BSP infusion was determined in four other studies conducted in women at term.

Analytical methods

Plasma. One ml of each plasma sample was mixed with 9 ml of 0.1 N KOH. The optical density was determined in a Beckman DU spectrophotometer set at 580 mμ. Plasma obtained before infusion of BSP was treated similarly and served as a blank. Standard BSP curves were prepared for each patient by addition of known amounts of BSP to plasma obtained before BSP infusion. Since blank samples of fetal cord blood could not be obtained, BSP concentration in fetal cord plasma was estimated by the method of Gaebler (11).

Infusion mixture and urine. Samples of infusion mixture and urine samples were diluted with distilled water and alkalized, and the optical density was determined at 580 mμ. For analyses of urine, samples obtained just before infusion of BSP were diluted to the same extent as the sample containing BSP and served as blanks.

Chromatography of BSP compounds in plasma. Equal volumes of plasma from each of the four plasma samples obtained during each hour of BSP infusion were pooled. BSP compounds were extracted from the pooled plasma and prepared for paper chromatography on Whatman 3MM paper as outlined by Carbone, Grodsky, and Hjelte (12). The chromatograms were developed in a descending system employing glacial acetic acid: water: *n*-propyl alcohol (1:5:10 by vol), for 16 to 18 hours. BSP bands were identified by exposing the dried papers to ammonia vapors. BSP was eluted from each band and the concentration determined by methods described in detail in previous publications (13, 14). One or two bands corresponding to BSP conjugates and a band containing free BSP were identified on the chromatograms. The proportion of BSP appearing as conjugated BSP was determined by dividing the sum of the optical den-

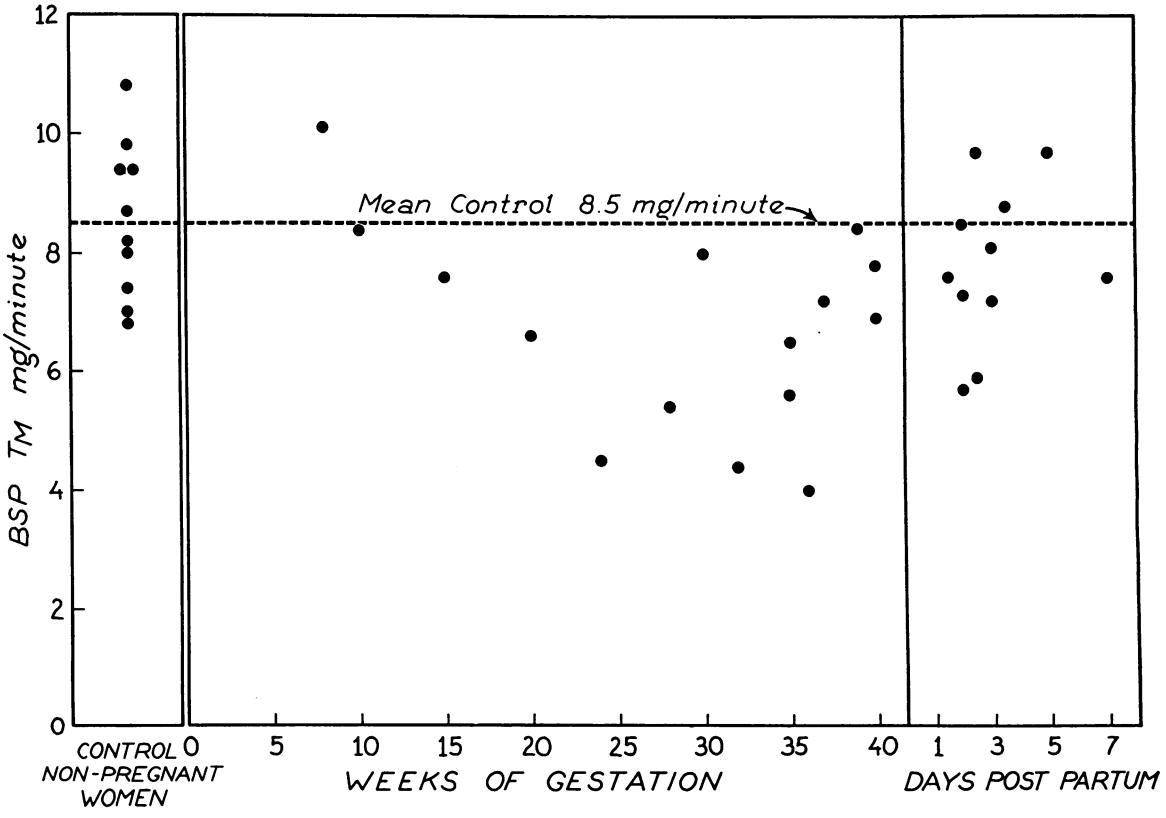

FIG. 1. ALTERATIONS IN MAXIMAL EXCRETORY RATE OF BSP INTO BILE (BSP T_m) DURING NORMAL PREGNANCY AND THE FIRST WEEK POSTPARTUM.

TABLE II

Hepatic BSP-removal mechanisms during normal pregnancy

Patient Age, years BSA, m^2 *	Weeks of gestation	Plasma volume	Hematocrit	Total blood volume	BSP						BSP retention 45 min	S	T_m
					Infusion period	Infusion rate	Average P	Distribution in plasma: Conjugated	Distribution in plasma: Free	Average ΔP			
		ml	%	*ml*		*mg/min*	*mg/100 ml*	%	%	*mg/100 ml/min*	%	*mg/mg/100 ml*	*mg/min*
L.S.					I	24.38	16.73	9.8	90.2	+.376			
33					II	8.37	22.57	28.4	71.6	−.002			
1.87	8	2,645	40.2	4,425	III	14.29	28.18	31.3	68.7	+.057		36.5	10.1
B.H.					I	16.00	5.21	3.6	96.4	+.102			
30					II	5.72	3.74	19.7	80.3	−.027			
1.65	10	3,050	36.1	4,775	III	9.53	3.97	12.8	87.2	+.004	1.4	46.3	8.4
A.L.					I	16.13	5.17	9.0	91.0	+.067			
26					II	5.63	3.27	35.3	64.7	−.007			
1.46	15	2,855	30.0	4,080	III	9.75	3.70	29.2	70.8	+.005	1.4	102.5	7.6
L.W.					I	19.06	8.08	12.6	87.4	+.156			
37					II	6.86	9.44	41.4	58.6	+.010			
1.84	20	3,600	35.4	5,575	III	12.56	12.22	36.7	63.3	+.062	7.3	46.1	6.6
C.T.					I	20.36	4.59	4.2	95.8	+.070			
23					II	7.05	3.85	23.5	76.5	+.009			
1.80	24	4,665	30.0	6,665	III	11.75	6.16	23.5	76.5	+.040	1.4	144.1	4.5
L.W.					I	23.78	6.72	6.6	93.4	+.125			
17					II	8.25	6.98	29.2	70.8	+.016			
1.81	28	3,625	31.3	5,275	III	13.70	11.56	27.9	72.1	+.086	3.3	98.7	5.1
L.J.					I	18.86	5.26	5.7	94.3	+.074			
29					II	6.56	4.29	15.7	84.3	−.011			
1.77	30	3,305	29.3	4,719	III	11.83	6.04	9.6	90.4	+.026	2.8	110.8	8.0
D.S.					I	20.00	5.83			+.088			
37					II	6.96	6.83			+.022			
1.75	32	5,520	31.2	8,125	III	11.44	9.95			+.045		107.0	4.4
J.M.					I	19.04	5.32			+.072			
30					II	6.21	3.95			−.001			
1.62	35	3,970	36.1	6,215	III	11.18	4.99			+.013		105.9	6.5
R.M.					I	14.5	4.42			+.062			
17					II	5.25	3.48			−.006			
1.68	35	2,940	41.0	4,985	III	8.58	5.07			+.018	6.1	91.5	5.6

* Body surface area based on prepregnant weight.

TABLE II—*Continued*

Patient Age, years BSA, m^2*	Weeks of gestation	Plasma volume	Hematocrit	Total blood volume	BSP Infusion period	BSP Infusion rate	BSP Average P	BSP Distribution in plasma: Conjugated	BSP Distribution in plasma: Free	BSP Average ΔP	BSP retention 45 min	S	T_m
		ml	%	*ml*		*mg/min*	*mg/100 ml*	%	%	*mg/100 ml/min*	%	*mg/mg/100 ml*	*mg/min*
P.S.					I	13.07	5.25			+.069			
26					II	4.56	4.51			−.002			
1.45	36	3,060	36.0	4,780	III	7.75	6.28			+.027		92.0	4.0
B.N.					I	17.60	7.60	9.1	90.9	+.111			
22					II	5.90	7.52	22.7	77.3	−.018			
1.60	37	3,595	36.0	5,615	III	9.84	10.01	24.9	75.1	+.035	10.8	56.3	7.2
M.K.					I	22.79	6.80	3.8	96.2	+.106			
16					II	7.75	6.46	17.3	82.7	+.010			
1.73	39	4,110	32.1	6,640	III	13.36	8.07	19.0	81.0	+.019	4.2	92.3	8.5
L.S.					I	16.2	4.22			+.081			
24					II	5.83	4.60			−.012			
1.69	40	3,750	32.2	5,530	III	9.78	5.49			+.031	5.5	71.6	6.9
K.R.					I	19.67	4.80			+.084			
23					II	6.42	4.54			−.015			
1.83	40	3,970	33.0	5,925	III	11.14	6.30			+.036	5.1	91.8	7.8

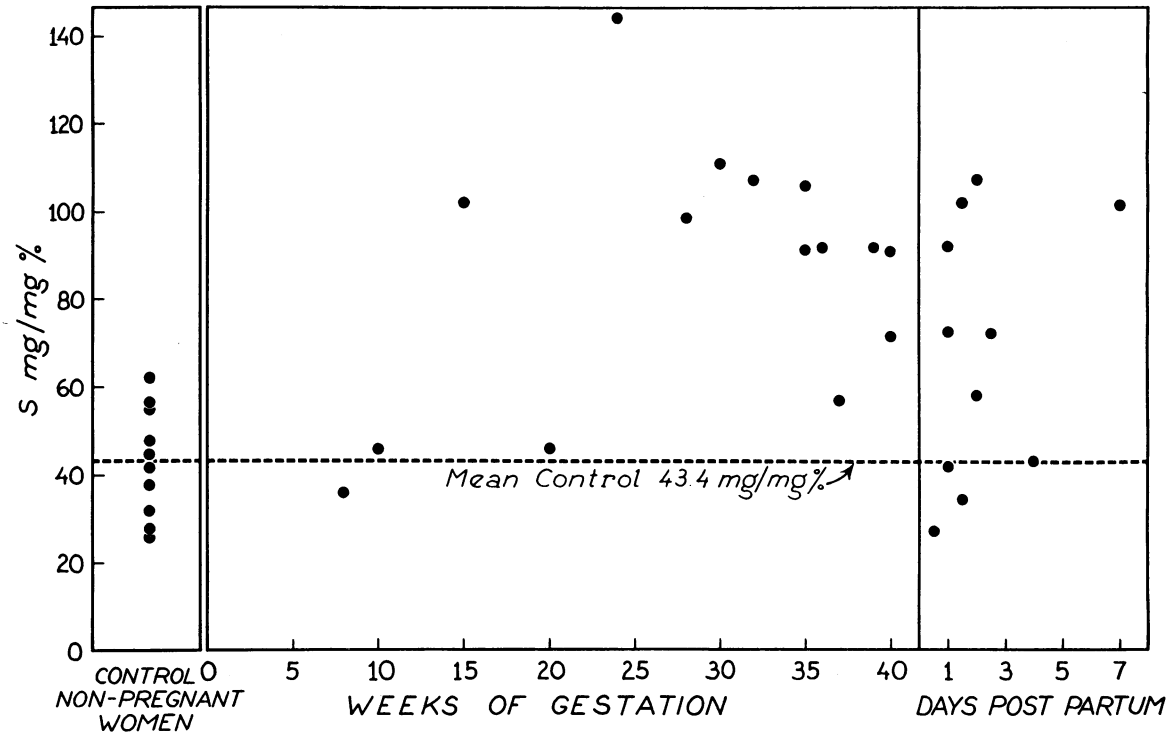

FIG. 2. ALTERATIONS IN HEPATIC RELATIVE STORAGE CAPACITY FOR BSP (S) DURING NORMAL PREGNANCY AND THE FIRST WEEK POSTPARTUM.

sities of the conjugate bands by the sum of the optical densities of the conjugate and free BSP bands. For these studies, it was assumed that the molecular extinction coefficient for free and conjugated BSP was identical.

Extraction of BSP from placenta. The amnion and chorion were stripped off, and the umbilical cord was cut at its juncture with the placenta. The placenta was weighed, cut into small pieces roughly 1-inch square, and homogenized in a Waring Blendor with a volume of distilled water equal to the weight of the placenta. Two-ml samples of homogenate, equivalent to 1 g of placenta, were pipetted in triplicate into glass-stoppered, conical centrifuge tubes. To extract BSP, 4 ml of 80% acetone was added to each tube. After thorough shaking, the tubes were centrifuged at 4,500 rpm for 10 minutes. The supernatant fluid was removed and the precipitate extracted twice with 3 ml of 80% acetone. The supernatant fluids were pooled and the volume recorded. Duplicate 3-ml samples of the combined acetone extract were mixed with 1 ml of 0.1 N KOH, and the optical density was determined by using 0.1 N KOH as a blank in a Beckman DU spectrophotometer set first at 580 mμ and then at 620 mμ. The alkalinized acetone extracts exhibited varying amounts of turbidity. The optical density at 580 mμ of similarly turbid alkalinized acetone extracts of placentas free of BSP averaged 1.10 times the optical density at 620 mμ. Thus, the BSP content of each placenta was calculated as follows: [OD 580 − 1.10 OD 620 (unknown)]/[OD 580 − 1.10 OD 620 (standard)] × concentration of standard solution in milligrams per 100 ml × volume of acetone extract in fractions of 100 ml × weight of the placenta in grams. Since recovery of known amounts of BSP homogenized with placentas averaged 62%, the value above was multiplied by 100/62.

RESULTS

1. Maximal excretory rate of BSP into bile, BSP T_m. In 10 control nonpregnant women, the average BSP T_m was 8.5 mg per minute ± 1.3 SD. The individual results are listed in Table I. T_m appeared to rise with increasing surface area, but the correlation coefficient of 0.5 was not statistically significant, $p > 0.05$. BSP T_m decreased during the latter part of normal pregnancy (Table II, Figure 1). The average T_m in the last half of pregnancy of 6.2 mg per minute ± 1.7 SD represented a fall of 27% below control, a statistically significant decrease, $t = 3.38$, $p < 0.01$. A rapid return of T_m to control values was observed during the first week postpartum (Table III, Figure 1).

2. Hepatic relative storage capacity, S. The average value for S in 10 control nonpregnant

TABLE III

Hepatic BSP-removal mechanisms during first week postpartum

Patient Age, years BSA, m^2*	Days post-partum	Plasma volume	Hemato-crit	Total blood volume	BSP									
					Infu-sion period	Infusion rate	Average P	Distribution in plasma: Conju-gated	Distribution in plasma: Free	Average ΔP	BSP reten-tion 45 min	S	T_m
		ml	%	*ml*		*mg/min*	*mg/100 ml*	%	%	*mg/100 ml/ min*	%	*mg/mg/ 100 ml*	*mg/min*
C.A.					I	19.20	7.70	11.0	89.0	+.228			
37					II	3.96	6.56	48.0	52.0	−.033			
1.60	½	2,435	38.2	3,940	III	11.06	8.97	29.9	70.1	+.029	4.7	27.3	7.6
M.T.					I	16.38	8.01	13.9	86.1	+.085			
20					II	5.86	6.89	36.9	63.1	−.003			
1.57	1	2,720	31.3	3,960	III	9.17	9.97	28.8	71.2	+.035	3.6	91.9	5.7
E.J.					I	17.63	5.98	12.9	87.1	+.147			
31					II	5.86	6.59	45.8	54.2	+.004			
1.63	1	2,910	29.0	4,100	III	10.12	8.67	46.0	54.0	+.013	5.3	41.5	7.3
E.J.					I	15.64	5.12	6.0	94.0	+.074			
24					II	5.44	3.18	27.5	72.5	−.028			
1.70	1	2,855	37.5	4,570	III	9.18	3.32	21.9	78.1	+.002		72.5	8.5
M.D.					I	20.38	14.04	9.7	90.3	+.220			
19					II	4.58	12.82	39.8	60.2	−.024			
1.55	1¼	3,070	38.5	4,990	III	8.02	13.42	41.7	58.3	+.038	14.5	34.9	5.9
D.J.					I	20.68	7.60	8.8	91.2	+.096			
18					II	7.02	6.64	26.7	73.3	−.021			
1.75	1½	2,885	37.8	4,640	III	12.28	6.77	36.9	63.1	+.008		101.9	9.7
E.M.					I	17.37	4.65	6.9	93.1	+.063			
23					II	5.79	3.03	26.6	73.4	−.016			
1.83	2	3,695	38.0	5,960	III	10.05	3.95	26.4	73.6	+.014	4.1	106.7	8.1
A.W.					I	17.55	8.53	9.4	90.6	+.124			
17					II	0.38	7.35	32.0	68.0	−.004			
1.70	2	2,670	27.3	3,675	III	10.37	8.92	27.9	72.1	+.030	4.7	58.3	7.2
D.W.					I	19.61	5.92	7.8	92.2	+.099			
23					II	7.51	4.90	31.2	68.8	−.013			
1.83	2½	3,620	38.1	5,850	III	11.27	6.25	29.2	70.8	+.026		72.0	8.8
J.H.					I	18.16	7.77	8.4	91.6	+.120			
21					II	6.85	6.84	31.0	69.0	−.030			
1.61	4	2,895	31.0	4,195	III	10.94	7.69	30.2	69.8	+.012	3.0	43.1	9.5
E.B.					I	17.63	6.10	3.9	96.1	+.083			
25					II	5.54	4.35	20.1	79.9	−.011			
1.72	7	2,315	43.0	4,130	III	1.10	5.46	20.4	79.6	+.014		101.4	7.6

* Body surface area based on prepregnant weight.

women was 43.4 mg per mg per 100 ml ± 12.3 SD (Table I). No correlation between S and surface area was apparent. S did not change during the early part of pregnancy (Table II, Figure 2). Later, however, S rose markedly and averaged 96.5 mg per mg per 100 ml ± 22.6 SD during the last half of pregnancy. The increase of 122% above control in the latter half of pregnancy was highly significant, $t = 6.56$, $p < 0.001$. After delivery, S tended to fall toward control values, although it still remained elevated as long as one week postpartum in some patients (Table III, Figure 2).

3. BSP concentration in maternal and fetal cord plasma. Constant infusions of BSP were administered to 14 women at term for a mean period of 73 minutes. During this time, an average of 1,177 mg of dye was removed from the circulation. The concentration of BSP in maternal plasma at the time of delivery averaged 9.2 mg per 100 ml. In fetal cord plasma, sampled at the same time, no BSP was detected in 10 specimens. BSP was found in the other 4. In these instances, the concentration of dye, in milligrams per 100 milliliters, in fetal cord and maternal plasma, respectively, was 0.08 fetal to 14.6 maternal; 0.16 to 5.8; 0.63 to 17.0; and 3.6 to 7.6.

4. BSP uptake by placenta. BSP was infused for an average of 70 minutes in 10 women at term. Of the 1,085 mg of BSP removed from blood during this period, an average of only 7.2 mg could be accounted for in the placentas. Since placentas contain approximately 50 ml of maternal plasma (15), the quantity of BSP to be expected in this volume of plasma (maternal plasma concentration of BSP in milligrams per 100 ml at time of delivery × 0.5) averaged 5.1 mg. Actual tissue content of BSP was approximately 2.1 mg of BSP per placenta, therefore.

5. Urinary excretion of BSP. An average of 1,526 mg of BSP was removed from blood during a mean infusion period of 83 minutes in four women at term. Only 8.1 mg of BSP was excreted into urine during this period, on the average.

DISCUSSION

Almost all of the results of the 45-minute BSP tests carried out during the course of normal pregnancy (Table II) fell within the range of values obtained in a group of control nonpregnant women. It seemed unlikely, therefore, that any alterations in hepatic BSP-removal mechanisms would be noted during the course of normal pregnancy. However, two significant changes were observed when the prolonged-infusion method of Wheeler and his associates (1, 2) was utilized. Thus, S increased markedly, whereas BSP T_m decreased during the last half of pregnancy. It is apparent that the Wheeler method provides an extremely useful tool for examining hepatic BSP-removal mechanisms. It not only permits an appraisal of both hepatic uptake and biliary excretion of BSP in quantitative terms, but obviously it is capable of detecting significant changes even when the sensitive clinical BSP test yields normal results.

The most impressive finding in the present studies was the marked rise in S during the last half of pregnancy. There are several possible explanations for this change. First, it was necessary to demonstrate that significant BSP removal from blood in sites other than the liver did not account spuriously for the higher values of S observed in the last half of pregnancy. Since S was as high at 24 weeks of gestation as at 40, it seemed unlikely that BSP uptake by the placenta or the fetus, which would enlarge considerably during this period of gestation, contributed in any significant way to BSP removal from plasma. This was corroborated by our studies which demonstrated that virtually no BSP is taken up by or crosses the placenta. Others have examined transplacental movement of BSP after administration of a single iv injection of 5 mg per kg. Freiheit detected no dye in cord blood of two infants delivered within half an hour of BSP administration (16). Smith, Moya, and Shnider (17) observed BSP in umbilical vein blood as early as 20 seconds after BSP injection, and low levels of BSP were found in nine of 15 subjects. No BSP was detected in umbilical vein blood of six infants. In our constant infusion studies in which cord blood was sampled, no measurable amount of BSP was found in 10 of 14 subjects. If a large quantity of BSP had passed into the fetal circulation, it seems likely that relatively high levels of BSP would have been detected,

since BSP removal from blood is delayed in the newborn infant (18–22). In the present studies, urinary loss of BSP was small in pregnancy. On the basis of earlier observations (1, 23–25), it seems unlikely that other extrahepatic sites accounted for a marked increase in BSP removal from blood, although this possibility is not excluded. Significant uptake of BSP by tissues other than the liver apparently does not explain the rise in S.

Before attributing increased S to an intrinsic change in the liver, it is necessary to consider other extrahepatic factors that might result in increased hepatic BSP storage in pregnancy. With the method used in these studies, it is assumed that the quantity of BSP taken up into storage for each increment in plasma concentration of 1 mg per 100 ml is the same over a limited range of plasma concentration (1). It is conceivable that the capacity of the storage mechanism differs when changes in plasma concentration occur at low and high plasma levels of BSP. If the mechanism accounting for storage is composed of units with different affinities for BSP, components with high affinity would take up BSP first, thus at low plasma levels. Units with progressively lower affinity would remain as the quantity of BSP stored increases during rising plasma concentration. Thus lower values for S would be expected at higher plasma levels. In the present studies, average plasma levels of BSP during each hour of BSP infusion were somewhat lower in women in the last half of pregnancy than in control nonpregnant women (5.53, 4.18, and 7.27 mg per 100 ml in pregnant women compared to 8.95, 7.01, and 8.88 mg per 100 ml in control nonpregnant women, Tables I and II). Apparently, in the dog, S is relatively constant over a range of BSP levels in plasma of at least 3 to 12 mg per 100 ml (26). The plasma concentrations attained in the present studies fell well within this range, and if S behaves in the human as it does in the dog, it seems unlikely that the small differences in plasma levels could account for the large changes in S observed during pregnancy.

The degree of protein-binding of BSP in plasma may exert an influence on uptake of BSP into storage. It is possible that the quantity of BSP stored in the liver is proportional to a small fraction of unbound dye in plasma rather than to total plasma concentration of BSP. Thus, increased unbound BSP in plasma of pregnant women might result in increased S. In preliminary studies (27), ultrafiltrates of plasma, obtained from both control and pregnant women, to which BSP had been added *in vitro* revealed no free BSP in plasma water with total BSP concentrations in plasma of up to 20 mg per 100 ml. These observations suggest, therefore, that differences in protein-binding over the range of plasma BSP concentrations attained in the present studies did not influence values of S.

A rise in S might be considered to result from a decrease in T_m. This seems unlikely, however, for in at least three situations—in patients with the Dubin-Johnson syndrome (2), after acute ligation of the common bile duct in dogs (26), and during administration of 17α-ethyl-19-nortestosterone (37)—S has remained normal when T_m is decreased markedly.

It seems reasonable to conclude, therefore, that an increase in S during the last half of pregnancy indicates that the relative storage capacity of the liver for BSP increases, and furthermore, that this alteration is due to an intrinsic change in the liver. Such an increase in S could be accounted for by an increase in hepatic mass, by increased storage capacity of each unit of liver tissue, or by both. As for increased hepatic mass in normal pregnancy in the human, few data are available for analysis. A review of autopsy information derived from women dying after a brief illness during pregnancy in our hospital revealed an average liver weight of approximately 1,700 g, with no significant difference in liver mass during early and late pregnancy. Thus, an increase in size of the liver does not explain the rise in S. Normally, BSP is concentrated in human liver three to four times above plasma concentration, on the average (2). Although the nature of the concentrating mechanism is not known—whether it is a liver protein with greater affinity for BSP than plasma protein, an active transport mechanism, or something else—, an increase in capacity of this mechanism in each unit of liver tissue is the most likely explanation for the rise in S during pregnancy.

The implication of this observation appears to extend beyond hepatic uptake of BSP. Obviously, the human liver is not usually exposed to BSP. A rise in storage capacity for this dye could hardly be considered a specific response, therefore. It seems reasonable to expect that increased hepatic storage capacity for a variety of compounds, including drugs, will be found during pregnancy.

The fall in T_m in the last half of pregnancy may also be accounted for by several possibilities. First, if the liver holds on to BSP more avidly, less BSP may be available for excretion. The observation that S remained increased at a time when T_m was normal in several postpartum patients suggests another mechanism, however. Second, interference with BSP conjugation might result in decreased T_m. Previous work indicated that conjugation of BSP with glutathione (28–30), a process catalyzed by a liver enzyme (31), does not influence the extent to which BSP is stored in the liver (32). BSP T_m is dependent on conjugation, however, since conjugated BSP is delivered into bile at a faster rate than free BSP (32). The components of the BSP-glutathione conjugating system have not been examined in human liver during pregnancy. In the rat, a decrease in BSP conjugating enzyme activity with normal levels of glutathione has been found during the last 5 days of gestation (14). When liver glutathione levels have been maintained, however, a moderate decrease in enzyme activity has not resulted in a decrease in BSP T_m in the rat (32). Whether conjugation is impaired as a result of a critical decrease in enzyme activity or glutathione content in the human liver must await further studies. An analysis of BSP compounds appearing in plasma was carried out in order to detect any major changes in conjugation that might occur in the present studies. BSP conjugates were observed in plasma during the prolonged infusions of BSP (Tables I to III). The proportion of total plasma BSP accounted for by BSP conjugates during each hour of infusion was almost the same in patients during the last half of pregnancy and in control nonpregnant women (5.8, 21.6, and 20.9% in pregnancy, as compared to 6.4, 26.4, and 24.4% in controls). The small differences between pregnant and control patients in the averages for each hour were not significant statistically. Since the quantity of BSP infused was approximately the same in pregnant and nonpregnant subjects, the data above suggest that major changes in BSP conjugation in pregnancy did not affect T_m.

Finally, decreased T_m may result from an impairment of the transport processes involved in the delivery of both conjugated and free BSP from liver cells to bile. In this regard, it is of interest that a syndrome of obstructive jaundice has been observed during the latter part of pregnancy (33–36). After delivery, the obstructive features disappear, and hepatic function returns to normal. Obstructive jaundice may recur with subsequent pregnancies. The nature of the intrahepatic disturbance appears to involve impairment of biliary secretory mechanisms. It is tempting to speculate that the decrease in BSP T_m observed in the present studies reflects a similar response of the liver, although obviously a less marked one, to changes occurring normally during pregnancy. According to this view, obstructive jaundice might result either from an increase in quantity of the factor that accounts for decreased BSP T_m in normal pregnancy, or from a greater sensitivity of biliary secretory mechanisms to normal amounts of this factor found in pregnancy.

Analysis of various mechanisms that might account for both the rise in S and the fall in T_m in pregnancy appears to indicate that alterations in two independent hepatic systems are involved. Furthermore, since the changes in hepatic BSP-removal mechanisms occur during the last half of pregnancy, and then return to or toward normal in the first week after delivery, it seems likely that they are mediated by increased hormone levels in pregnancy, probably of estrogens, progesterone, or both. With decrease in hormone levels after delivery, BSP-removal mechanisms return toward normal.

SUMMARY

Hepatic sulfobromophthalein sodium (BSP)-removal mechanisms have been appraised quantitatively during pregnancy and the first week postpartum in terms of *1*) the relative storage ca-

pacity for BSP, S, defined as the number of milligrams of dye taken up into storage for each increment of plasma concentration of 1 mg per 100 ml and *2*) the maximal excretory rate of BSP into bile, BSP T_m, in milligrams per minute. S rose 122% during the last half of pregnancy, then returned to or toward normal during the first week postpartum. In contrast, BSP T_m decreased 27% in the last half of pregnancy, then rapidly increased to normal levels after delivery. These changes appear to reflect alterations in two independent hepatic mechanisms.

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