Bromsulfalein Retention Resulting from Liver Damage by a Carbon-Free Filtrate of India Ink.* (21125)

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India ink has been widely used to block the reticuloendothelial system(2). Since bromsulfalein clearance was impaired by India ink in a manner roughly proportional to the dose of ink, Mills and Dragstedt(3) proposed the use of the BSP clearance test as a measure of reticuloendothelial blockade. The interpretation of these experiments was challenged by Brauer and Pessotti(4) who suggested on the basis of *in vitro* experiments that the primary effect of India ink on the liver consisted of circulatory impairment. Subsequent work in our laboratory(5) suggested that the effect of India ink on liver function was neither the result of reticuloendothelial blockade nor of circulatory impairment. Apparently the observed results were not produced by the carbon of this preparation. Consequently, we inferred the presence of some toxic component in the aqueous phase of the India ink. The idea that India ink contains a soluble liver poison has been suggested by Victor *et al.*(6) on the basis of preliminary experiments with vital stains. The present investigation offers additional evidence that the action of India ink on BSP excretion is the result of the toxicity of the dispersing medium rather than the blocking effects of particulate matter.

Methods. India ink filtrate was prepared by repeatedly passing 8% Higgins India ink in 0.9% saline (v/v) through a Seitz filter under pressure, until the filtrate was water clear to the eye. The filtrate appeared slightly yellow and its pH was equal to that of the original ink. We observed initially that when ink was filtered by employing suction instead of pressure the pH of the filtrate was less than that of the ink and this method of preparation was discarded. The carbon suspension was prepared as follows: 400 mg of Cabot Elf-O carbon black was suspended in 50 ml of water which was agitated in an ultrasonic generator‡ for 5 minutes subsequent to the addition of carbon. Five ml of 8% gelatin were added to stabilize the colloid and this was followed by 0.9 g NaCl. The resulting mixture was diluted to 100 ml with water. The carbon suspension thus produced contained approximately the same amount of carbon as 8 ml of undiluted India ink and the

TABLE I. BSP Concentration at Various Intervals before and after Carbon Administration.

	BSP concentration (mg %)										
Dog wt, kg	Min. after 1st dose of BSP				Min. after carbon administration			Min. after 2nd dose of BSP			
	2	4	6	30	10	20	30	2	4	6	30
9.3	4.83	2.76	2.04	.94	.89	.55	.43	4.78*	3.60*	2.99*	1.02
19.5	4.44	2.44	1.40	.41	.30	.23	.20‡	4.33*	2.82*	1.89*	.67
10.7	7.86	5.11	4.54	2.55	2.23	2.00	1.84	7.95	6.59	5.61	3.76
11.1	5.42	3.18	2.05	1.94	1.76	1.68	1.54	9.34	7.14	5.69	3.17
6.8	6.83	4.38	3.22	2.60	2.38	2.19	2.03	7.46	5.10	3.70	3.36
Avg	5.88	3.57	2.65	1.69	1.51	1.33	1.21	7.16	5.28	4.11	2.40
S.E.†	.64	.51	.55	.44	.40	.40	.38	.73	.70	.67	.64

* These values are 3, 5 and 7 min. after BSP instead of 2, 4 and 6 min. Averages of these columns are calculated from the estimated 2, 4 and 6 min. values for these 2 dogs.

† Stand. error.

‡ Estimated from the 20 min. value.

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‡ General Electric Catalogue No. 8665966g3, 300 kc.

TABLE II.
BSP Concentration at Various Intervals before and after India Ink Filtrate Administration.

Dog wt, kg	BSP concentration (mg %) Min. after 1st dose of BSP 2	4	6	30	Min. after filtrate administration 10	20	30	% of inj. dose released*	Min. after 2nd dose of BSP 2	4	6	30
7.3	6.21	3.60	2.45	.84	2.59	2.77	2.55	17.1	12.2	11.7	10.9	8.22
18.0	6.16	3.66	2.36	.52	1.71	2.02	2.10	15.8	14.5	12.5	12.2	7.38
8.2	4.70	3.28	1.51	.12	.56	.62	1.22	11.0	10.2	8.86	7.45	2.83
9.5	7.59	4.25	2.68	1.16	2.39	2.82	2.92	17.6	14.3	12.8	12.2	7.60
11.3	6.44	3.08	1.34	.60	1.95	2.92	2.26	16.6	13.3	12.6	11.5	7.25
Avg	6.22	3.57	2.07	.65	1.84	2.23	2.21	15.6	12.9	11.7	10.9	6.66
S.E.†	.46	.20	.27	.17	.36	.43	.28	1.2	.79	.72	.88	.92

* Calculated on the basis of a 5% plasma volume.
† Stand. error.

microscopic appearance of this suspension was quite similar to that of the 8% India ink. The dogs used in the experiments were anesthetized with Na pentobarbital. Ink filtrate, carbon emulsion or BSP were injected into the femoral vein of one leg and blood samples were obtained from the opposite leg. The blood BSP concentration was determined by the method of Gaebler(7).

Results and discussion. The 5 dogs in Table I were injected with BSP (5 mg/kg) and their plasma levels were determined 2, 4, 6, and 30 minutes after BSP injection (columns 2-5). At the 30-minute interval 100 cc of carbon suspension was given intravenously over a period of 4-5 minutes. This carbon disappeared rapidly from the blood stream and most of it appeared to be in the liver when the animal was autopsied. The center 3 columns of Table I give the plasma BSP levels 10, 20, and 30 minutes after the end of the carbon injection. In all animals the BSP levels continued to fall throughout this period. The last 4 columns of Table I present the plasma BSP levels after a second dose of BSP which was administered immediately following the last 30-minute blood sample. The previous administration of carbon obviously had little or no effect on the rate at which BSP was removed from the circulation.

Table II gives the data for similar experiments except that India ink filtrate was injected instead of the carbon suspension. Two striking differences between these and the above results are apparent: 1) during the 30-minute period following the administration of filtrate 15.6% of the injected BSP was released into the circulation and the BSP concentration in the blood increased instead of showing a decrease; 2) the removal of the second dose of BSP was markedly impaired after the dose of ink filtrate. Thus, it appears that the carbon-free India ink filtrate produced the same effects as were previously obtained with whole India ink(5).

Our observation(5) that a dose of India ink which markedly inhibits BSP excretion has no measurable effect on the rate of removal of colloidal gold from the bloodstream throws considerable doubt on the assumption (3) that doses of India ink which interfere with dye excretion block the Kupffer cells of the liver. The demonstration that a carbon-free filtrate of India ink inhibits BSP excretion whereas carbon in a gelatin stabilized suspension has no inhibitory effect on BSP clearance is further evidence that some factor other than the carbon particles of India ink is toxic to the hepatic excretory system.

Victor *et al.*(6) observed that a combined alcoholic extract and acid supernatant of India ink interfered with the excretion of brilliant vital red into the biliary system. These authors suggested that some component of India ink might interfere with the transfer of dye from the liver phagocytes to the bile. However, their hypothesis does not explain the fact that both India ink and India ink filtrate caused the release of BSP already stored in the liver. If India ink merely blocked the transfer of BSP from liver cells to the bile canaliculi, one would not expect such a release of dye. However, the liberation

of BSP from the liver by India ink would appear plausible if India ink contained some substance which diminished the BSP-concentrating ability of the liver cells.

Our observations that the effect of India ink on BSP clearance is not produced by gelatin stabilized carbon particles and that doses of India ink sufficient to inhibit BSP excretion do not influence the phagocytic process(5) indicate that BSP clearance is not a suitable index of Kupffer cell activity.

Summary. The administration of a gelatin-stabilized carbon suspension did not affect bromsulfalein (BSP) clearance in dogs. However, a carbon-free filtrate of Higgins India ink markedly inhibited BSP uptake and effected the release of previously stored BSP from the liver. It appears that the effect of India ink on BSP excretion is not due to reticuloendothelial blockade but rather to a toxic effect of some soluble component of the ink on the liver. It is suggested that in dogs BSP clearance is not a suitable index of Kupffer cell activity.

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Effect of Quinidine on Papillary Muscle.* (21126)

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The effect of quinidine on the excitability of heart muscle has been studied in experimental animals as well as in man(1). The inhibition by quinidine is manifested by a depression of contraction and an increased refractory period. Although evidence is scanty it is generally believed that the effect of quinidine on the heart is due to a slowing of recovery processes resulting from impaired metabolism. In the present work simultaneous changes in contraction and oxygen uptake of the isolated cat papillary muscle following the administration of quinidine were observed. The results were further analyzed in the light of the effects of quinidine on the metabolism of heart slices.

Method. The method described previously (2) was used for the simultaneous observation of contraction and oxygen consumption of the muscle. The oxygen consumption and anaerobic glycolysis of cat heart slices were determined manometrically by a conventional Warburg method. The thickness of the slices

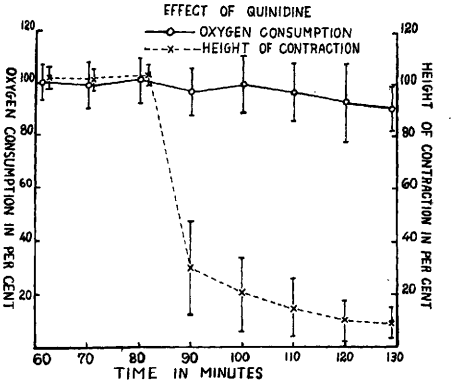

FIG. 1. Effect of quinidine (1:10000) on oxygen consumption and height of contraction of papillary muscles (7 exp.). In this and following figures, the 99% confidence limits of each mean are presented graphically by the vertical lines.

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