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In Vitro Adsorption Characteristics of Bile Salt Anions by Activated Carbon Beads for Oral Administration[1)]

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Adsorption characteristics of bile salt anions by activated carbon beads consisting of about 67% activated carbon powder in agar were studies *in vitro* and compared with those of a cholestyramine preparation from the standpoint of usefulness for sequestering bile salts in the intestine. Although the rate of adsorption of bile salts by the activated carbon powder in the beads was somewhat reduced as compared with that of the naked powder, the adsorption capacity was essentially retained in the beads, and was enhanced by the presence of physiologic anions. The interfering effect of physiologic anions on the adsorption by cholestyramine resin preparation was greater for trihydroxy bile salts than for dihydroxy analogs. Thus, the beads showed a greater capacity for trihydroxy bile salts than the resin preparation. For dihydroxy analogs the opposite relationship was observed, *i.e.*, the capacity of the resin preparation was greater than that of the beads. In the presence of triolein, the adsorption of bile salts by the powder was greatly impaired, whereas that by the beads was only slightly reduced. Encapsulation of the powder by agar apparently permitted selective adsorption of bile salts in the presence of the lipid.

Keywords——activated carbon preparation; activated charcoal; activated carbon; agar-encapsulated activated carbon; oral activated carbon beads; bile salt adsorption; cholestyramine; hyperlipidemia; physiologic anions; triolein

In spite of the great drug adsorptive power of activated carbon powder, its oral use even in the treatment of acute drug intoxication is limited because of the handling and palatability problems associated with the fine black powder. We have, thus, prepared[2)] activated carbon beads for oral administration by drying spherical agar beads in which activated carbon powder is finely dispersed. The beads are not only easy to handle but were demonstrated,[3)] in human subjects, to inhibit effectively the absorption of theophylline and phenytoin from the intestinal tract.

The present communication deals with the results of an *in vitro* study of the adsorption of endogeneous substances, bile salts, by the beads to investigate their possible usefulness in the treatment of hypercholesteremia, in the relief of pruritus, *etc.*, where adsorption of bile salt anions in the gastrointestinal tract is desired. For this purpose, an anion-exchange resin (cholestyramine) which exchanges chloride ions for bile salt anions has been widely used for nearly 20 years in the United States and European countries.[4)] It will shortly be introduced in Japan.[5)] Thus, we have compared the bile salt adsorption characteristics of the beads with those of a cholestyramine preparation.

A hypolipidemic effect of orally administered activated carbon was reported by Friedman *et al.* in patients[6)] with renal insufficiency and in uremic and diabetic rats.[7)] Although the mechanism is not clear, adsorption of bile salt anions in the intestine (as in the case of the anion-exchange resin) is considered to be responsible: promotion of fecal excretion of bile salts interrupts the normal enterohepatic circulation, which in turn induces an increase

in the rate of conversion of cholesterol to bile salts in the liver and lowers the blood cholesterol level. In this connection, Krasopoulos *et al.*[8] studied the adsorption of bile salts by activated carbon powders and showed that it is comparable to that by cholestyramine.

Experimental

Materials——Two preparations of activated carbon powder were employed. Powder A was purchased from Wako Pure Chemical Industries, Osaka, and is a chemically activated wood charcoal. Powder B was obtained from Inuhinode Seiyaku, Co., Osaka and is a wood charcoal of JP X grade, activated by steam. These charcoal powders were dried to constant weight before use. The activated carbon beads, beads A and B, were prepared as described previously[2] from powders A and B, respectively. For the equilibrium adsorption study, beads of mesh size 28/48 were exclusively used, and for the rate study, beads of mesh size 48/250 were also employed. A cholestyramine preparation (lot no. QTS22) was kindly supplied through Bristol Banyu Pharmaceuticals, Tokyo. It contains 4 g of anhydrous cholestyramine in 9 g of the preparation. Cholic acid (a product of Wako Pure Chemical Industries) and chenodeoxycholic acid (a gift from Eisai Co., Tokyo) were converted to sodium cholate (C) and sodium chenodeoxycholate (CDC), respectively, by addition of sodium hydroxide. The following bile salts were purchased in sodium salt form: glycocholate (GC, Nakarai Chemicals, Kyoto); deoxycholate (DC, Tokyo Kasei Kogyo Co., Tokyo); glycodeoxycholate (GDC, Calbiochem, San Diego, California); and taurodeoxycholate (TDC, Sigma Chemical Co., St. Louis, Missouri). These bile salts were dried to constant weight prior to use. Their purities were reported by the manufacturers to be better than 98%. All other chemicals were purchased from Wako Pure Chemical Industries and were used without further purification.

Adsorption Studies——Equilibrium adsorption studies were carried out at 37 °C as described previously[2] in three media: (1) water, pH adjusted to 7.0—7.5 when necessary by addition of sodium hydroxide or hydrochloric acid, (2) medium 2, JP X Disintegration Test, pH 6.8—6.9, and (3) 0.05 M bicarbonate buffer containing 0.15 M NaCl, pH 8.3—8.5. The time course of adsorption was studied in medium 2 also as described previously.[2] The amounts of sorbent preparation weighed were 20 mg for activated carbon powders, 30 mg for the beads, and 45 mg for the resin preparation. The adsorption data were treated according to the Langmuir equation:

$$D_b = \frac{D_b^m a\, D_f}{1 + a\, D_f}$$

where D_b is the amount (mol) of bile salt anions adsorbed per gram of sorbent in the preparation, D_b^m is the maximum amount (mol) of drug adsorbed per gram of activated carbon, D_f is the concentration of unbound bile salt anions in M, and a is the affinity constant in M^{-1}. The concentration of unbound bile salt anions was analyzed spectrophotometrically by forming sulfuric acid chromogens in concentrated H_2SO_4,[9] for C and GC in 94% H_2SO_4 after reaction for 1 h at 37 °C, and for all dihydroxy bile salts in 65% H_2SO_4 after reaction at 60—80 °C for 20—60 min. The analytical wavelengths employed were the wavelengths of maximum absorbance of each reaction mixture in the range of 380—390 nm. All measurements were made on a Shimadzu model UV-240 double-beam spectrophotometer.

Results

Cholate Adsorption Characteristics of Powders and Beads

Table I lists the Langmuir constants calculated for the binding of C to powders A and B and the corresponding beads in medium 2. The maximum binding capacity of powder A is nearly twice that of powder B. However, the affinity of powder B was higher than that of powder A. In bead form, the affinity was less than that of the corresponding powders. The binding capacity of powder A was essentially retained in beads A and is nearly equal to that for acetaminophen in the same medium.[2]

Comparison of Adsorption Characteristics of Beads A and a Cholestyramine Preparation in Medium 2 at 37 °C

Adsorption isotherms for the binding of various bile salts are presented for beads A (Fig. 1a) and a cholestyramine preparation (Fig. 1b). For beads A, the Langmuir constants are summarized in Table II. Both the capacity and affinity constants are greater for unconjugated bile salts than for the conjugated ones and the affinity constant for the taurine-conjugate of DC was particularly small. The capacity constants for dihydroxy bile salts were greater than

TABLE I. Comparison of Cholate Binding Parameters of Two Different Activated Carbons and the Corresponding Activated Carbon Beads (Mesh Size 28/48) in Medium 2, pH 6.8—6.9 at 37 °C

	$D_b^m \times 10^{4\,a)}$ (mol/g)	$a \times 10^{-4\,b)}$ (M^{-1})	$r^{c)}$	$n^{d)}$
Powder A	10.1	3.79	0.998	8
Beads A	9.94	1.81	0.999	13
Powder B	5.13	12.5	0.999	15
Beads B	3.61	2.50	0.997	17

a) Maximum amount (mol) of cholate adsorbed per gram activated carbon.
b) Affinity constant.
c) Correlation coefficient.
d) The number of data employed for calculation of the parameters.

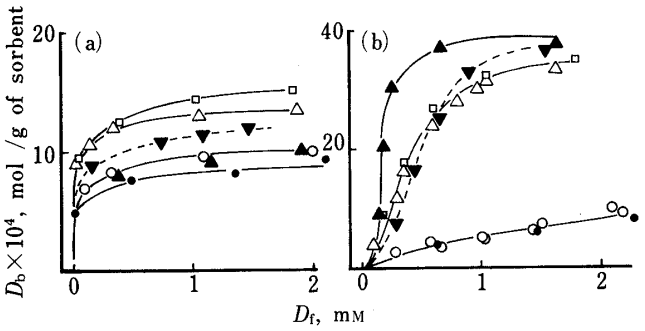

Fig. 1. Adsorption Isotherms for the Binding of Bile Salt Anions to (a) Beads A, Mesh Size 28/48 and (b) Cholestyramine Preparation at 37 °C in Medium 2

○, C; ●, GC; □, CDC; △, DC; ▼, GDC; ▲, TDC.

TABLE II. Langmuir Constants for the Binding of Bile Salt Anions to Beads A (Mesh Size 28/48) at 37 °C in Medium 2

Bile salt anion	$D_b^m \times 10^{4\,a)}$ (mol/g)	$a \times 10^{-4\,b)}$ (M^{-1})	$r^{c)}$	$n^{d)}$
C	9.94	1.81	0.999	13
GC	9.40	1.18	0.995	5
CDC	15.8	1.56	0.999	4
DC	13.6	3.10	0.999	5
GDC	12.0	1.84	0.999	5
TDC	11.2	0.534	0.999	4

a—*d*) As for Table I.

those for trihydroxy analogs. These results indicate the importance of the hydrophobicity of the bile salt anions in the adsorption by activated carbon. The adsorption isotherms for the cholestyramine preparation (Fig. 1b) showed sigmoidal curves for all bile salts investigated. Thus, no attempt was made to calculate the Langmuir constants. All dihydroxy bile salts were better adsorbed by the resin preparation than by beads A at higher concentrations, but trihydroxy bile salts were better adsorbed by beads A.

Effect of Media

The Langmuir constants summarized in Table III indicate that in the buffers both the capacity and affinity constants are higher than in water for both powder A and beads A. In Fig. 2, the adsorption isotherms of beads A and the cholestyramine preparation are compared for C and DC in water and the bicarbonate buffer, which is more similar to the intestinal

TABLE III. Effect of Media on the Binding Parameters of Cholate and Deoxycholate to Powder A and Beads A (Mesh Size 28/48)

	Medium	Bile salt anion	$D_b^m \times 10^{4\,a)}$ (mol/g)	$a \times 10^{-4\,b)}$ (M^{-1})	$r^{c)}$	$n^{d)}$
Powder A	Water	C	5.81	2.48	0.996	11
	Medium 2	C	10.1	3.79	0.998	8
	Water	DC	9.14	0.623	0.994	5
	Medium 2	DC	14.1	3.68	0.999	6
Beads A	Water	C	6.46	0.737	0.996	7
	Bicarb. buff.	C	8.47	1.42	0.997	5
	Medium 2	C	9.94	1.81	0.999	13
	Water	DC	8.21	0.743	0.999	5
	Bicarb. buff.	DC	11.0	2.69	0.999	4
	Medium 2	DC	13.6	3.10	0.999	5

a—*d*) As for Table I.

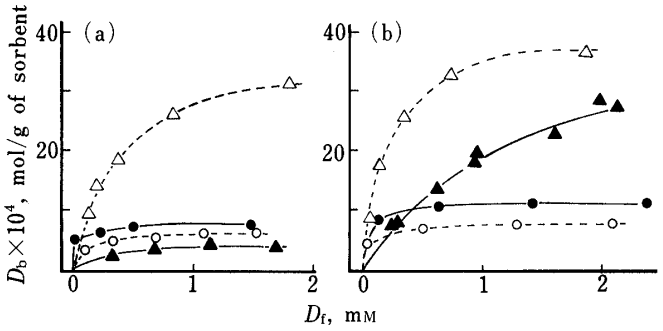

Fig. 2. Effect of Media on the Adsorption of (a) Cholate and (b) Deoxycholate at 37 °C

○, beads A in water; ●, beads A in bicarbonate buffer, pH 8.3—8.5; △, cholestyramine preparation in water; ▲, cholestyramine preparation in bicarbonate buffer.

TABLE IV. Effects of Various Substances on the Binding of Cholate in Water at 37 °C

Additive	% adsorbed[a)]		
	Powder A	Beads A mesh 28/48	Cholestyramine preparation
—	54±4	54±6	93±1
0.1 M NaCl	78±1	73±1	49±1
0.1 M Na_2SO_4	79±2	75±1	39±1
2 mM salicylate	59±1	57±1	89±3
1% BSA	73±1	74±3	59±1
2 mM theophylline	50±3	48±3	94±1
2.4 mM triolein	20±4	52±3	82±8

a) An amount of preparation equivalent to 20 mg of sorbent was equilibrated with 10 ml of 2 mM cholate in the presence and absence of an additive in water, pH 7.0—7.5. Mean ± S.D. (of 2 or 3 determinations).

fluid than medium 2. The high capacity of the resin in water for adsorption of C was dramatically reduced in the bicarbonate buffer. In the case of DC, the capacity of the resin preparation was not so much reduced by the presence of the inorganic salts.

Effects of Various Additives on the Adsorption of C

The effects of various ionic and nonionic substances were investigated on the adsorption of C by the three preparations in water. The results presented in Table IV show that the

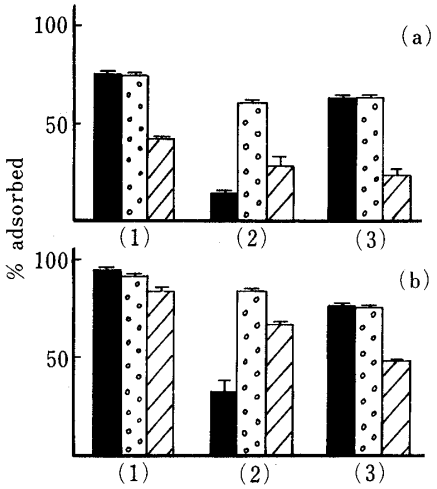

Fig. 3. Effects of Triolein and BSA on the Adsorption of (a) Cholate and (b) Deoxycholate at 37 °C in Bicarbonate Buffer, pH 8.3—8.5

The percent adsorbed was determined for an amount of each preparation equivalent to 20 mg of sorbent equilibrated with 10 ml of the buffer containing 20 μmol of bile salt.

(1), control; (2), in the presence of 2.4 mM triolein; (3), in the presence of 1% BSA.

■, powder A; ⊡, beads A, mesh size 28/48; ▨, cholestyramine preparation.

The vertical bar represents the standard deviation of the mean of 2 or 3 determinations.

TABLE V. Time Course of Adsorption from Medium 2 at 37 °C

Adsorbate	% adsorbed[a]			
	Time (min)	Beads A mesh 48/250	Beads A mesh 28/48	Powder A
Salicylate	5	94.2[b]	—	96.7[b]
	15	97.1[b]	—	99.4[b]
Cholate	10	71.1	49.6	88.9
	30	91.1	70.4	93.8
	60	94.1	83.5	95.7

a) An amount of preparation equivalent to 20 mg of sorbent was equilibrated with 10 ml of 2 mM cholate in medium 2.
b) Ref. 2.

adsorption by the resin preparation was greatly reduced by the presence of ionic substances, including bovine serum albumin (BSA) which exists as anions. On the other hand, the adsorption by activated carbon was enhanced by the presence of ionic substances including BSA, which is expected to bind bile salt anions,[10] and thereby to interfere with the adsorption by activated carbon. Theophylline, present as molecular species in water, did not greatly reduce the adsorption of C by activated carbon, in spite of the fact that powder A (or beads A) has an even greater adsorption capacity for theophylline than for C.[11] Triolein reduced the adsorption of C by powder A, but the adsorption by beads A and the resin preparation was not greatly influenced.

Figure 3 shows the effects of triolein and BSA on the binding of C and DC in the bicarbonate buffer. As expected from the preceding results, BSA reduced the binding of both bile salts to the resin preparation, but the adsorption by activated carbon was not increased by the presence of BSA. We have observed (results not shown) that the effect of BSA on the binding of C in water was pH-dependent, less binding being observed with increasing pH for both powder A and beads A. As for the effect of triolein, the adsorption of C and DC by powder A was greatly reduced by the lipid, which is well known to be adsorbed by activated carbon, probably through competitive binding. However, beads A essentially retained the adsorption capacity for bile salt anions in the presence of triolein.

Adsorption Rates

Adsorption rates of C are compared with those of salicylate in Table V. The rates of

adsorption of the larger anion, C (mol. wt. 409), were less than those of the smaller salicylate ion (mol. wt. 138) and the effect was more pronounced in beads A, the rate being inversely related to the size of the beads.

Discussion

The effects of physiologic anions on the adsorption of bile salt anions by activated carbon and cholestyramine appears to be opposite: they enhance the binding by the former, but retard that by the latter. In the case of cholestyramine–bile salt interactions, electrostatic interaction is reinforced by a secondary hydrophobic interaction,[12] and thus the retarding effect of physiologic anions is less for more hydrophobic dihydroxy bile salts than for the trihydroxy analogs. In the case of activated carbon, hydrophobic forces are considered to be mainly operative, and thus the dihydroxy analogs are more effectively adsorbed than the trihydroxy analogs. The anion-enhanced adsorption may be a kind of salting-out of bile salt anions at the surface of charcoal by physiologic anions. A similar effect was observed previously with adsorption of salicylate on activated carbon.[2,13,14] This is in agreement with the observation that for trihydroxy bile salts, beads A is a better sorbent preparation in the bicarbonate buffer than the cholestyramine preparation, while for dihydroxy analogs, the opposite relation holds.

Adsorption of bile acids (molecular form) is expected to be better by the beads than by the resin preparation. Thus, for the treatment of gastritis caused by the reflux of duodenal contents into the stomach, so-called bile reflux gastritis,[15] the beads are expected to be the agent of choice since Lindenbaum and Higuchi showed[16] that only strongly acidic taurine-conjugated bile acids are significantly adsorbed by cholestyramine at the gastric pH.

Although agar-encapsulated activated carbon powder generally retained the bile salt adsorption capacity of the powder, the rate of adsorption was less for the beads, being inversely related to the bead size. The reduction, however, is not so serious as to make the beads impractical for oral use. A definite advantage of the beads over the powder is that the hydrophilic agar confers selectivity for bile salts in the presence of a lipid which strongly interferes with bile salt adsorption by the powder.

The use of orally administered activated carbon for the treatment and prevention of atherosclerosis represents an entirely new, interesting, and safe approach. A palatable preparation of charcoal powder such as the beads reported here may serve as an alternative to cholestyramine therapy since its bile salt-sequestering mechanism is different from that of cholestyramine and the side-effects such as constipation and hyperchloremic acidosis associated with cholestyramine[4] are unlikely to occur with the agar-entrapped activated carbon.

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