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Endotoxin Binding by Charged and Uncharged Resins[1] (38895)

JAMES P. NOLAN, JOHN J. McDEVITT, AND GWENDOLYN S. GOLDMANN
(Introduced by C. Bishop)

Department of Medicine, The State University of New York at Buffalo and the Buffalo General Hospital, 100 High Street, Buffalo, New York 14203

Our laboratory has previously reported that cholestyramine resin tightly binds *E. coli* endotoxin, impeding its absorption through the isolated gut sac and modifying its toxicity (1). Since hemoperfusion through resin columns has been shown to be safe and effective in treating drug overdoses, the present study was undertaken to examine the capabilities of various exchange resins to bind endotoxin *in vitro*, and to prevent its systemic toxicity, with the anticipation that *in vivo* studies of the removal of circulating endotoxin could be attempted with the most suitable material.

Methods. Resins. (1) Analytic grade (A.G.) Dowex 1 chloride ion resins were obtained from Bio-Rad Laboratories, Richmond, CA. The X1, X2, X4, and X8 designations in this series reflect the percentage of divinylbenzene cross-linkage. The mesh size refers to the particle size range of the dry copolymer beads before ionic groups are attached as measured by U.S. Standard Mesh screens. (2) Amberlite XAD-2 resin was kindly supplied by Rohm and Haas, Philadelphia, PA. The mesh size was 20–50. (3) The activated charcoal used in these experiments was manufactured by Merck & Co., Inc., Rahway, NJ.

Endotoxin. For the binding studies, the same lot of purified lipopolysaccharide (*Escherichia coli* 026:B6, Difco Laboratories, Detroit, MI) was used throughout. The endotoxin was reconstituted to a 1.0 mg/ml solution in pyrogen-free isotonic saline and labeled with ^{51}Cr after the method of Braude (4). For the toxicity studies, in addition to unlabeled *E. coli* 026:B6 endotoxin, purified *Salmonella typhosa* 0901 lipopolysaccharide (Difco) was reconstituted and used.

Rats. Female Holtzman rats weighing 150–175 g were used for the toxicity studies. Food and water were allowed until the injection of the filtrate, at which time both were removed until the time of sacrifice 24 hr later.

Design of study. (a) *In vitro resin binding of labeled endotoxin.* Five grams of resin was weighed and swelled in isotonic saline overnight at room temperature. The fines were decanted twice with fresh saline. After the second wash, the resin was equilibrated with 9.0 ml isotonic saline to a temperature of 37° and 1 ml of the 1.0 mg/ml ^{51}Cr-labeled endotoxin was added. The mixture was incubated and agitated for the specific time and immediately filtered through Whatman No. 1 paper. The separated resin was placed in plastic tubes and counted in a gamma scintillation counter. The eluate and dampened filter paper were similarly counted. The amount of endotoxin bound was calculated as a percentage of the counts per minute (cpm) in the resin tubes to the total counts recovered. (The counts recovered consistently equaled 96–98% of the total counts added). The percentage unbound was calculated as the combined cpm in the eluate and filter paper. Each particular resin was tested separately twice and the results obtained were uniformly within 3% of each other.

(b) *Toxicity studies.* In order to demonstrate binding of the toxic moiety of endotoxin, unlabeled lipopolysaccharides were batched with 5 g of Dowex 1-X2 (200–400) and 5 g of activated charcoal as described in (a). After incubation and passage through the filter paper, 1 ml of supernatant was injected intraperitoneally to each of a group of rats. A milliliter of endotoxin solution not mixed with resin was injected into each of a control group of animals. At the end of 24 hr, the rats were etherized lightly, bled by

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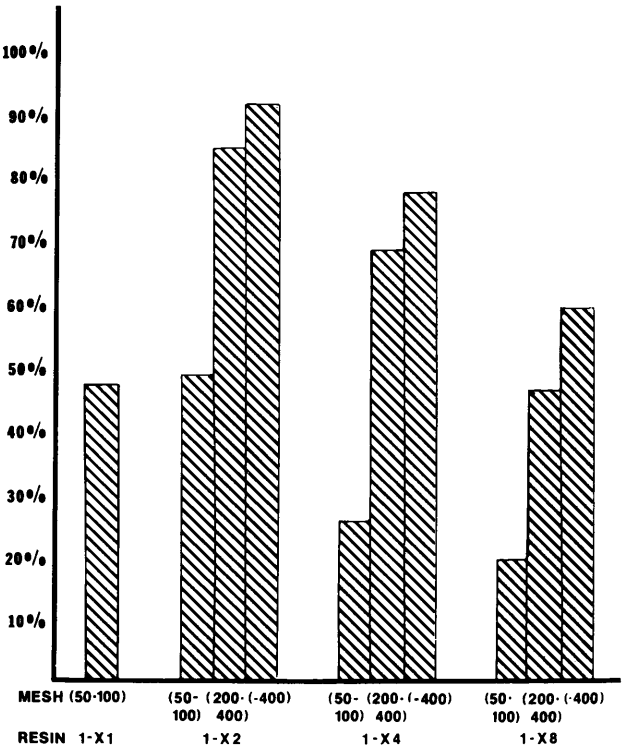

FIG. 1. Endotoxin binding by Dowex 1 resins. The bars represent the percentage of total cpm of labeled endotoxin recovered in the resin fraction after 15-min incubation at 37°. The mesh size for each percentage of cross-linkage is compared in its binding ability.

aortic puncture, and sacrificed. Serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) levels were measured in Sigma-Frankel Units.

Results. Figure 1 compares the binding ability of the Dowex 1 series. It can be seen that while some binding occurs in all the resins, the most affinity for endotoxin occurs when there is 2% cross-linkage with divinylbenzene (91% with 1-X2 (-400 mesh) and that avidity decreases to 77% and 59% as the cross-linkage percentage is increased to 4% and 8%. In addition, mesh size seems critical, with the 50–100 mesh binding the least amount and the finer -400 mesh binding the most. Dowex 1-X2, however, binds almost as effectively at the 200–400 mesh as at the -400 mesh (84% and 91%). Figure 2 depicts the binding ability of the 1-X2 resin with two other materials known to have strong binding properties. Amberlite XAD-2 is a polystyrene resin that like charcoal is felt to cause molecular adsorption by hydrogen binding. When compared to a strong anion-exchange resin like Dowex 1-X2, the XAD-2 is only about 30% as effective in binding the lipopolysaccharide, but the activated charcoal shows equal avidity for this heterogeneous molecule, both binding approximately 90% of the endotoxin in 15 min. Table I shows that almost all the binding to the 1-X2 occurs within 15 min, with less than an additional 10% being bound after a 60-min incubation. In the 1-X8 series, the added incubation time significantly improves binding with the finer meshes. In Table II, the capacity of varying amounts of resin to bind 1 mg of endotoxin in 10 ml of saline is compared. While 0.5 g binds only 28%, 2 g binds 87% and 5 g 91%. The amount of endotoxin removed by 5 and 10 g of Dowex 1-X2 resin is virtually the same. Table III compares the toxicity of *E. coli* and *S. typhosa* endotoxin before and after incubation with Dowex 1-X2 resin and

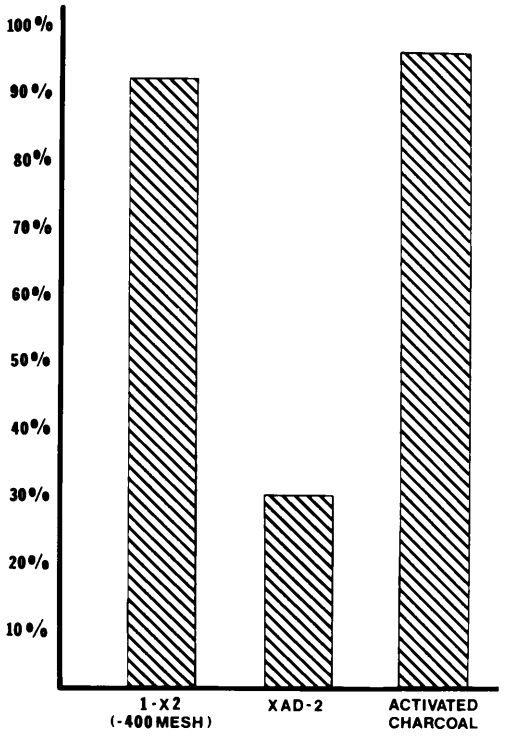

FIG. 2. Comparison of endotoxin binding by Dowex 1-X2, XAD-2, and activated charcoal. The bars represent the percentage of endotoxin bound after 15-min incubation at 37°.

TABLE I. EFFECT OF TIME OF INCUBATION ON RESIN BINDING OF ENDOTOXIN.[a]

Resin (5 g dry wt)	% Bounds (min) 15	60
1-X2 (50–100)	48%	54%
(200–400)	84%	89%
(−400)	91%	95%
1-X4 (50–100)	25%	35%
(200–400)	68%	76%
(−400)	77%	83%
1-X8 (50–100)	19%	19%
(200–400)	46%	67%
(−400)	59%	86%
Activated charcoal	95%	95%
XAD-2	29%	33%

[a] Percent removal of 1 mg of labeled *E. coli* 026 endotoxin in 10 ml of isotonic saline after 15 min and 60 min of resin incubation at 37°.

TABLE II. EFFECT OF RESIN WEIGHT ON ENDOTOXIN BINDING BY DOWEX 1-X2.[a]

Resin amount	Endotoxin added	Incubation time	% Bound
0.5 g	1 mg	15	28
1.0 g	1 mg	15	51
2.0 g	1 mg	15	87
5.0 g	1 mg	15	91
10.0 g	1 mg	15	97

[a] The ability of varying wet weights of Dowex 1-X2 (−400 mesh) resin to bind 1 mg of labeled *E. coli* 026 endotoxin in 10 ml of isotonic saline.

activated charcoal. The mean SGOT and SGPT values are significantly lower for both endotoxin solutions when pretreatment with the resins are carried out prior to injection, confirming their ability to effectively remove endotoxin from solution and prevent its toxicity.

Discussion. Dowex 1 resin is a strongly basic anion-exchange resin composed of quarternary ammonium exchange groups attached to a styrene-divinylbenzene polymer lattice, and is capable of exchanging anions including those of acidic, basic, and neutral salts. The 1-X2 resin (cholestyramine) has been shown to bind effectively many substances including bile acids (5) and drugs (6). In our previous study (1), not only was intraintestinal binding of endotoxin demonstrated, but importantly, this binding prevented the toxic tissue effects of the lipopolysaccharide. When a mixture of 0.25 mg of *E. coli* endotoxin in 1 ml of saline and 50 mg of cholestyramine was injected intraperitoneally into a series of rats, the average serum glutamic pyruvate transaminase value at sacrifice 24 hr later was 76 units. A similarly treated group in which the endotoxin was mixed with Dowex 1-X8 resin (50–100) mesh) produced an average transaminase value of 466 units. The present study confirms that Dowex 1-X2 is superior to 1-X4 and 1-X8 in causing *in vitro* binding of an aqueous solution of labeled endotoxin. Finer particle diameter as reflected by mesh size increased the binding of endotoxin regardless of the percentage of cross-linkage. It has similarly been shown that increased milling of cholestyramine increases the binding sites available for bile acids (7). Activated charcoal appears to bind endotoxin extremely

TABLE III. TOXICITY IN RATS OF RESIN-TREATED ENDOTOXIN SOLUTIONS.[a]

Resin	Endotoxin	Dose (mg/ml)	No. rats	Mean SGOT	Mean SGPT
1-X2 (200–400)	*E. coli* 026	0.75	6	76[b]	13[b]
None	*E. coli* 026	0.75	6	1620	872
Charcoal	*E. coli* 026	0.75	6	88[b]	35[b]
None	*E. coli* 026	0.75	6	1620	872
1-X2 (200–400)	*S. typhosa* 0901	1.0	5	75[c]	16[c]
None	*S. typhosa* 0901	1.0	5	812	955
Charcoal	*S. typhosa* 0901	1.0	5	170[c]	93[c]
None	*S. typhosa* 0901	1.0	5	812	955

[a] Serum transaminase elevations 24 hr after ip injection of endotoxin solutions treated and not treated with Dowex 1-X2 and charcoal.

[b] $P < 0.025$.

[c] $P < 0.1$.

well also but XAD-2 is relatively ineffective. Toxicity studies in rats confirm the ability of resins to bind labeled endotoxin *in vitro*. Transaminase values are significantly lower in rats given the filtrate incubated with Dowex 1-X2 and charcoal. In addition, these studies demonstrate that the ability to bind endotoxin is not limited to *E. coli*, but is just as effective in lipopolysaccharide from *S. typhosa*.

The techniques of hemoperfusion with both Dowex 1 resins and activated charcoal are well established. Nealon and his colleagues used anion-exchange resins to treat barbiturate poisoning in dogs (2) and Rosenbaum *et al.* used resin hemoperfusion for acute drug intoxication (3). Willson and his associates perfused Dowex 1 with benefit in acute liver failure in dogs, presumably by removing toxic protein-bound metabolites (8). Hemoperfusion through activated charcoal has been shown to remove water-soluble molecules of intermediate size better than standard hemodialysis (9), and has been used for the treatment of uremia (10) and for fulminant hepatic failure (11).

Endotoxemia, particularly of intestinal origin, may be of critical importance in the manifestations of septic and irreversible hemorrhagic shock. The results of the present study would suggest that Dowex 1-X2 of suitable mesh size and/or activated charcoal may be excellent materials to study the effects of binding circulating endotoxin.

Summary. Cholestyramine (Dowex 1-X2), a strongly basic anion-exchange resin, has previously been shown to bind bacterial endotoxin, preventing both its toxicity and intestinal absorption. Because hemoperfusion through charged and uncharged resins is practical, a study was undertaken to test the endotoxin-binding characteristics of a number of resins. The resin to be tested was washed and swelled overnight, and 1 mg/ml of ^{51}Cr-labeled endotoxin was added and the mixture agitated and incubated at 37° for a specific time period. In the Dowex 1 series, the 1-X2 was superior to the 1-X4 and 1-X8 in its ability to bind *E. coli* endotoxin, removing about 90% from solution in 15 min. Increasing mesh size seemed to offer more binding sites for each Dowex 1 resin. Activated charcoal adsorbed about 90% of the endotoxin also, but Amberlite XAD-2 showed little binding capacity. Injection of filtrate from unlabeled *E. coli* and *S. typhosa* resin-treated solution into rats, demonstrated that both Dowex 1-X2 and activated charcoal prevented the transaminase rise noted in animals injected with solutions not so treated. It is concluded that Dowex 1-X2 resin and activated charcoal efficiently remove endotoxin *in vitro*, and may offer a unique method for removing circulating endotoxin *in vivo*.

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