Interaction of Antihistamines with Hydrocolloids

By HORACE D. GRAHAM and YEDE MARIE BAKER†

Antihistamines of various types form highly insoluble complexes with carrageenan and other sulfated hydrocolloids. The carboxylic acid type hydrocolloids and the proteins, casein and gelatin, formed less insoluble complexes; the neutral polysaccharides and agar (supposedly sulfated) formed gels in the presence of the antihistamines. Pyrathiazine, chlorcyclizine, pyrilamine, and pyrrobutamine formed more insoluble complexes than thenyldiamine, methapyriline, benadryl, and thonzylamine when mixed with carrageenan. The interaction was accelerated by increased temperature up to 75°, was not appreciably affected by pH within the range of pH 2.75 to 6.55 and, at 45°, reaction times of 5–70 minutes produced no essential difference in the amount of benadryl hydrochloride bound per unit of carrageenan (Seakem type 5).

HYDROCOLLOIDS of various types are being added to pharmaceutical products with increasing frequency. Since many of these hydrocolloids are polyanionic, they can enter into strong interactions with cationic drugs such as many of the antihistamines and may affect drug insolubilization, appearance, stability, and prolongation of activity.

Kennon and Higuchi (1) have studied the interaction of benadryl and pyribenzamine hydrochlorides with sodium carboxymethylcellulose (CMC), one of the relatively weaker binders. These studies were undertaken to examine the interaction of a broad spectrum of hydrocolloids with several antihistamines, since any insoluble product formed through such interactions may be of value as sustained release form of the drugs, a topic which has been receiving much attention recently (2–5).

EXPERIMENTAL

Antihistamines.—Pyrrobutamine, pyrilamine maleate, WY 2149, benadryl, pyrathiazine, chlorcyclizine, phenergan, thenyldiamine, methapyrilene, thonzylamine, triprolidine, antistine, and pyribenzamine hydrochlorides were used as received from the various manufacturers and were made up in double distilled water.

The hydrocolloids, other reagents, and equipment and the experimental details have been previously described (5). Hypnean was prepared according to Smith and Montgomery (6).

Where precipitation did not occur, the equilibrium dialysis technique (1, 7) was employed and binding data were obtained using the technique of Hughes and Klotz (8).

The degree of dissociation of the polyelectrolytes and the significance of the Donnan effects were ascertained by the technique of Kennon and Higuchi (1).

Quantitative Determination of the Antihistamines.—For determining the amount of free antihistamine in the experimental systems, the color reactions of Osol and Sideri (9) and the ultraviolet absorption characteristics outlined by Kleckner and Osol (10) were exploited.

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RESULTS AND DISCUSSION

The sulfated and carboxylic acid type polysaccharides and the proteins formed insoluble complexes with all of the antihistamines tested, while the neutral polysaccharides and agar (supposedly sulfated) produced a gel rather than a precipitate. The antihistamines varied in their ability to precipitate the hydrocolloids and—if carrageenan (Seakem type 5) is taken as the representative hydrocolloid—the relative tendencies of benadryl, pyrrobutamine, pyrathiazine, chlorcyclizine, phenergan, pyrilamine, thenyldiamine, methapyriline, and thonzylamine to form precipitates with this hydrocolloid were calculated to be 1.0, 3.65, 3.54, 1.98, 2.54, 0.96, and 0.28, respectively. Table I and Fig. 1 show the maximum binding capacities of several hydrocolloids for many of the antihistamines used. The high binding capacity of carrageenan is clearly indicated. The maximum binding capacity of furcellaran is slightly less than one-half that of carrageenan, probably a reflection of the sulfate content of these two phytocolloids. CMC, sodium alginate, pectin, and quince seed mucilage differed only slightly in their maximum binding capacities for the antihistamines listed. Of the hydrocolloids listed, locust bean gum and gum guar exhibited the lowest binding capacities for the antihistamines.

Figures 2 and 3 show the extent of the Donnan effects on the binding of several of the antihistamines by CMC and carrageenan (Seakmen type 5). The degree of dissociation of CMC was found to be 61.6% and agrees well with the value of 60% reported by Kennon and Higuchi (1). Carrageenan (Seakmen type 5) was found to be 79.5% dissociated. Harwood (11) calculated the ionization of a 1.5% solution of this hydrocolloid to be 59% and, assuming a molecular weight of 1000, calculated the degree of ionization of a 0.01 *N* solution to 63.4% at 25°. The drastic deviations from the theoretical Donnan-only behavior, in all cases, suggest that the drug cations are bound by the hydrocolloid anions by other mechanism(s). This is substantiated by the gross observations where turbidity occurred when most of the antihistamines were mixed with the hydrocolloids and by the data (Table I) for the maximum binding capacities. When dilute systems of gum tragacanth and sodium alginate were similarly treated, corresponding great deviations from the theoretical curve were observed, again supporting the gross observations and the binding data. Since the antihistamines are cationic in nature, salt formation is probably the most pre-

TABLE I.—MAXIMUM BINDING CAPACITY OF HYDROCOLLOIDS FOR ANTIHISTAMINES, MICROMOLES OF ANTIHISTAMINE BOUND PER GRAM OF HYDROCOLLOID

	Antihistamine				
	Benadryl HCl	Pyrrobutamine	Pyrathiazine HCl	Phenergan HCl	Pyrilamine Maleate
Carrageenan	5,520	20,000	18,640	9,840	13,800
Furcellaran	3,000	9,800	8,987	5,675	6,800
CMC	820	2,680	2,365	1,300	2,020
Sodium alginate	960	2,600	2,300	1,656	2,600
Pectin	801	2,400	2,265	2,160	2,350
Gum tragacanth	662	1,987	1,740	1,060	1,232
Gum arabic	340	1,086	884	640	740
Quince seed mucilage	878	2,260	1,984	1,590	1,960
Locust bean gum	320	301	304	267	470
Gum guar	300	304	265	285	340

dominant reaction occurring between the polyanionic polysaccharides and the drugs, but hydrogen bonding and van der Waals forces probably also contribute to the total binding.

Figure 4 shows the effect of pH and electrolytes on the binding of benadryl hydrochloride by carrageenan (Seakem type 5). Determinations of the amount of benadryl hydrochloride bound by carrageenan (Seakem type 5) in the presence of several salts revealed that the order of interference may be summarized as: trivalent > divalent > monovalent. Iron and aluminum are particularly interferring and will actually precipitate the hydro-

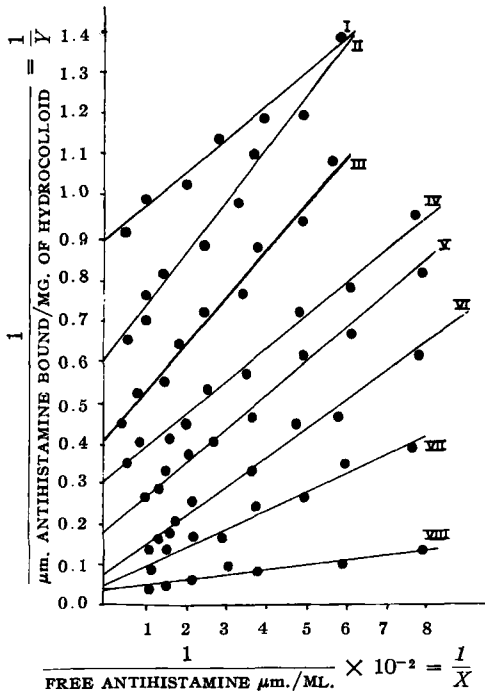

Fig. 1.—Plot of *1/y vs. 1/x* for limiting binding capacity of hydrocolloids for antihistamines. Data from studies at 27°C. in aqueous solutions. Key: I, pectin-benadryl hydrochloride; II, gum tragacanth-phenergan; III, sodium alginate—phenergan hydrochloride; IV, furcellaran—benadryl hydrochloride; V, carrageenan (Seakem type 5)—benadryl hydrochloride; VI, carrageenan (Seakem type 5)—pyrilamine maleate; VII, carrageenan (Seakem type 5)—pyrathiazine hydrochloride; VIII, carrageenan (Seakem type 6)—pyrrobutamine.

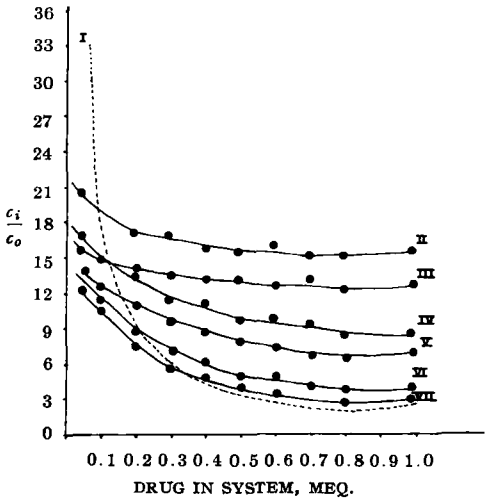

Fig. 2.—Illustration of the binding tendency of CMC toward antihistamines in aqueous solutions at 27°C. Key: I, Donnan; II, pyrrobutamine; III, pyrilamine; IV, pyrathiazine; V, phenergan; VI, benadryl; VII, pyribenzamine.

colloids. Reduced binding of the antihistamines by the hydrocolloids in the presence of electrolytes must be mainly because of the competition for the reactive sites on the antihistamine molecule.

The pH of the medium influences the interaction of the antihistamines with the hydrocolloids. In the alkaline range, the antihistamines will produce a turbidity in the absence of the hydrocolloids. Between pH 2.75 to 6.55 and, in distilled water, only slight differences in the amount of antihistamine bound per milligram of carrageenan (Seakem type 5) were observed. This constancy over such a wide range of pH would probably not hold for hydrocolloids such as CMC whose ionization and stability are severely affected at low pH levels of the medium. In studies on the effect of pH, hydrochloric acid was used to attain the desired acidity. If phosphate buffer is used, then the maximum strength (final molarity of the salt in the medium) must be 0.05 *M*. Beyond this, the amount of antihistamine bound per unit of hydrocolloid decreased, presumably because of the electrolyte effect (Fig. 4).

As the temperature of the interaction medium increased, the reaction between the hydrocolloid and the antihistamine increased, as gauged by the amount of antihistamine bound per milligram of

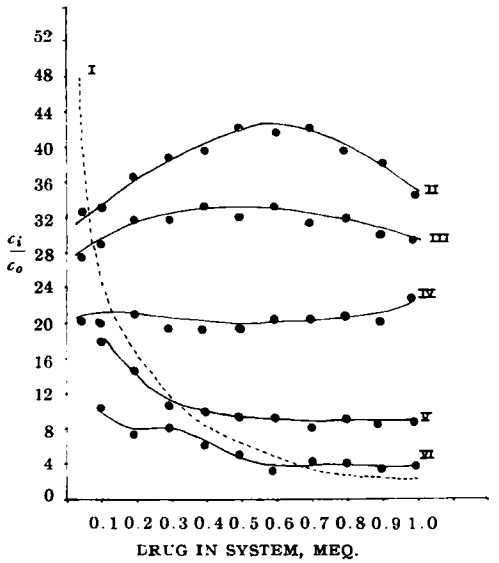

Fig. 3.—Illustration of the binding tendency of carrageenan (Seakem type 5) toward antihistamines in aqueous solutions at 27°C. Key: I, Donnan; II, pyrrobutamine; III, pyrathiazine; IV, pyrilamine; V, phenergan; VI, benadryl.

hydrocolloid. This held true up to 75°. Between 75–85°, there was no measurable difference in binding. Although 45° was not the optimum interaction temperature, it was selected to minimize the possibilities of hydrolysis if the reaction was carried out at higher temperatures.

At 45°, reaction times of 5–70 minutes produced no essential difference in the amount of benadryl hydrochloride bound per milligram of carrageenan (Seakem type 5).

Sustained or timed release of drugs from complexes has been receiving much attention recently (2, 4, 5); the interaction products of hydrocolloids and antihistamines may be of significance in this respect. Table II indicates the solubility of the benadryl-carrageenan and pyrilamine-carrageenan complexes in sodium chloride and hydrochloric acid. These data point to their promise as sustained-release forms of the drugs.

Antihistamines have a great number of pharmacological actions and, though *in vitro* studies may be inconclusive, they may throw some light on *in vivo* mechanisms. Judah (12), in his studies on the interaction of antihistamines *in vitro*, suggested that they prevented mitochondrial swelling by interactions with the mitochondrial membrane. Conceivably, substances closely related to some of the hydrocolloids used in these studies (*e.g.*, heparin, proteins, or chondroitin sulfate) could have been involved. Kobayashi (13) has discussed the binding of histamine by heparin with particular attention to the importance of the sulfate moiety of this compound in the binding process. Since the antihistamines are "histamine antagonists," the strong binding of the antihistamines by the sulfated hydrocolloids reflect the possible role of such types of compounds in the mechanism of action of the antihistamines. The role of proteins in the binding

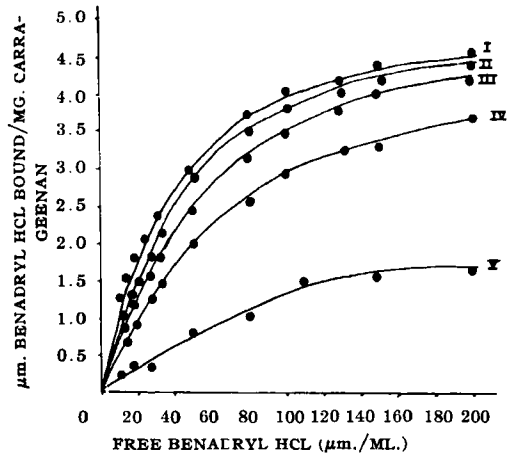

Fig. 4.—Effect of pH and electrolytes on the binding of benadryl hydrochloride by carrageenan (Seakem type 5) at 27°C. Key: I, distilled water; II, pH 2.75 to 6.55; III, sodium chloride (10^{-2} mole); IV, barium chloride (10^{-3} mole); V, ferric chloride (10^{-5} mole).

TABLE II.—SOLUBILITY OF BENADRYL- AND PYRILAMINE-CARRAGEENAN[a] COMPLEXES IN SODIUM CHLORIDE AND HYDROCHLORIC ACID AT 27°C.

	Benadryl Complex			Pyrilamine Complex		
	Bound antihistamine (%) released after					
	1 hr.	6 hr.	24 hr.	1 hr.	6 hr.	24 hr.
Sodium Chloride (Final Molarity)						
0.0	14.7	16.8	17.5	11.0	11.2	15.3
0.2	43.7	73.4	74.0	49.3	72.2	82.5
0.4	75.0	75.2	75.0	64.3	80.8	83.0
1.6	75.8	75.5	76.6	65.1	64.2	82.5
2.4	75.8	76.2	77.7	66.0	79.2	82.5
3.2	76.6	77.2	77.7	66.7	84.3	82.5
4.0	78.0	79.0	79.4	68.7	84.3	84.3
Hydrochloric Acid (Final Normality)						
0.00	14.7	15.8	17.5	10.2	10.5	15.0
0.01	29.5	57.6	68.4	58.0	58.0	71.0
0.10	63.2	66.8	71.7	62.0	63.0	84.0
0.20	72.2	72.4	72.2	67.0	67.3	84.0
0.50	72.0	72.2	71.7	75.0	71.0	81.0
1.00	71.8	72.0	70.0	75.0	75.0	89.0
2.00	72.0	72.4	70.0	74.0	75.0	89.0

[a] Seakem type.

of antihistamines is also of interest. Maietta (14, 15) has exploited such interactions in using the combined antigen-antihistaminic technique in shortening the treatment of hay fever.

These studies demonstrate that a wide variety of hydrocolloids and antihistamines form highly insoluble complexes which probably can serve as sustained-release forms of these drugs. Conceivably, soluble complexes which are probably formed in most of the systems studied also may be of pharmacological importance.

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Effectiveness of Antibacterial Agents Presently Employed in Ophthalmic Preparations as Preservatives Against *Pseudomonas aeruginosa*

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Seven chemical substances or combinations of these substances presently employed as preservatives in ophthalmic solutions were studied to note their effectiveness as antibacterial agents against 13 different strains of *Pseudomonas aeruginosa*. New *in vitro* methods were devised. Among the latter were techniques to (*a*) differentiate between bacteriostatic and bactericidal activities and (*b*) determine the sterilizing time for each antibacterial agent. The methods presented here have several advantages over those previously employed and those now in use. An *in vivo* procedure was also employed in evaluating these chemical agents to note whether the findings were in agreement with the final results obtained in the *in vitro* studies. The following chemicals were examined: chlorobutanol, benzalkonium chloride, thimerosol, combinations of methyl and propylparaben, phenylmercuric nitrate, phenylethyl alcohol, and polymyxin B sulfate.

BECAUSE OF THE incidence and seriousness of *Pseudomonas aeruginosa* (*Ps. aeruginosa*) infections resulting from the use of contaminated ophthalmic solutions (1–11), various workers have critically investigated the antibacterial agents employed as preservatives in such preparations. The findings of these workers have been contradictory (12–18). The *in vitro* procedures employed in evaluating the effectiveness of the antibacterial agents have been challenged. The following observations are noted. There is need for methods which will determine the effectiveness of (*a*) antibacterial agents used as preservatives in ophthalmic solutions against *Ps. aeruginosa;* and (*b*) substances which are capable of inactivating or inhibiting the antibacterial action of the preservatives used.

The purpose of this paper is to report on studies which were performed in an effort to develop *in vitro* methods more effective than those employed at present and which will establish the efficiency of these antibacterial agents as preservatives in ophthalmic solutions against *Ps. aeruginosa.*

GENERAL CONSIDERATIONS

In devising methods for the evaluation of the effectiveness of antibacterial agents as preservatives in ophthalmic solutions, it is important to develop a technique which will determine the time required for such agents to produce sterility. Most methods which have been used to date did not always take this into consideration. Indeed, they usually measured only the bacteriostatic activity of preservatives.

Until recently, most workers employed a dilution technique to differentiate between the bactericidal and bacteriostatic activities of antibacterial agents. The basis for this procedure is the dilution of the

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