Interaction of Carrageenan and Other Hydrocolloids with Alkaloids II

Equilibrium Dialysis Studies

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The extent of binding of the alkaloids, quinine hydrochloride, hyoscyamine hydrochloride, caffeine, and theophylline by the hydrocolloids carrageenan, furcellaran, sodium carboxymethylcellulose, gum tragacanth, sodium alginate, pectin, locust bean gum, and gum acacia was studied. Data were obtained employing varying pH levels of the reaction system and temperatures of 4, 27, and 37°. A Langmuir-type plot of the data to obtain the limiting binding capacity of the hydrocolloids at infinitely high concentrations of the alkaloids, indicates that quinine hydrochloride was bound to the greatest extent by most of the hydrocolloids used. The order of binding was as follows: carrageenan > sodium carboxymethylcellulose > furcellaran > sodium alginate. Locust bean gum, pectin, gum tragacanth, and gum acacia bound this alkaloid considerably less. Approximately the same trend was exhibited with hyoscyamine hydrochloride, whereas caffeine and theophylline were bound to a much smaller, but nonetheless significant, extent than the other alkaloids tested.

IN A PREVIOUS communication, Graham and Thomas (1) demonstrated that several natural and some synthetic hydrocolloids are precipitated by alkaloids of different classes. However, in some cases, no visible precipitation was noted although, on a structural basis, significant interactions could have occurred. Such subtle reactions can be studied best by equilibrium dialysis, a technique which has been used extensively to elucidate interactions between proteins and other substances (2, 3), between anionic polysaccharides and detergents (4), and for studying several other reactions (5–13).

This report summarizes the data obtained from studies on the interaction of several alkaloids with hydrocolloids which are commonly used in pharmaceutical and food formulations. Evidence has been obtained that, even in cases where no visible turbidity was evident, considerable binding of the alkaloids by the hydrocolloids occurred.

EXPERIMENTAL

Materials

Alkaloids.—Quinine hydrochloride (2%), hyoscyamine hydrochloride (1%), caffeine (1%), and theophylline (0.5%).

All samples were prepared in double distilled water. Where necessary, solution was accomplished by gentle heating in a water bath. Representative alkaloids were selected on the basis of the following characteristics: Quinine hydrochloride: this alkaloid reacts strongly with most hydrocolloids producing visible turbidity in most cases. Hyoscyamine hydrochloride: at low concentrations, this alkaloid produces little or no visible turbidity when mixed with the hydrocolloids used (1); however, at high concentrations (2–4%) ready precipitation occurs, especially with some of the sulfated hydrocolloids. Caffeine and theophylline: these alkaloids do not produce visible turbidity with any of the hydrocolloids used.

Hydrocolloids.—These are listed in Table I. All samples were prepared and dialyzed as previously described (1). All calculations are expressed on a dry weight basis. The moisture content of each hydrocolloid was determined by drying each sample to constant weight in a vacuum oven at 80°.

Equipment

Beckman DU spectrophotometer, Beckman pH meter, model G, 30-ml. Pyrex, glass-stoppered test tubes, 50-ml. acid buret, Burrell wrist-action shaker, refrigerated room at 4°, dialysis membrane—27/32 dialysis tubing, seamless cellulose, Fisher Scientific Co.

Procedure

Preparation of the Dialysis Bags.—Twelve-inch strips of the dialysis membrane were cut with as much uniformity as possible and 12-in. lengths of string were used to tie the bags. The strips of dialysis membrane and string were thoroughly washed in tap water, soaked in, and finally rinsed several times with distilled water. Dialysis bags were constructed by tying one end as tightly as possible, placing the hydrocolloid dispersion in the bag and then tying the open end securely.

Dialysis and Determination of the Amount of Alkaloid Bound by the Hydrocolloids.—The experimental regimen was essentially that employed by Klotz, *et al.* (12), and more recently by Patel and Kostenbauder (10). A 25-ml. quantity of the stock solution containing 10–1000 mg. of the particular hydrocolloid was placed in the dialysis bag, the open end was tied securely, and the bag placed in a wide-

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TABLE I.- LIMITING BINDING CAPACITY (μM./GM.) OF HYDROCOLLOIDS FOR VARIOUS ALKALOIDS

Hydrocolloids	Alkaloids: Quinine HCl	Hyoscyamine HCl	Caffeine	Theophylline
Carrageenan (SeaKem type 5)	303.0	189.0	58.8	54.6
Sodium carboxymethylcellulose	232.0	111.1	53.0	38.4
Furcellaran	200.0	142.8	44.8	37.0
Sodium alginate	200.0	140.0	40.0	35.0
Pectin	169.1	136.8	38.0	33.0
Gum tragacanth	88.0	46.0	26.0	30.4
Locust bean gum	50.5	44.4	38.0	33.0
Gum acacia	47.0	26.0	16.0	18.6

mouth bottle containing 75 ml. of the particular alkaloid. The bottles were placed on a Burrell wrist-action shaker and reaction allowed to take place for 36 hours, an interval sufficient for the attainment of equilibrium. Experiments conducted using 300-ml. Erlenmeyer flasks, 50 ml. of the hydrocolloid dispersion, and 50 ml. of outside solution gave essentially similar results. However, the use of wide-mouth bottles as described offers the possibility of keeping the bathing fluid in better contact with the bag surface. Moreover, during the shaking, such bottles are less subject to breaking as was experienced with the flasks in some instances.

At the end of the reaction period, the outside solution was poured into a 100-ml. volumetric flask and the contents made up to volume with double distilled water. One milliliter of this solution was then used for determination of the free alkaloid in the solution on the outside of the dialysis bag. In several instances, the solution inside the dialysis bag was also analyzed for its content of both free and total alkaloid. In such cases, the solution was also accurately made up to volume. A control, which differed from the primary bag only in that the former contained distilled water instead of hydrocolloid, was included. This permitted corrections for any alkaloid bound by the dialysis membrane.

The amount of alkaloid bound by a given quantity of hydrocolloid was calculated by subtracting the sum of the free alkaloid in the system and the alkaloid bound by the bag from the total amount of alkaloid added.

Methods Used in Determining the Amount of Free Alkaloid

Routinely, the amount of free alkaloid was determined by the colorimetric dichromate-sulfuric acid method of Graham and Thomas (14). Essentially, the procedure is as follows: 1 ml. of the solution to be assayed is placed in a Pyrex, ground glass-stoppered test tube, 1 ml. of a 5% solution of potassium dichromate is added, and the mixture is incubated for 5 minutes in a constant temperature water bath at 30°. At the end of this incubation period, 8 ml. of concentrated sulfuric acid is added, the tube is swirled gently to mix the contents well, and then placed in an ice water bath for 20 minutes. Duplicate control tubes containing all ingredients except the alkaloid are included and are treated similarly. The color developed is measured at 650 mμ with a Coleman Universal spectrophotometer, model 14, or with a Beckman DU spectrophotometer, and the amount of alkaloid present determined by reference to a standard curve.

Since the alkaloids used absorb strongly in the ultraviolet region, the concentration of alkaloid in the solutions both inside and outside the dialysis bag, after dilution with distilled water, was determined, in some cases by ultraviolet spectrophotometry using the Beckman DU spectrophotometer. Quinine hydrochloride was determined at 331 mμ (9), caffeine at 272 mμ (15), and theophylline at 276 mμ (16).

Under the experimental conditions employed, excellent agreement was obtained whether the concentration of alkaloid was determined only in the solution outside the dialysis bag or both in the solution inside and outside of the dialysis bag. Similar excellence in agreement of values obtained by analyzing both the solution inside the dialysis bag and the solution outside the dialysis bag, has been noted by Patel and Kostenbauder (10) and by Ehrenpreis (3). Additionally, as seen from Table II, good agreement in results were obtained by both the colorimetric dichromate-sulfuric acid method and the ultraviolet absorbance method. In view of these facts, the dichromate-sulfuric acid method was used routinely.

Equilibrium Dialysis Calculations

Essentially, the method of calculation and the assumptions involved are similar to those outlined by Patel and Kostenbauder (10) in their studies on the binding of parahydroxybenzoic acid esters by Tween 80. In principle, the thin semipermeable

TABLE II.- AMOUNT OF ALKALOIDS BOUND BY HYDROCOLLOIDS AT 27° AS DETERMINED BY THE DICHROMATE-SULFURIC ACID (DS) METHOD AND BY THE ULTRAVIOLET (UV) SPECTROPHOTOMETRIC METHOD

Hydrocolloid	μm. of Alkaloid Bound per Gm. of Hydrocolloid at 27°: Quinine Hydrochloride DS Method	Quinine Hydrochloride UV Method	Caffeine DS Method	Caffeine UV Method	Theophylline DS Method	Theophylline UV Method
Carrageenan (SeaKem type 5)	256.0	262.0	40.0	42.2	48.6	48.0
Sodium carboxymethylcellulose	206.0	216.0	33.6	35.6	36.4	35.8
Furcellaran	198.0	189.4	38.0	40.4	29.6	29.0
Sodium alginate	175.0	186.2	26.8	29.6	24.8	24.2
Pectin	150.1	153.2	20.0	24.5	23.2	24.8
Gum tragacanth	88.0	74.6	26.0	24.8	21.6	20.7
Locust bean gum	50.0	48.2	25.0	26.2	23.4	22.9
Gum acacia	42.0	43.4	14.4	15.4	18.0	17.6

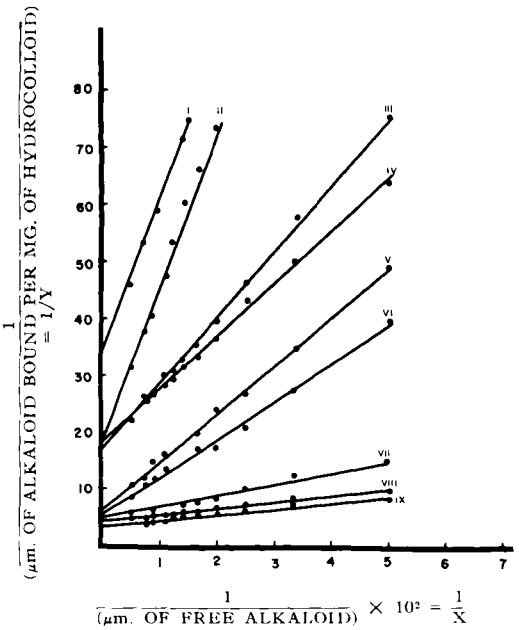

Fig. 1.—Plot of $1/y$ *vs.* $1/x$ for limiting binding capacity of hydrocolloids for various alkaloids, data from studies done at 27° in aqueous solutions. I, Locust bean gum-theophylline; II, sodium carboxymethylcellulose-caffeine; III, carrageenan-caffeine; IV, carrageenan-theophylline; V, furcellaran-hyoscyamine hydrochloride; VI, carrageenan-hyoscyamine hydrochloride; VII, sodium alginate-quinine hydrochloride; VIII, sodium carboxymethylcellulose-quinine hydrochloride; IX, carrageenan-quinine hydrochloride.

membrane permits free passage of the alkaloid but not of the hydrocolloid. The alkaloid placed on the outside of the bag will diffuse inwardly to react with the hydrocolloid placed on the inside of the bag. At equilibrium, the activity of the alkaloid will be equal on both sides of the membrane. For a sufficiently dilute solution it can be assumed that the concentration of the alkaloid on both sides of the membrane will be essentially the same. Therefore, if the macromolecule is placed on one side of the membrane and the concentration of alkaloid on the opposite side is determined, it becomes possible to determine the amount of free or unbound alkaloid in equilibrium with the hydrocolloid. The procedure employed is outlined symbolically: let A_t = total alkaloid added; let A_b = alkaloid actually bound by the hydrocolloid; let A_m = alkaloid bound by membrane; let $A_{f.o.}$ = free alkaloid outside of bag; let $A_{f.i.}$ = free alkaloid inside of bag = $A_{f.o.}$ Then, $2A_{f.o.}$ = total free alkaloid in system. Then $A_b = A_t - (2A_{f.o.} + A_m)$. In these calculations, the Donnan effects were neglected.

Influence of Temperature and pH on the Binding Process

In order to characterize the reactions more thoroughly, the influence of temperature and pH on the binding of alkaloids by the hydrocolloids was investigated.

A constant quantity of the particular hydrocolloid was allowed to equilibrate for 36 hours against an excess of each alkaloid according to the general procedure at 4, 27, and 37°. For the experiments at 4°, all reagents were preincubated at this temperature; the dialysis systems were set up and removal of aliquots for determination of the free alkaloid on the outside was done in the cold room. This precluded any experimental errors due to temperature changes which would result through removal of the flasks from the cold room.

Possible variations in the extent of binding at 27° caused by differences in the pH of the system were investigated by dialyzing 200 mg. of each hydrocolloid against an excess of each alkaloid. The alkaloids and hydrocolloids were suspended in 0.02 *N* phosphate buffers of varying pH levels. At the end of the dialysis period, the pH of the solutions inside the bag and outside the bag was checked with a Beckman model G pH meter. Quinine hydrochloride precipitates readily in the presence of salts, hence the quinine hydrochloride system was not investigated.

RESULTS AND DISCUSSION

Estimation of the Degree of Binding from the Equilibrium Dialysis Data.—Variations in the degree of binding of the various alkaloids by the hydrocolloids are shown in Table I and Fig. 1. The limiting or maximum binding capacity per unit of hydrocolloid at infinitely high levels of the alkaloids was ascertained by plotting the reciprocal of the amount of alkaloid bound per mg. of hydrocolloid (i/y) *vs.* the reciprocal of the amount of free alkaloid present (i/x). The y intercept is the reciprocal of the limiting quantity of the alkaloid bound per mg. of hydrocolloid. This approach has been used extensively by Kennon and Higuchi (9) and Patel and Kostenbauder (10) in studying the interaction of drugs with polyelectrolytes.

Figure 2 is a plot of the amount of alkaloid bound *vs.* the free alkaloid in the system at 27° in aqueous solutions. Carrageenan, carboxymethylcellulose, furcellaran, sodium alginate, and pectin bound quinine hydrochloride and hyoscyamine hydrochloride to a considerably greater extent than gum tragacanth, locust bean gum, and gum acacia. The so-called xanthine alkaloids, caffeine and theophylline, when mixed with the hydrocolloids used, produced no turbidity whatsoever. However, as shown by Fig. 2 and Table I, binding occurred. In defense of this observation, the work of Guttman and Athalye (17) may be cited. Here, quite stable complexes were formed between riboflavin and caffeine and theophylline. They postulated that the imidazole ring of the xanthine nucleus must be strongly involved. As in their studies, it may be postulated that although hydrogen bonding could possibly provide a primary linkage between the interactants, reinforcement by secondary van der Waals interactions involving other parts of the molecule could also have occurred. Studies on interactions of xanthine molecules with bovine serum albumin by Eichman, *et al.* (18), also indicate the capability of complex formation by caffeine and theophylline with organic molecules.

It is evident that the complexes formed by caffeine and theophylline with the hydrocolloids used are weaker than those complexes formed by quinine hydrochloride and hyoscyamine hydro-

chloride with the same hydrocolloids. This is particularly so with carrageenan, furcellaran, sodium carboxymethylcellulose, sodium alginate, and pectin, in which cases the interaction is primarily a salt formation.

Between 4° and 27°, the amount of each alkaloid bound by the hydrocolloids varies slightly. However, at 37° considerably greater binding was exhibited between the alkaloids quinine hydrochloride and hyoscyamine hydrochloride and the hydrocolloids carrageenan and sodium carboxymethylcellulose. See Table III. The increased binding at the higher temperatures could be due to a weakening of the sodium gegenion field around the polymer so that more drug-polymer interaction occurs. The reaction may be a complex one and besides ionic interactions, hydrogen bonding and secondary van der Waals interactions may occur. However, if van der Waals forces were the only or main factor involved, increased temperature would cause a decrease in interactions.

The interaction with caffeine and theophylline showed less variation with temperature within the range of 4–37°. This probably reflects the weak interactions which occurred as contrasted with quinine hydrochloride and hyoscyamine hydrochloride.

As seen from Table IV, the pH of the system had no marked effect on the extent of binding of the alkaloids by carrageenan. However, beyond either

TABLE III.—INFLUENCE OF TEMPERATURE ON THE BINDING OF ALKALOIDS BY HYDROCOLLOIDS

Hydrocolloid	μm. Alkaloid Bound per Gm. of Hydrocolloid 4°	27°	37°
	Quinine Hydrochloride		
Carrageenan (SeaKem type 5)	248.4	253.8	401.2
Carboxymethylcellulose	200.1	205.0	250.8
Gum tragacanth	91.6	90.4	94.6
Locust bean gum	48.4	49.2	49.8
	Hyoscyamine Hydrochloride		
Carrageenan (SeaKem type 5)	112.6	118.0	162.8
Carboxymethylcellulose	97.4	96.2	140.6
Gum tragacanth	40.8	45.2	51.6
Locust bean gum	38.2	39.35	40.8
	Caffeine		
Carrageenan (SeaKem type 5)	40.0	42.2	43.4
Carboxymethylcellulose	33.0	32.6	37.5
Gum tragacanth	26.4	27.2	30.2
Locust bean gum	25.2	25.8	28.8
	Theophylline		
Carrageenan (SeaKem type 5)	37.2	38.0	37.6
Carboxymethylcellulose	36.0	37.2	36.8
Gum tragacanth	31.6	31.4	32.6
Locust bean gum	24.0	23.4	22.8

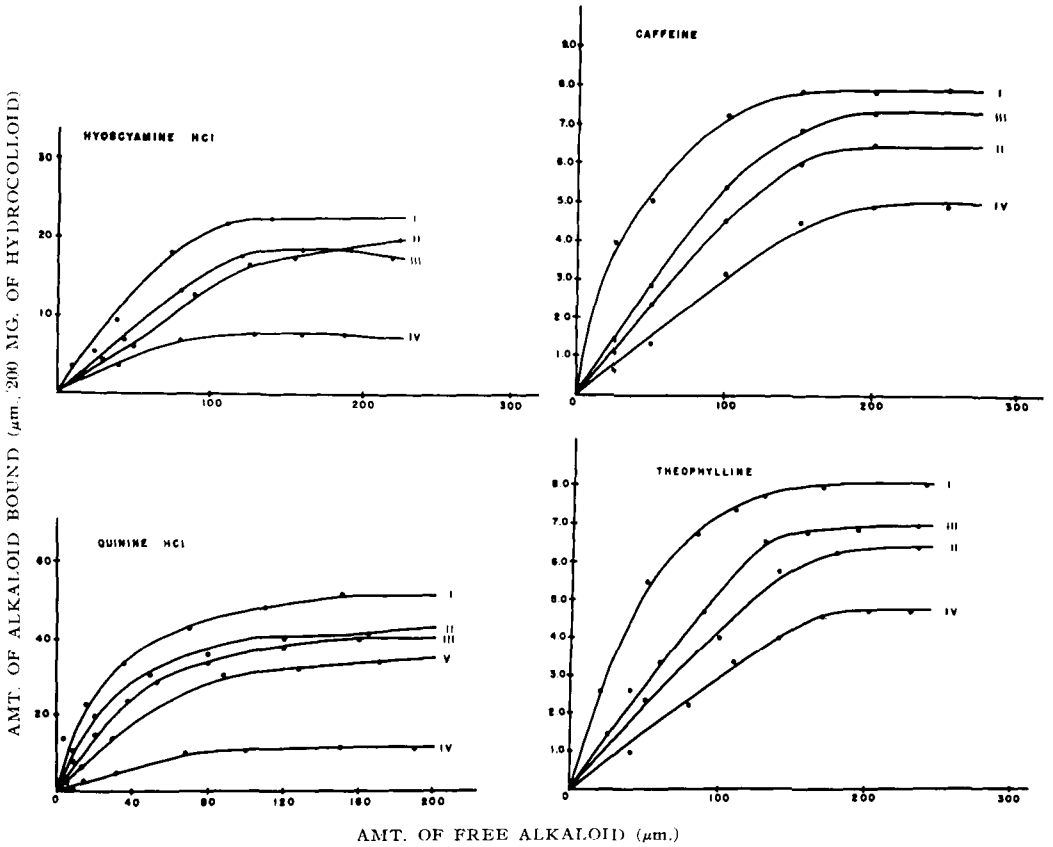

Fig. 2.—Binding of alkaloids by hydrocolloids at 27°. I, Carrageenan (SeaKem type 5); II, sodium carboxymethylcellulose; III, furcellaran; IV, locust bean gum; V, sodium alginate.

TABLE IV.—INFLUENCE OF pH ON THE BINDING OF ALKALOIDS BY HYDROCOLLOIDS AT 27°

	μm. Alkaloid Bound per Gm. of Hydrocolloid				
	pH 3.0	5.4	6.4	8.4	10.1
			Hyoscyamine Hydrochloride		
Carrageenan (SeaKem type 5)	105.8	118.6	120.0	115.6	110.0
Carboxymethylcellulose	84.6	94.2	97.6	93.8	90.0
Gum tragacanth	43.0	45.2	46.8	42.6	40.0
Locust bean gum	34.0	40.0	38.4	37.6	37.0
			Caffeine		
Carrageenan (SeaKem type 5)	40.6	42.8	40.3	41.4	39.8
Carboxymethylcellulose	30.4	33.0	32.8	30.6	32.5
Gum tragacanth	25.8	26.8	27.6	27.0	26.8
Locust bean gum	25.8	25.2	26.2	26.2	25.8
			Theophylline		
Carrageenan (SeaKem type 5)	37.8	40.0	38.4	39.2	39.0
Carboxymethylcellulose	37.4	35.0	35.4	34.6	38
Gum tragacanth	32.00	31.2	30.6	32.6	31.8
Locust bean gum	23.2	23.6	24.0	23.6	22.8

extreme, the stability of the hydrocolloid and, perhaps of the alkaloid, must be taken into serious consideration. Carboxymethylcellulose, in particular, would be relatively more severely affected than the sulfated hydrocolloids like carrageenan and furcellaran. At pH levels of about 3.0 or below, the ionization of sodium carboxymethylcellulose would be severely repressed and the hydrocolloid would precipitate (19). Data obtained at pH 3.0 for sodium carboxymethylcellulose may therefore be somewhat equivocal. As previously stated, studies in buffer solutions are complicated by precipitation of certain alkaloids, for example, quinine hydrochloride. Such side reactions, even if not visible, must not be ignored.

These studies bear significantly upon the problems which may arise when alkaloids and hydrocolloids are both incorporated into pharmaceutical and other preparations. Where rapid effects are required, the concentration of "free alkaloid" in the system and not the total alkaloid, is important. However, in many areas of nutritional (20) and pharmaceutical (21, 22) therapy, gradual or sustained release of the medicinal or nutritional constituent is desirable. Economy and convenience are two of the many advantages claimed for such sustained release. In this respect, hydrocolloids incorporated into the dosage forms may enhance the desired properties. Alternate effects, such as the inactivation of antimicrobial and other agents, could also be caused by the presence of such hydrocolloids as carrageenan and carboxymethylcellulose, as suggested by Eisman, *et al.* (23).

If binding data are available, it will be possible to approximate and perhaps determine the required concentration of a particular alkaloid or alkaloid-like agent necessary for eliciting a particular physiological function in the presence of specific hydrocolloids incorporated into the medicating mixture for emulsifying or other effects. Wagner (22), in an excellent review entitled "Biopharmaceutics: Absorption Aspects," paid critical attention to the interaction effects of adjuvants, such as gum acacia, when they are used in tablets and other preparations. Alterations in the surface area of the drug, if in solid forms, and inhibition of absorption through formation of a less soluble compound were cited as possibilities. The opposite of these effects may also be possible, hence the significance of such interactions in formulation work should not be overlooked.

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