Complex Formation Between Hydrocolloids and Tranquilizers and Hypotensive Agents

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Highly insoluble complexes are formed between hydrocolloids such as carrageenan and sodium carboxymethylcellulose and sodium alginate, and tranquilizers and hypotensive agents such as promazine hydrochloride, chlorpromazine hydrochloride, and reserpine. Meprobamate and hexamethonium chloride form much weaker complexes as evidenced by their failure to produce turbidity with several hydrocolloids tested. Using the promazine hydrochloride–carrageenan (Seakem type 5) interaction as the experimental system, it was shown that maximum interaction occurred at 45° and at a pH of 4.95 in a buffered medium. After intramuscular injection of the carrageenan–promazine hydrochloride complex into rabbits, free promazine was detected in the blood up to six days after administration as contrasted to the disappearance of the free form, similarly administered, after only 24 hours.

RECENTLY, chlorpromazine has gained much attention, not only because of its tranquilizing effect but also because of its interaction with sulfated mucopolysaccharides such as chondroitin sulfate and other biochemical compounds (1–4). Since chondroitin sulfate occurs in dentin and enamel and is produced in excessive quantities in abnormal calcification of the arteries and bone (5, 6), such interactions are important pathologically. In one specific study, Sobel and Burger (6) exploited it to study the mechanism of dentin formation. DeLuca and Kostenbauder (7) mentioned that drugs such as chlorpromazine, promethazine, and tetracaine hydrochlorides are bound by nonionic surfactants but, as far as can be ascertained, a comprehensive study of the binding of tranquilizers and hypotensive agents by hydrocolloids has apparently not been made.

Many tranquilizers, when given in large doses, may cause several undesirable side effects. On the other hand, if small compatible doses are given, the treatment must be repeated at frequent intervals. For these reasons, sustained release dosage forms of many medicinals have become of primary importance.

Chemically, sulfated hydrocolloids like carrageenan, furcellaran, fucoidan, etc., are similar to chondroitin sulfate, are readily available, and are very reactive. Therefore, it was decided to study the interactions of these natural compounds with tranquilizers and hypotensive agents. It was considered that if highly insoluble complexes could be obtained, these studies would provide some basis for the use of some of these hydrocolloids in the development of sustained release forms of the drugs. Since the hydrocolloids are natural, readily available, and highly innocuous products, their introduction into biological systems would present no medico-legal problems.

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EXPERIMENTAL

Reagents

Hydrocolloids.—These are listed in Table I. Unless otherwise stated, all dispersions were prepared as described by Graham and Thomas (8). All calculations were made on a dry weight basis.

Tranquilizers and Hypotensive Agents.—Promazine hydrochloride, 0.5% in distilled water; chlorpromazine hydrochloride, 0.5% in distilled water; Serpasil phosphate (reserpine), 0.1% in distilled water; veratrine sulfate, 0.5% in distilled water; Veriloid, 0.1% in dilute acetic acid; and veratrine alkaloid, 0.1% in dilute acetic acid.

Equipment

The following were used: a Coleman Universal spectrophotometer, model 14; a Beckman pH meter, model G; Pyrex glass-stoppered test tubes; and dialysis membrane, 27/32 dialysis tubing, seamless cellulose.[1]

Procedure

General Survey of the Interaction of Hydrocolloids with Tranquilizers and Hypotensive Agents.—In order to obtain some idea of the reaction of a broad spectrum of hydrocolloids with several tranquilizers and hypotensive agents, a screening program was undertaken. For this, 1–5 ml. of each hydrocolloid suspension was mixed with 2 ml. of the stock solution of each tranquilizer or hypotensive agent and the development or nondevelopment of turbidity noted. The results of this study are summarized in Table I.

Relative Reactivity of Hydrocolloids with Tranquilizers and Hypotensive Agents.—Since in some

[1] From Fisher Scientific Co

cases only slight precipitation was observed in the screening program, more information was sought on the relative reactivity of the hydrocolloids with tranquilizers and hypotensive agents. The sulfated hydrocolloids were previously observed to be precipitated most readily by the drugs in question. Moreover, the turbidity developed was shown to be proportional to the amount of hydrocolloid present. Based on this premise, a scheme was developed for comparing the reactivities of the hydrocolloids.

One to five ml. containing 1–10 mg. of the particular hydrocolloid was mixed with 2 ml. of the stock solution of the particular tranquilizer or hypotensive agent at 45°. The total volume in all cases was 10 ml., attained by adding distilled water where necessary. The reaction was allowed to proceed for 15 minutes, the tubes were cooled to room temperature and the turbidity developed measured at 400 mμ, with a Coleman Universal spectrophotometer, model 14. Since some solutions were appreciably viscous, accompanying control tubes were included by substituting 2 ml. of distilled water for the solution of tranquilizer or hypotensive agent. The actual turbidity (optical density) developed, therefore, was calculated as "the turbidity measured minus the turbidity (if any) of the accompanying control."

In order to compare the reactivities of the hydrocolloids, the turbidity produced by 1 ml. of carrageenan (Seakem type 5) with a particular tranquilizer or hypotensive agent was assigned an arbitrary value of 10. The turbidity produced by 1 mg. of any other hydrocolloid through interaction with the same tranquilizer or hypotensive agent was then compared with the value obtained for carrageenan (Seakem type 5) to give values recorded in Table II.

Influence of Variables on the Interaction.—Several variables such as temperature, pH, buffer normality, interaction time, and the presence of inorganic salts could possibly influence the binding of the drugs by the hydrocolloids. Therefore, these variables were systematically investigated. Unless otherwise stated, the carrageenan (Seakem type 5)-promazine hydrochloride system was used throughout the investigations on variables.

The influence of temperature was assessed by reacting 10 mg. of the hydrocolloid with 2 ml. of the drug solution at 30, 45, and 65° in a total volume of 10 ml. (Where reactions were done at 45 and 65°, the tubes were immediately cooled in an ice water bath after the desired reaction time.) After adjusting to room temperature, the turbidity (optical density) developed was measured at 400 mμ with the Coleman Universal spectrophotometer, model 14. For the establishment of the optimum

TABLE I.—SURVEY OF THE INTERACTION OF HYDROCOLLOIDS WITH TRANQUILIZERS AND HYPOTENSIVE AGENTS[a]

Hydrocolloids	Tranquilizers		Hypotensive Agents			
	Promazine Hydrochloride	Chlorpromazine Hydrochloride	Reserpine	Veriloid	Veratrine Alkaloid	Veratrine Sulfate
Group I.—Sulfated polysaccharides						
Carrageenans (Seakem types 4, 5, 7, 402)	+	+	+	+	+	+
Furcellaran	+	+	+	+	+	+
Sodium kappa carrageenan	+	+	+	+	+	+
Sodium lambda carrageenan	+	+	+	+	+	+
Gigartina acicularis	+	+	+	+	+	+
Gigartina pistillata	+	+	+	+	+	+
Eucheuma cottonii	+	+	+	+	+	+
Eucheuma spinosum	+	+	+	+	+	+
Fucoidan	+	+	+	+	+	+
Agar	+	±	−	−	±	+
Hypnean	+	+	+	+	+	+
Iridophycan	+	+	+	+	±	+
Gelcarin	+	+	+	+	+	+
Group II.—Carboxylic acid type polysaccharides						
Gum karaya	+	+	−	−	±	−
Gum arabic	−	−	−	−	±	±
Gum tragacanth	−	−	−	−	±	+
Gum ghatti	−	−	−	−	+	±
Pectin	+	+	−	−	±	+
Sodium carboxymethylcellulose	+	+	+	+	±	+
Sodium alginate	+	+	+	+	±	+
Group III.—Neutral polysaccharides						
Locust bean gum	−	−	−	−	−	−
Gum guar	−	−	−	−	±	−
Potato starch	−	−	−	−	−	−
Group IV.—Proteins						
Casein	+	+	−	−	+	+
Gelatin	−	−	−	−	±	±
Group V.—Other compounds						
Heparin	+	+	+	+	+	+
Chondroitin sulfate	+	+	+	+	+	+
Polyglucose sulfate	+	+	+	+	+	+

[a] + = precipitation; − = no precipitation; and ± = gelation.

TABLE II.—RELATIVE REACTIVITY OF THE HYDROCOLLOIDS WITH TRANQUILIZERS AND HYPOTENSIVE AGENTS

Hydrocolloids	Tranquilizers: Promazine Hydrochloride	Tranquilizers: Chlorpromazine Hydrochloride	Hypotensive Agents: Reserpine	Hypotensive Agents: Veriloid	Hypotensive Agents: Veratrine Sulfate
Group I.—Sulfated polysaccharides					
Carrageenan Seakem type 5	10.00	10.00	10.00	10.00	10.00
Carrageenan Seakem type 402	10.62	11.20	9.85	14.00	11.40
Furcellaran	5.39	5.80	6.70	5.4	6.2
Sodium kappa carrageenan	10.55	10.20	9.98	10.6	10.8
Sodium lambda carrageenan	16.00	15.80	14.79	13.2	14.6
Viscarin	9.74	10.4	11.94	10.8	11.97
Agar	0.08	0.11	0.46	0.08	0.40
Gelcarin	12.95	11.20	9.98	11.2	9.92
Group II.—Carboxylic acid type polysaccharides					
Gum karaya	1.03	0.98	0.06	0.04	0.26
Gum arabic	0.03	0.01	0.09	0.01	0.04
Gum tragacanth	0.00	0.09	0.00	0.00	0.00
Gum ghatti	0.09	0.08	0.16	0.01	0.01
Pectin	0.04	0.04	0.15	0.04	0.04
Sodium carboxymethylcellulose	0.84	0.82	6.18	2.76	0.06
Sodium alginate	0.48	0.47	0.70	0.82	0.36
Group III—Neutral polysaccharides					
Locust bean gum	0.01	0.04	0.71	...	0.03
Gum guar	0.34	0.05	0.33	...	0.60

interaction time at 45°, mixtures of the hydrocolloid and the drug in a total volume of 10 ml. were allowed to interact for the periods shown in Table III and the optical density measured as described above. Studies on the effects of pH were done by adding 2 ml. of 0.1 *M* phosphate buffers of varying pH levels to test tubes containing a suspension of 10 ml. of the hydrocolloid and 2 ml. of the drug solution. The total volume was always 10 ml. The mixtures were incubated at 45° for 15 minutes and, after cooling to room temperature, the turbidity that developed was measured at 400 mμ. The final pH of each system was checked with a Beckman model G pH meter. Since the drugs gave precipitates with buffer salts in the alkaline range, all experimentation was restricted to the acid range. Any influence of buffer molarity was ascertained by adding varying amounts of 1.0 *M* phosphate buffer, pH 4.95, to test tubes containing 1 ml. of the hydrocolloid suspension and 2 ml. of the drug solution. After making the volume 10 ml., the turbidity developed at 45° for 15 minutes was measured as described above.

Since pharmaceutical formulations may contain certain salts, the influence of some of the more common salts on the intensity of the interaction was

TABLE III.—INFLUENCE OF VARIABLES ON THE INTERACTION OF CARRAGEENAN[a] WITH PROMAZINE HYDROCHLORIDE

Variable	Absorbance at 400 mμ: Immediately Measured	Absorbance at 400 mμ: After Cooling and Brought to Room Temperature
Temp., (° C.)		
30	0.153	0.155
45	0.220	0.324
65	0.142	0.318
Reaction Time, Min. at 45° C.		
0	0.180	0.300
5	0.200	0.320
10	0.205	0.320
15	0.215	0.320
30	0.215	0.325
36	0.210	0.370
45	0.195	0.370
90	0.240	0.330
Buffer Molarity		
0.01	...	0.198
0.05	...	0.198
0.10	...	0.200
0.30	...	0.185
0.50	...	0.181
0.70	...	0.145
pH	Promazine Hydrochloride Bound μM/mg. of Carrageenan (Seakem type 5)	
5.50 (unbuffered)	...	1.40
2.55	...	1.15
2.85	...	1.18
4.45	...	1.20
4.95	...	1.55

[a] Seakem type 5.

TABLE IV.—INFLUENCE OF ADDED SALTS ON THE INTERACTION OF CARRAGEENAN[a] WITH PROMAZINE HYDROCHLORIDE AT 45° C.

Salt Added as Chloride	Tolerance Level, *M*
Monovalent	
Li^+	4.25×10^{-4}
K^+	3.0×10^{-4}
NH_4^+	6.4×10^{-4}
Na^+	5.0×10^{-4}
Divalent	
Mg^{++}	2.5×10^{-4}
Ba^{++}	5.0×10^{-4}
Cd^{++}	1.1×10^{-4}
Zn^{++}	1.4×10^{-4}
Sr^{++}	1.4×10^{-4}
Mn^{++}	2.5×10^{-4}
Ca^{++}	1.9×10^{-4}
Trivalent	
Al^{+++}	1.2×10^{-8}
Fe^{+++}	1.0×10^{-4}

[a] Seakem type 5.

assessed. This was done by adding varying amounts of each salt solution to the reaction tubes and measuring the optical density as described above. Duplicate control tubes containing no added salt were included in each experiment. The tolerance level of each salt was established in a manner similar to that employed by Graham and Thomas (8). It was taken as "That maximum final concentration (the concentration of salt in the 10 ml. of reaction medium) of salt which did not cause a significant difference (in this case ±1%) in the absorbance as compared to a control tube to which no salt was added." The results are summarized in Table IV.

Maximum Binding Capacity of Hydrocolloids for the Tranquilizers and Hypotensive Agents.—More detailed quantitative data on the binding process were obtained by determining the maximum binding capacity of representative hydrocolloids for promazine hydrochloride and reserpine. For this, 5 ml. of the suspension containing 50 mg. of the particular hydrocolloid was allowed to react at 45° for 15 minutes with increasing quantities of the tranquilizer or hypotensive agent, in a centrifuge tube, and with a total volume of 10 ml. After cooling to room temperature, the reaction mixture was centrifuged at 1500 r.p.m. for 10 minutes. One milliliter of the supernatant was placed in 9 ml. of methyl alcohol and this alcohol mixture again centrifuged as above. One milliliter of this supernatant was then used for the colorimetric determination of the particular tranquilizer or hypotensive agent. The amount of the drug bound was calculated as "the amount added minus the amount found free in the supernatant."

Colorimetric Methods for Determination of Tranquilizers and Hypotensive Agents. Promazine was determined according to the method of Leach and Crimmin (9). Reserpine was determined by the method of Indemans, *et al.* (10).

Calculation of the Maximum Binding Capacity.—The maximum amount of promazine hydrochloride and reserpine bound per gram of three representative hydrocolloids was established from a Langmuir type plot as employed by Kennon and Higuchi (11) and Patel and Kostenbauder (12). By plotting the reciprocal of the amount of drug bound per unit of hydrocolloid on the Y axis *vs.* the reciprocal of the amount of free drug in the system, and extrapolating to zero concentration of the drug, a line which cuts the Y axis was obtained. The Y intercept gives a measure of the maximum binding capacity (13). The results are summarized in Table V and representative graphs are shown in Figure 1.

Solubility of the Promazine Hydrochloride–Hydrocolloid Complexes.—Since the promazine hydrochloride–carrageenan complex was so readily formed and seemed highly insoluble, it was deemed useful to study its solubility in solutions of sodium chloride and of hydrochloric acid. The method used was similar to that employed by Antonopoulous, *et al.* (14), in studying detergent complexes and Bridger, *et al.* (15), in studying metal ammonium phosphate complexes. For this, 5 ml. of a dispersion containing 50 mg. of carrageenan (Seakem type 5) and 5 ml. of a 0.5% solution of promazine hydrochloride were allowed to react at 45° for 30 minutes in 15-ml. graduated centrifuge tubes. After cooling, the mixture was centrifuged at 2500 r.p.m. for 10 minutes and the supernatant carefully poured off. The precipitate was washed five times with distilled water and centrifuged after each washing. The precipitate from the centrifuge tubes was added to consecutive 200 × 15 Kimax test tubes and quantities of 4 M sodium chloride or 2 N hydrogen chloride added to give the final normalities shown in Table VI. The final volume was 20 ml. in all cases attained by adding distilled water where necessary. The tubes were shaken on a Burrell wrist action shaker at 27° and, at the intervals shown, 3 ml. of the

Table V.—Maximum Binding Capacity of Hydrocolloids for Promazine Hydrochloride and Reserpine at 45° C.

Hydrocolloids	μM of Drug Bound/Gm. of Hydrocolloid: Promazine Hydrochloride	Reserpine
Carrageenan (Seakem type 5)	1500	666.0
Na carboxymethylcellulose	244	540.5
Pectin	14.3	238.0

Table VI.—Solubility of the Promazine Hydrochloride-Carrageenan[a] Complex in Sodium Chloride and Hydrochloric Acid at 27° C.

	% of Bound Promazine Released After 1 Hr.	6 Hr.	12 Hr.	24 Hr.
	Sodium Chloride (Final Molarity)			
0.0 (H_2O)	4.000	4.01	4.17	4.87
0.2	26.52	29.46	30.55	29.17
0.4	29.65	31.63	34.72	38.88
1.6	38.74	40.42	48.61	43.75
	Hydrochloric Acid (Final Normality)			
0.0 (H_2O)	4.00	4.01	4.17	4.87
0.01	7.00	7.82	8.33	13.18
0.1	18.46	20.01	23.61	30.00
0.2	25.20	26.52	30.55	36.11
0.5	24.62	29.80	38.19	58.33
1.0	40.80	48.60	57.08	65.29
2.0	56.40	60.00	79.17	83.33

[a] Seakem type 5.

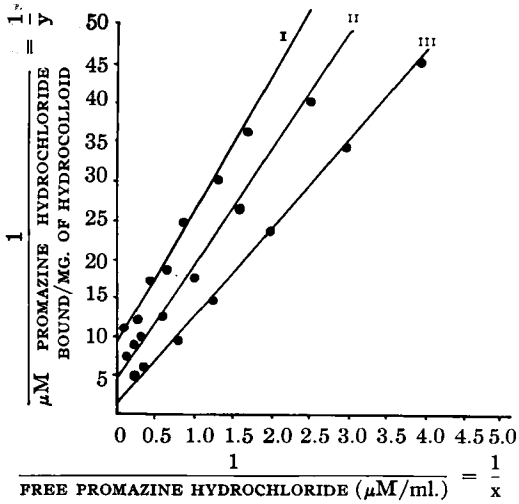

Fig. 1.—Langmuir type plot of data to determine maximum binding capacity of hydrocolloids for promazine hydrochloride. I = pectin; II = sodium carboxymethylcellulose; and III = carrageenan (Seakem type 5).

mixture centrifuged and one ml. of the supernatant assayed for promazine released from the complex. The results are shown in Tables VI and VII. For comparison, the promazine hydrochloride–sodium carboxymethylcellulose complex was also studied.

Experiments on the Promazine Hydrochloride–Carrageenan Complex.—Batch quantities were prepared by using a 200-ml. suspension containing 200 mg. of carrageenan (Seakem type 5), preincubated at 45° for 30 minutes. One hundred milliliters of a solution containing 2 Gm. of promazine hydrochloride was similarly preincubated. At the end of the preincubation period, both the hydrocolloid suspension and the promazine hydrochloride solution were mixed in a 1000-ml. Erlenmeyer flask and allowed to interact for 30 minutes at 45°. The mixture was placed in a cold room at 4° overnight and filtered. The filtrate was collected and carrageenan added to precipitate any promazine in it. If precipitation occurred, the cooling and filtration processes were repeated. The precipitates were pooled and washed with distilled water to remove any unreacted hydrocolloid. This was then followed by washing 5 times with absolute methyl alcohol. The precipitate was then collected, placed in a Petri dish and dried overnight in a vacuum oven at 75°. After this, the mass was pulverized in a mortar and stored in a vial.

Titration of the Complex.—In order to determine the promazine content of the complex 10 mg. was placed in a 25-ml. volumetric flask and enough concentrated sulfuric acid added to dissolve it. Water was added to make it up to volume. From this, 1 ml. was taken, further appropriately diluted with distilled water and the promazine content determined colorimetrically by the method of Leach and Crimmin (9).

Sustained Release of the Promazine from the Complex *in vivo*.—In order to ascertain if sustained release of the promazine from the carrageenan (Seakem type 5)–promazine complex could be achieved, free promazine, and the suspending medium, respectively, was injected intramuscularly into individual rabbits. Sterile physiological saline and a 1:5:4 mixture of ethyl alcohol, glycerin, and water were used as suspending media in the separate experiments. Samples of blood were drawn at intervals from 4 hours to 6 days and analyzed for promazine by the method of Leach and Crimmin (9). Simultaneously, changes in the picture of the white blood cells were followed. The results are summarized in Table VIII.

TABLE VII.—SOLUBILITY OF PROMAZINE HYDROCHLORIDE-SODIUM CARBOXYMETHYLCELLULOSE COMPLEX IN SODIUM CHLORIDE AND HYDROCHLORIC ACID AT 27° C.

	% of Bound Promazine Released After— 1 Hr.	6 Hr.	12 Hr.	24 Hr.
Sodium Chloride (Final Molarity)				
0.0 (H_2O)	10.6	11.4	12.6	14.3
0.2	68.0	71.4	78.0	88.4
0.4	72.0	78.0	87.0	94.3
0.8	75.2	81.6	90.0	98.0
1.6	80.2	87.6	91.2	98.4
2.0	81.6	90.2	94.3	98.7
Hydrochloric Acid (Final Normality)				
0.0 (H_2O)	10.6	11.4	12.6	14.3
0.01	20.6	25.0	28.6	30.2
0.10	28.0	38.0	40.8	53.0
0.20	40.8	46.0	65.8	76.5
0.50	68.7	85.6	93.0	94.6
1.00	72.4	86.2	94.5	96.2
2.00	76.0	87.0	94.3	96.4

RESULTS AND DISCUSSION

From Tables I and II it is clear that the sulfated polysaccharides are the most reactive hydrocolloids. The low reactivity of agar may be partly explained by the fact that its sulfation is still not well established (16, 17). The carboxylic acid type polysaccharides are next in order of reactivity. Of these, sodium carboxymethylcellulose is one of the more reactive. In the experiments with sodium carboxymethylcellulose, all reagents were dissolved in distilled water to avoid precipitation of the hydrocolloid itself at low pH levels of the medium (17). The neutral polysaccharides were the least reactive.

Of the several variables which affect the interaction of promazine hydrochloride with carrageenan (Seakem type 5), the pH of the interaction medium is the most important. In the alkaline range, the tranquilizer will precipitate in the absence of the hydrocolloid, hence only the acid range was investigated. Maximum interaction occurs at pH 4.95, and as the pH decreases the amount of promazine bound per mg. of the hydrocolloid decreases. At a final pH of 2.25, binding of the drug by the hydrocolloid was still appreciable since ionization of the sulfate group is not drastically impeded even at this low final pH. A similar pattern would be expected for the other sulfated hydrocolloids. However, the carboxylic acid type hydrocolloids would be more severely influenced since at pH levels of 2.5–3.0, sodium carboxymethylcellulose, in particular, would precipitate in the absence of the tranquilizer.

The temperature has a great influence on the interaction as exemplified by the promazine hydrochloride–carrageenan (Seakem type 5) interaction. In the acid range (pH 4.95) turbidity is a good measure of the intensity of the interaction and it was used to assess this influence. At 30°, the turbidity developed was lower than at 45° or at 65°. Apparently, at this temperature (30°) ionization of both interactants may not be complete. The great sensitivity of the system to temperature as indicated

TABLE VIII.—DETECTION OF PROMAZINE IN BLOOD OF RABBIT AFTER INTRAMUSCULAR INJECTION OF PROMAZINE HYDROCHLORIDE AND THE CARRAGEENAN-PROMAZINE COMPLEX

Time Lapse After Injection, Hr.	Promazine Detected in Blood After Injection of Carrageenan[a] Promazine Complex μg./ml. of Blood	Promazine Hydrochloride μg/ml. of Blood
0.0	0.0	0.0
4.0	2.0	2.64
12.0	3.07	4.5
18.0	...	1.9
24.0	3.55	0.0
48.0	3.47	...
72.0	1.29	...
96.0	0.95	...
144.0	0.47	...

[a] Seakem type 5.

TABLE IX.—EFFECT OF THE INTRAMUSCULAR INJECTION OF THE CARRAGEENAN[a]-PROMAZINE COMPLEX ON THE PICTURE OF THE WHITE BLOOD CELLS OF THE RABBIT

Time After Injection, Hr.	Variations in Count of White Blood Cells				
	Neutrophils, %	Monocytes, %	Basophils, %	Eosinophils, %	Lymphocytes, %
0.0	26.8	12.0	0	5.0	54.0
4.0	38.0	12.5	0	3	46.0
12.0	19.0	34	0	0	46.0
18.0	...	...	...	...	...
24.0	45.0	29.5	1.0	2	22.5
48.0	29.0	30.5	0	0	20.5
72.0	30.0	32.0	2	0	36.0
96.0	41.0	19.0	2	3	36
144.0	...	...	...	...	...

[a] Seakem type 5.

by the measurements made immediately (before cooling) and those made after cooling to room temperature indicate that, apparently, forces other than the ionic type can participate in the binding process. Since the turbidity developed at 45° was essentially the same as that at 65° (after cooling), 45° was chosen for all later experiments.

Under the experimental conditions employed, at 45° the maximum interaction occurred after 30 minutes. Despite this, a heating time of 15 minutes was selected to avoid any possibility of hydrolysis of the hydrocolloid due to prolonged heating, and to facilitate rapidity of experimentation when many samples were involved. The degree of interaction at 45° for 15 minutes remained constant for as long as 4 hours, hence comparisons based on the time–temperature selection are not invalid.

Final buffer molarities of 0.01–0.10 resulted in no drastic change in the interaction as evidenced by the turbidity measurements. Therefore, where buffers were used, a final molarity of 0.01 was selected.

Table IV summarizes the influence of salts on the promazine hydrochloride–carrageenan (Seakem type 5) interaction. On a molar basis, the order of tolerance of the added salts was found to be monovalent > divalent > trivalent. Severe interference of the salts is due to competition for the binding sites of the hydrocolloids. The trivalent cations will actually precipitate carrageenan and other hydrocolloids, even at relatively low concentrations.

Although only the promazine hydrochloride–carrageenan (Seakem type 5) system was investigated in detail, it is to be expected that the other systems would be similarly affected by the variables listed. As noted with the precipitation with alkaloids (8), lambda carrageenan was always more reactive than kappa carrageenan. This is in line with chemical data which show that the lambda fraction is much richer in ester sulfate content than the kappa fraction (17).

The solubility data recorded in Tables VI and VII indicate that the promazine hydrochloride complexes of carrageenan (Seakem type 5) and sodium carboxymethylcellulose are highly insoluble in sodium chloride and hydrochloric acid. Relatively, the sodium carboxymethylcellulose complex is more soluble than the carrageenan complex in both sodium chloride and hydrochloric acid. This insolubility renders them potentially suitable as sustained release forms of this and possibly other drugs. The need for complexing agents for the development of such depot forms of drugs has recently been clearly emphasized by Hirscher and Miller (18).

Analysis of the data recorded in Table VIII indicate that the promazine hydrochloride–carrageenan (Seakem type 5) complex can serve as a depot form of the drug. The prolonged presence of the drug in the blood of the rabbit leads to the conclusion that the drug was released from the complex. Contrastingly, the disappearance of drug administered as the "free form," after 24 hours, indicates the further superiority of the complex where sustained release is desired. Le Blanc (19) reported that a single injection of chlorpromazine caused a drop in the eosinophils, leucocytes, and platelets in the blood of male albino rats. This effect, in their opinion, was not a direct result of hypothermia. Korst (20) has reviewed several cases of agranulocytosis caused by phenothiazine derivatives including chlorpromazine and promazine. It was noted that in some cases the neutrophils decreased drastically or even vanished and that the total leucocyte count severely decreased. Since promazine hydrochloride is very closely related to chlorpromazine hydrochloride, it was decided to investigate the blood picture changes, if any. The variations in the blood cell picture shown in Table IX, may be considered as further evidence that the promazine was being released from its hydrocolloid complex. Besides the possibility of their use for development of sustained release forms of drugs, the effect of these hydrocolloids on the availability of drugs and other chemicals in pharmaceutical preparations must also be emphasized. It has been shown that gums (hydrocolloids) will lower the availability of tetracycline antibiotics.

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Technical Articles

Technique of Implanting Permanent Electrodes in Cats for Chronic Stimulation and Observation of EEG and Behavioral Effects

By ZOLA P. HOROVITZ and MAY-I CHOW

A procedure for preparing cats with permanently implanted electrodes is described. This technique, which has been used to successfully prepare over 50 of these animals, employs fixation with dental cement and the use of a direct contact connection on the top of the head. The procedure is quick, inexpensive, and very productive. This makes it quite useful in both industrial and academic pharmacological, physiological, and psychological research.

SINCE Hess' pioneer work on stimulation of the unrestrained, unanesthetized cat (1), techniques for stimulating and lesioning or for recording of electrical and behavioral phenomena in the active animal have become useful to the pharmacologist, physiologist, and psychologist. Bradley and Elkes (2), Horovitz and Chow (3), and many others, have used cats with permanently implanted electrodes (P.I.E. cat) for correlating the effects of drugs upon electrical and behavioral responses. This type of preparation has also been used for various types of chronic stimulation by Killam and Killam (4), Doty (5), and Horovitz, *et al.* (6).

The literature, unfortunately, reveals only a few complete descriptions of the techniques involved in preparing these P.I.E. animals. The techniques used by Hess, ingenious for his time, have been outdated by technical advancements. Knowles (7) in 1951 described a method of implantation that employed a base-plate assembly. This technique requires a large craniotomy and the building of a complicated base-plate head assembly for each cat. Bradley and Elkes (8) in 1953 described an excellent procedure for implanting deep and cortical electrodes but the leads were threaded under the skin of the neck and out the animal's back. This required the animal to carry a bulky harness on its back. Delgado (9) has described a method of implanting multipolar needle electrodes; unfortunately his technique also requires a bulky harness. Delgado (10) has expertly reviewed various implantation procedures and the effects of long term stimulation and recording in animal brains.

Our laboratory has explored various aspects of many techniques for preparing the P.I.E. cat. We feel that the procedure described below is the quickest, easiest, and most productive we have tested. It requires very little laboratory preparation; every item is commercially available, and the animal requires very little maintenance care. We have successfully used this procedure to prepare over 50 P.I.E. cats within the past two years.

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