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Aggregation of Antihistamines in Aqueous Solution: Effect of Counterions on Self-Association of Pyridine Derivatives

D. ATTWOOD [x] and O. K. UDEALA

Abstract □ The effects of electrolytes on the self-association of the antihistaminic drugs, tripelennamine hydrochloride, thenyldiamine hydrochloride, pyrilamine maleate, pheniramine maleate, chlorpheniramine maleate, and brompheniramine maleate, in aqueous solution were examined by light-scattering methods. The concentration dependence of the light scattering from tripelennamine hydrochloride and thenyldiamine hydrochloride in 0.154 mole of sodium chloride/kg and 0.150 mole of sodium maleate/kg indicated a micellar pattern of aggregation. Higher aggregation numbers and lower CMC's were determined in the presence of the maleate ion. No significant discontinuity in the concentration dependence of the light scattering of the remaining compounds in either of the two electrolytes was evident, and the aggregation of these compounds was treated using a stepwise association model. Values of the association constants and the limiting number of associating species were, in general, increased by the addition of electrolyte in the order water < sodium chloride < sodium maleate. An apparently nonmicellar pattern of aggregation could be induced by chemically changing the counterion from chloride to maleate.

Keyphrases □ Antihistamines—pyridine derivatives, aggregation in aqueous solution, effect of counterions on self-association □ Aggregation—antihistaminic pyridine derivatives, aqueous solution, effect of counterions on self-association □ Counterions—effect on self-association of antihistaminic pyridine derivatives in aqueous solution □ Pyridine derivatives—antihistamines, aggregation in aqueous solution, effect of counterions on self-association □ Electrolytes—effect on self-association of antihistaminic pyridine derivatives in aqueous solution

The self-association in aqueous solution of some antihistamines containing a pyridine nucleus was investigated by light-scattering methods previously (1). No significant discontinuity in the concentration dependence of the light scattering, attributable to a critical micelle concentration (CMC), could be detected. In all cases, however, the scattering intensity exceeded that calculated for the unassociated monomer.

The scattering behavior of three of these compounds, [pyrilamine (mepyramine) maleate, brompheniramine maleate, and chlorpheniramine maleate] could be simulated using a nonmicellar model of association which assumed aggregate growth by stepwise addition of monomers. The intensity of the light scattered by the other compounds studied (pheniramine maleate, tripelennamine hydrochloride, and thenyldiamine chloride) was not of sufficient intensity to establish if association conformed to a micellar or nonmicellar pattern.

Other reports in this series (2–4) concerned antihistamines containing a diphenylmethane nucleus (diphenhydramine hydrochloride, bromodiphenhydramine hydrochloride, chlorcyclizine hydrochloride, and diphenylpyraline hydrochloride). Such compounds have been shown to exhibit typical colloidal behavior in aqueous solution.

This study examined the light scattering of the antihistamines containing a pyridine nucleus in the presence of sodium chloride and sodium maleate. The effect of chemically changing the counterion was investigated for tripelennamine and pyrilamine in an attempt to isolate the cause of their nonmicellar behavior.

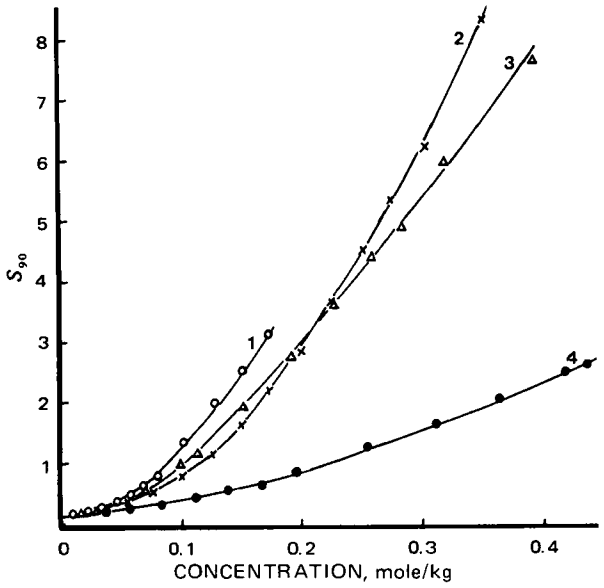

Figure 1—*Light-scattering plots in 0.154 mole of sodium chloride/kg at 303° K. Scattering at 90° to the incident beam, S_{90}, is plotted as a function of molal concentration. Key: 1, brompheniramine maleate; 2, pyrilamine maleate; 3, chlorpheniramine maleate; and 4, pheniramine maleate.*

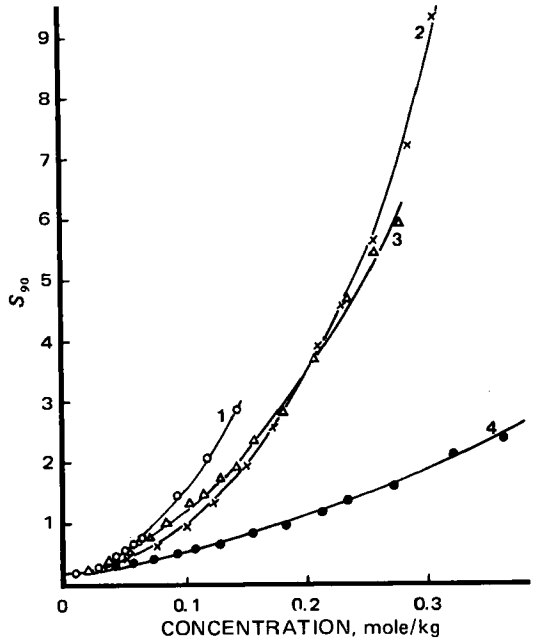

Figure 2—*Light-scattering plots in 0.150 mole of sodium maleate/kg at 303° K. Scattering at 90° to the incident beam, S_{90}, is plotted as a function of molal concentration. Key: 1, brompheniramine maleate; 2, pyrilamine maleate; 3, chlorpheniramine maleate; and 4, pheniramine maleate.*

EXPERIMENTAL

Materials—The following drugs were used as received: pyrilamine maleate[1] {*N*-[(4-methoxyphenyl)methyl]-*N'*,*N'*-dimethyl-*N*-2-pyridinyl-1,2-ethanediamine butenedioate}, tripelennamine hydrochloride[2] BP [*N*,*N*-dimethyl-*N'*-(phenylmethyl)-*N'*-2-pyridinyl-1,2-ethanediamine monohydrochloride], thenyldiamine hydrochloride[3] [*N*,*N*-dimethyl-*N'*-2-pyridinyl-*N'*-(3-methylthienyl)ethylenediamine hydrochloride], pheniramine maleate[4] [*N*,*N*-dimethyl-3-phenyl-3-(2-pyridinyl)propylamine butenedioate], chlorpheniramine maleate[5] [3-(4-chlorophenyl)-*N*,*N*-dimethyl-3-(2-pyridinyl)propylamine butenedioate], and brompheniramine maleate[6] [3-(4-bromophenyl)-*N*,*N*-dimethyl-3-(2-pyridinyl)propylamine butenedioate].

Tripelennamine maleate was prepared by the addition of maleic acid to tripelennamine base in anhydrous ether. The salt was recrystallized from methanol–ether and dried over phosphorus pentoxide under vacuum, mp 132°.

Anal.—Calc. for $C_{20}H_{25}N_3O_4$: C, 64.67; H, 6.78; N, 11.31. Found: C, 64.7; H, 7.1; N, 11.3.

Pyrilamine hydrochloride was prepared by passing dried hydrogen chloride gas into a solution of pyrilamine base in anhydrous ether. The salt was recrystallized from methanol–ether and dried over phosphorus pentoxide under vacuum, mp 138–140° [lit. (5) mp 143°].

Anal.—Calc. for $C_{17}H_{24}ClN_3O$; C, 63.44; H, 7.52; Cl, 11.02; N, 13.06. Found: C, 59.2; H, 7.2; Cl, 12.6; N, 12.2.

Sodium chloride was Analar grade, sodium maleate was reagent grade, and the water was double distilled from glass.

Light Scattering—The light-scattering instrument[7] was thermostated at 303° K, and measurements were made at a wavelength of 546 nm. Solutions were clarified by ultrafiltration[8] until the ratio of the light scattering at angles of 30 and 150° did not exceed 1.10. The refractive index increments of the micellar species were measured at 546 nm using a differential refractometer.

RESULTS

The light-scattering plots for pyrilamine maleate, chlorpheniramine maleate, brompheniramine maleate, and pheniramine maleate in 0.154 mole of sodium chloride/kg and 0.15 mole of sodium maleate/kg are shown in Figs. 1 and 2. The scattering curves are similar in appearance to those previously obtained in the absence of electrolyte (1). The scattering at 90°, S_{90}, increased continuously with concentration, with no apparent discontinuity normally associated with a CMC.

The scattering data were treated using a proposed nonmicellar model of association (6), which was previously applied to the aggregation of these systems in water. Aggregate growth was assumed to occur by a stepwise addition of monomers (Scheme I).

$$M_{N-1} + M_1 \rightleftharpoons M_N$$

Scheme I

The weight fraction, x, of compound existing as the monomer was determined by graphical integration of the light-scattering data according to:

$$\ln x = \int_0^c [(M/M_w) - 1]\, d \ln c \qquad \text{(Eq. 1)}$$

where M is the monomer molecular weight, M_w is the apparent weight-average aggregate weight, and c is the weight concentration (grams per cubic decimeter). Stepwise equilibrium constants, K_N, were estimated, as described previously, from:

$$[(M_w/xM) - 1]/(xc/M) = 4K_2 + 9K_2K_3\,(xc/M) \ldots + N^2 \left(\prod_2^N K_N\right) (xc/M)^{N-2} \qquad \text{(Eq. 2)}$$

This method was derived for ideal systems and does not take into account the interactions between charged aggregates. Such interactions are reduced in the presence of a swamping electrolyte. No corrections were applied to the refractive index increments measured in sodium chloride to allow for the presence of mixed counterions.

Errors in the calculation of K_N are cumulative, and only the lower values are quoted in Table I. Also included in Table I are approximate values of l, the degree of association of the highest molecular species existing in solution in significant amount, which were derived as described previously. The K_N and l values were, in general, increased by the addition of electrolyte in the order water < sodium chloride < sodium maleate.

The concentration dependence of the scattering from tripelennamine hydrochloride and thenyldiamine hydrochloride in the two electrolytes is shown in Fig. 3. The curves are typical of those normally

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[6] A. H. Robins & Co., Horsham, Sussex, United Kingdom.
[7] Fica 42,000 photogoniodiffusometer.
[8] Millipore filters, 0.1 μm.

Table I—Self-Association of Antihistamines in Water, 0.154 Mole of Sodium Chloride/kg, and 0.150 Mole of Sodium Maleate/kg

Compound	Solvent	Stepwise Association Constants, dm³/mole K_2	K_3	Limiting Aggregation Number, l
Pheniramine maleate	Water[a]	1.61	5.2	7
	Sodium chloride	1.58	4.0	10
	Sodium maleate	1.58	5.1	8
Chlorpheniramine maleate	Water[a]	2.03	9.9	11
	Sodium chloride	5.00	35.3	22
	Sodium maleate	6.13	39.2	21
Brompheniramine maleate	Water[a]	3.50	21.0	15
	Sodium chloride	3.10	35.6	22
	Sodium maleate	6.30	62.2	>30
Pyrilamine maleate	Water[a]	2.00	7.2	10
	Sodium chloride	2.50	17.8	>30
	Sodium maleate	2.90	17.7	>30
Tripelennamine maleate	Water[a]	0.75	6.30	14

[a] Values from Ref. 1.

obtained for surfactant solutions, showing clearly defined inflections at the CMC. The scattering below the CMC could be represented by theoretical lines calculated for the scattering of unassociated monomers, and accurate values of the CMC (Table II) were determined from the intersection of these lines with those representing the scattering at higher concentrations.

Where systems involved mixed counterions, *e.g.*, tripelennamine hydrochloride in sodium maleate, the relative refractive index increments used in the calculation of the theoretical lines were determined as a function of the solution concentration using equations

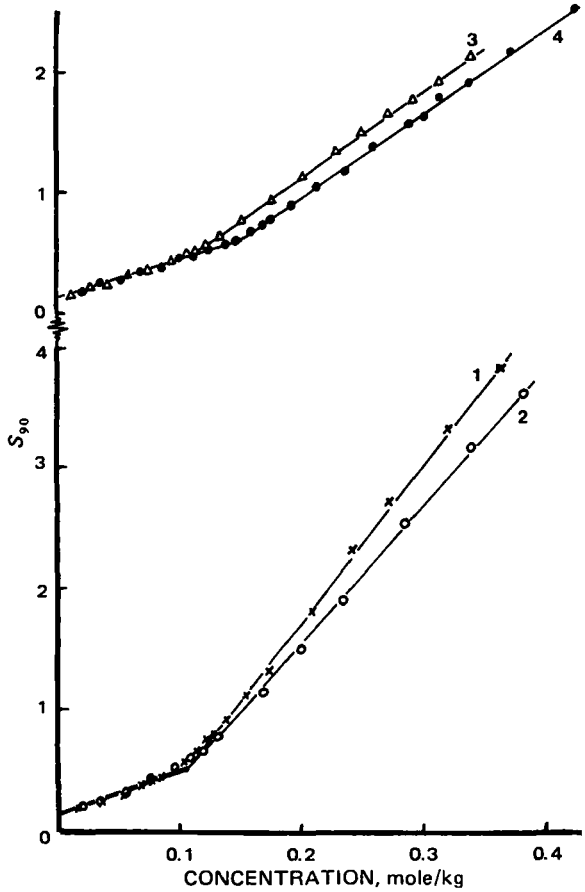

Figure 3—*Light scattering of tripelennamine and thenyldiamine hydrochloride in 0.154 mole of sodium chloride/kg and 0.150 mole of sodium maleate/kg at 303° K. Key: 1, tripelennamine hydrochloride in sodium maleate; 2, thenyldiamine hydrochloride in sodium maleate; 3, tripelennamine hydrochloride in sodium chloride; and 4, thenyldiamine hydrochloride in sodium chloride.*

proposed previously (7), in which the refractive index gradient of the solute, dn/dm_2, is given by

$$dn/dm_2 = (a + b)^{-1}\, a(dn/dm_{D\ \text{maleate}}) + b(dn/dm_{D\ \text{HCl}}) \quad \text{(Eq. 3)}$$

where a and b are the molalities of the sodium maleate and the hydrochloride salt of the drug, respectively; and $dn/dm_{D\text{maleate}}$ and $dn/dm_{D\ \text{HCl}}$ are the refractive index increments of the maleate and hydrochloride salts of the drug, respectively.

The refractive index increment of tripelennamine maleate could be determined experimentally. Since thenyldiamine maleate was not available, a value of $dn/dm_{D\ \text{maleate}}$ was calculated from the refractive index increment of thenyldiamine hydrochloride as suggested previously (7). A solution that was a molal in sodium maleate and b molal in thenyldiamine hydrochloride was regarded as being b molal in thenyldiamine maleate, b molal in sodium chloride, and $(a-b)$ molal in sodium maleate. The measured refractive index difference between a b molal solution of thenyldiamine hydrochloride and the sodium maleate solvent was corrected by adding the quantity b $(dn/dm_{\text{Na maleate}} - dn/dm_{\text{NaCl}})$ to give the corresponding refractive index difference for thenyldiamine maleate. A literature value (8) of 0.0104 kg/mole was used for the refractive index increment of sodium chloride, dn/dm_{NaCl}. A value of 0.0290 kg/mole was experimentally determined for the refractive index increment of sodium maleate.

The micellar aggregation numbers were calculated using equations proposed previously (9):

$$p = 2fm_3B \pm (8m_3B)^{1/2}A^{-1}(2 - fA)^{-1} \quad \text{(Eq. 4)}$$

$$N = p(p + 1)A(2m_3B + pA^2)^{-1} \quad \text{(Eq. 5)}$$

where A and B are the intercept and slope, respectively, of plots of Km_2/R_{90} against the molal concentration of micelles, m_2. The R_{90} is the Rayleigh ratio of the solution in excess of that of a solution at the CMC; $K = 2\pi^2 n_0^2 (dn/dm_2)_{m_3}^2 V^0/L\lambda^4$; n_0 is the refractive index of the solvent; V^0 is the volume of solution containing 1 kg of water; L is the Avogadro number; λ is the wavelength of the incident light; m_3 is the molality of supporting electrolyte; and $f = (dn/dm_3)_{m_2}/(dn/$

Table II—Properties of Micelles of Tripelennamine Hydrochloride and Thenyldiamine Hydrochloride in 0.154 Mole of Sodium Chloride/kg and 0.150 Mole of Sodium Maleate/kg

Compound	Solvent	CMC, mole/kg	Aggregation Number, N
Tripelennamine hydrochloride	Sodium chloride	0.11	3.3
	Sodium maleate	0.10	4.5
Thenyldiamine hydrochloride	Sodium chloride	0.13	2.9
	Sodium maleate	0.11	3.6

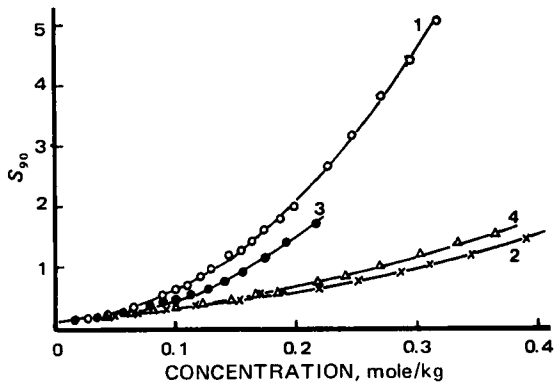

Figure 4—*Effect of change of counterion on the light scattering of pyrilamine and tripelennamine in water at 303° K. Key: 1, pyrilamine maleate; 2, pyrilamine hydrochloride; 3, tripelennamine maleate; and 4, tripelennamine hydrochloride. Light-scattering data for pyrilamine maleate and tripelennamine hydrochloride are from Ref. 1.*

$dm_2)_{m_3}$. Then $(dn/dm_2)_{m_3}$ and, hence, K vary with surfactant concentration according to Eq. 3.

To simplify the computational procedure, plots of m_2/R_{90} *versus* m_2 rather than Km_2/R_{90} *versus* m_2 were drawn, and the value of K appropriate at the CMC was applied to the intercept and slope to give values of A and B. Since the B values were too low to be determined accurately, N was equated with A^{-1}. Aggregation numbers are given in Table II.

A change of the counterion associated with tripelennamine, from hydrochloride to maleate, increased the intensity of scattering and gave a scattering curve similar in appearance to that observed with the other compounds with maleate counterions. The data were treated according to Eqs. 1 and 2 and Scheme I, and values of K_N and l are included in Table I. Changing the counterion of pyrilamine from maleate to chloride reduced the scattering intensity to a level similar to that of tripelennamine hydrochloride. Although the S_{90} value exceeded that calculated for unassociated monomers, it was not sufficiently high to indicate clearly the type of association.

DISCUSSION

The effect of the structure of the hydrophobic group on the pattern of aggregation was discussed previously (10). Hydrophobic groups containing aromatic ring structures are thought to aggregate by a continuous stepwise association process. Such ring structures are usually planar, and association involves a face-to-face stacking of monomers. Antihistamines with a diphenylmethane hydrophobic group have been shown (2–4) to aggregate by a micellar process rather than by stepwise association. The hydrophobic groups of such compounds, although aromatic, are neither planar nor rigid structures. Rotation around the central C atom of the diphenylmethane nucleus would clearly hinder the stacking process.

In contrast, the antihistamines under investigation have a single planar hydrophobic ring only. With the exception of thenyldiamine hydrochloride and tripelennamine hydrochloride, their aggregation may be adequately represented by a stepwise association model both in water (1) and in the presence of excess electrolyte (Figs. 1 and 2). The aggregation of thenyldiamine hydrochloride and tripelennamine hydrochloride in the presence of electrolyte is apparently a micellar process, as indicated by Fig. 3. Although it was not possible to establish by light-scattering methods the type of association for these two compounds in the absence of electrolyte (1), recent surface tension measurements (11) indicated that this process is also micellar.

The reason for the difference between the mode of aggregation of these two compounds and the remaining pyridine derivatives is not clear but may be associated with the nature of the counterion. Figure 4 shows that chemically changing the counterion of tripelennamine from chloride to maleate not only resulted in increased scattering but also in an apparently nonmicellar pattern of aggregation.

It is interesting to compare the effects of changing the counterions by addition of electrolyte with those of changing the counterions by chemical synthesis. The addition of a relatively high concentration of sodium maleate to tripelennamine hydrochloride and thenyldiamine hydrochloride, although increasing the scatter, did not result in the nonmicellar aggregation typical of the compounds with a maleate counterion. Similarly, the addition of sodium chloride to pyrilamine, chlorpheniramine, brompheniramine, and pheniramine maleates did not induce a micellar pattern of aggregation. A major difference between these two types of systems is that, in solutions with mixed counterions, the ratio of the chloride and maleate ions is continually changing as the solution concentration is altered; consequently, the two systems are not directly comparable.

Table III—Free Energies of Micelle Formation of Antihistamines (kJ/mole)

Compound	Water	0.154 Mole of Sodium Chloride/kg	0.150 Mole of Sodium Maleate/kg
Pheniramine maleate	−11.21	−11.95	−11.77
Chlorpheniramine maleate	−13.69	−16.15	−16.75
Brompheniramine maleate	−15.19	−16.42	−17.49
Pyrilamine maleate	−13.84	−15.31	−15.69
Tripelennamine maleate	−13.03	—	—

The average free energy change for the monomer, ΔG_m, for the formation of the N-mer is given by (10):

$$N\,\Delta G_m = -RT \ln \beta \qquad \text{(Eq. 6)}$$

where β is the product of all stepwise association constants K_2, K_3, . . . K_l, *i.e.*, $\beta = \Pi_2^l K_N$. Application of Eq. 6 to those antihistamines showing nonmicellar aggregation gave the ΔG_m values of Table III. Included in this table are ΔG_m values in the absence of electrolyte calculated from previously reported K_N and l values. For systems in which a complete analysis of the equilibrium constants was not possible, *i.e.*, brompheniramine maleate in sodium maleate and pyrilamine maleate in sodium chloride and sodium maleate, approximate ΔG_m values were calculated from available K_N values.

The value of ΔG_m may be considered to be composed of electrical and hydrocarbon contributions, designated ΔG_e and ΔG_h, respectively. In a rigorous examination of the thermodynamics of micellization of the cationic surfactant dodecyltrimethylammonium bromide, Anacker (12) noted an increase in $-\Delta G_m$ on the addition of electrolyte, which was shown to result from a reduction in ΔG_e. A similar increase in $-\Delta G_m$ is noted in Table III, the greater effect being produced by sodium maleate. From the data of Kauzmann (13), the free energy change, ΔG_h, associated with the formation of a micelle in which a phenyl ring is completely shielded from the aqueous environment would be −17kJ/mole.

The value of $-\Delta G_m$ is generally slightly less than $-\Delta G_h$ since the electrical contribution is small and positive. The approximate ΔG_m values calculated assuming the applicability of the stepwise association model are thus of a reasonable magnitude. If a similar ΔG_e is assumed for each compound, the ΔG_m values reflect an increased hydrophobicity in the order pheniramine $\ll$ chlorpheniramine $<$ brompheniramine, as might be expected. For pyrilamine maleate, ΔG_m in water is slightly larger than that of tripelennamine maleate, reflecting the increased hydrophobicity conferred by the methoxyl group on the phenyl ring of pyrilamine, the two compounds having otherwise identical structures.

The greater aggregating power of the maleate counterion as compared to that of the chloride ion is evident from the aggregation numbers of tripelennamine hydrochloride and thenyldiamine hydrochloride (Table II), from the magnitude of K_N and l values of Table I, and from a comparison of the scattering intensity of the hydrochlorides and maleates of tripelennamine and pyrilamine (Fig. 4). These observations are in agreement with previously reported investigations of the effect of organic counterions on the micellization of both anionic and cationic surfactants. In general, a greater depression of the CMC (14–16) and a greater increase in aggregation number (17) have been noted with organic counterions than with inorganic ions; these findings have been explained in terms of hydrophobic bonding between the micelle surface and the organic counterions.

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Binding Characteristics of Drugs to Synthetic Levodopa Melanin

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Abstract □ To define binding characteristics of drugs, levodopa melanin was prepared with the aid of mushroom tyrosinase. The binding of radiolabeled substances was studied with increasing concentrations of melanin in a fixed volume of potassium phosphate buffer (pH 7.4) at 37°. The affinity and capacity of the drug binding were calculated according to Langmuir's adsorption isotherm. The affinity constant of various sympathomimetic amines such as (−)-amphetamine, (+)-amphetamine, (−)-ephedrine, (±)-octopamine, and (+)-norepinephrine ranged from 1.1 to 2.8 × 10^5 M^{-1}. The binding capacity for the amines ranged from 1.4 to 3.2 × 10^{-9} mole/mg. Although the capacity of (±)-cocaine for binding was similar to that of the amines, the affinity was slightly higher, 8.9 × 10^5 M^{-1}. The binding of atropine to the synthetic melanin appeared to be a saturable process with the affinity and capacity values of 0.2 × 10^5 M^{-1} and 7.6 × 10^{-9} mole/mg, respectively. Although the binding lacks stereoselectivity, the drugs vary in their capacity and affinity to bind with melanin. The observed differential pharmacological and toxicological properties of drugs in the pigmented tissues may in part be related to their differential binding characteristics.

Keyphrases □ Melanin, synthetic—prepared by action of mushroom tyrosinase on levodopa, affinity and capacity of drug binding evaluated, various sympathomimetic amines □ Levodopa melanin—affinity and capacity of drug binding evaluated, various sympathomimetic amines □ Binding, drug-melanin—affinity and capacity evaluated, various sympathomimetic amines □ Sympathomimetic amines—binding to synthetic melanin, affinity and capacity evaluated □ Pigments—synthetic melanin, affinity and capacity of drug binding, various sympathomimetic amines

Several tissues such as iris, skin, hair, inner ear, and substantia nigra contain melanin. Many phenothiazines (1, 2), chloroquine (3), cocaine (4), antibiotics (5), and sympathomimetic drugs (6, 7) are markedly accumulated and retained for a long time by the pigmented tissue. The nonpigmented tissues from albino or nonalbino animals accumulate very little drug, and the accumulated substance is rapidly lost from the tissue (4, 6, 7).

In the pigmented tissue, the accumulation presumably occurs by the pigment cells and their constituent melanin granules. These granules consist of a lipoid membrane, a protein, and the melanin derived from levodopa (8). Since little is known about the binding characteristics of drugs by melanin, synthetic levodopa melanin was prepared and the affinity and capacity for drug binding were studied.

EXPERIMENTAL

Preparation of Synthetic Levodopa Melanin—Melanin was prepared by a modification of a reported method (2, 9). Ten grams of levodopa (L-dihydroxyphenylalanine)[1] and 66 mg of mushroom tyrosinase[2] (polyphenol oxidase) were placed in 3 liters of 0.1 *M* potassium phosphate buffer, pH 7.4, and stirred in a water bath at 37° for 4 hr. Buffers were prepared from reagent grade chemicals. Oxygen gas (95% oxygen–5% carbon dioxide) was bubbled through the preparation at room temperature for 90 min. The suspension was then stirred for an additional 4 hr. The mixture was then allowed to stand overnight (14 hr) with continual bubbling of oxygen.

The resultant precipitate of melanin was collected after centrifuging (19,000 rpm) at −2° and washed by resuspending in distilled water. The washings, followed by centrifugation, were performed until a clear supernatant solution was obtained. The washed precipitate thus obtained was freeze dried. The yield of melanin was approximately 1.41 g. The material exhibited a characteristic free radical signal in the electron spin resonance spectrometer[3] (like that seen in the natural melanins).

Methodology for Binding Studies—In all binding studies, the general procedure used was as follows. Aliquots of 1.0, 2.0, 4.0, 6.0, and 8.0 mg of synthetic melanin were placed in five 25-ml erlenmeyer flasks. Amounts of radiolabeled substances were added to each flask in the concentrations of 2 nCi/ml for ^{14}C-labeled materials and 6

[1] Merck Sharp & Dohme Research Laboratories, West Point, Pa.
[2] Sigma Chemical Co., St. Louis, Mo. (3690 units/mg).
[3] The spectra was supplied through the courtesy of Dr. L. Malspeis, College of Pharmacy, Ohio State University.