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STUDIES ON THE MECHANISM OF DRUG-BINDING TO MELANIN

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Abstract—The binding to melanin of chlorpromazine, chloroquine, paraquat and Ni^{2+} has been studied *in vitro* with pigment from beef eyes. The results showed a marked influence of the ionic environment on the ability of the organic substances to bind to melanin, indicating that electrostatic forces between the cationic forms of the substances and anionic sites on the melanin polymer (presumably carboxyl groups) are important for the complex formation. An analysis of the binding by the method of Scatchard showed that more than one binding class must be implicated in the binding of both the organic substances and Ni^{2+} to melanin. Several concordances were found for the data of the paraquat- and Ni^{2+}-binding, indicating a dominant influence of electrostatic forces for the melanin-binding of paraquat. However, several indications were found that non-electrostatic contributions must be added to form the binding-sites for chlorpromazine and chloroquine. It is possible that such contributions may be provided by van der Waals forces occurring at the conjunctions of the aromatic rings in the substances and the aromatic indole-nuclei of the melanin. Experiments with chlorpromazine indicated that the positive ion radical of the substance had a very high melanin-affinity. It is suggested that melanin may be able to oxidize chlorpromazine to a positive ion radical, explaining the firm binding of the substance to melanin and the evidence in the literature favouring this possibility are discussed.

Melanin has a capacity to accumulate many chemically and pharmacologically unrelated drugs [1–5]. The long-term administration of drugs with a high melanin affinity—such as chlorpromazine and chloroquine—may induce toxic lesions in the melanin-containing tissues [2, 4]. The molecular binding mechanism between drugs and melanin is not known in detail. Melanin possesses a stable free radical [6, 7] and it has been proposed that drugs which are good electron donors would be able to participate in a charge–transfer complex with the free radical of melanin [2, 8]. However, it has recently been shown that the free radicals of melanin are inaccessible to attacks by chemical reagents [9].

We have recently shown that the bisquaternary ammonium compound paraquat, which is widely used as a herbicide, is bound to melanin *in vivo* and *in vitro* [10, 11]. The results obtained indicated that ionic binding plays an important role in the interaction of paraquat and melanin. It is known that metal ions show a strong affinity for melanin, which can be ascribed to a cation-binding activity of anionic groups on the melanin-polymer [12–14]. This cation-binding activity also seems to be important for the binding of paraquat to melanin. Many drugs with known melanin-affinity are present as cations at physiological pH and electrostatic forces may be involved more generally in the interaction of such drugs and melanin.

The present investigation is intended to elucidate the mechanism of drug-binding to melanin. We have chosen to study the binding of chlorpromazine and chloroquine. Experiments with paraquat are also included. In addition data on the melanin-binding of an inorganic cation, Ni^{2+}, are presented and compared with the results obtained with the organic substances.

MATERIALS AND METHODS

Materials. Chlorpromazine chloride was obtained from AB Leo, Helsingborg, Sweden; chloroquine diphosphate from Bayer AG, Leverkusen, Western Germany; paraquat dichloride from Imperial Chemical Industries Ltd., Macclesfield, England. [^{35}S]Chlorpromazine chloride, sp. act. 20.3 μCi/mg, [^{14}C]chloroquine diphosphate (ring-3-^{14}C), sp. act. 58.8 μCi/mg, [^{14}C]paraquat dichloride [*N,N'*-dimethyl-4,4'-dipyridylium dichloride (^{14}C-methyl)], sp. act. 125 μCi/mg, and $^{63}NiCl_2$, sp. act. 10.4 mCi/mg Ni^{2+}, were obtained from the Radiochemical Centre, Amersham, England. Other chemicals used in the study were of analytical grade and purchased from regular commercial sources.

Pigment preparation. Pigment from beef eyes was prepared as described by Potts [2]. The uveal tissue was ground in a mortar with washed sea sand. The pigment granules—in this way released—were then isolated from other cellular components by repeated centrifugations in distilled water in a Sorvall SS-1 centrifuge. The final pigment granule suspension was adjusted to contain 10 mg by dry weight of pigment granules per ml suspension. This suspension was stored at +2° until used.

Melanin-binding. The binding to melanin of chlorpromazine, chloroquine, paraquat, and Ni^{2+} was studied mainly as described by Potts [2]. The substances were added to melanin suspensions in plastic centrifuge tubes. Usually 1 ml of the pigment granule suspension containing 10 mg of pigment granules was added to 6 ml incubation medium. In some cases a lower amount of pigment granules was used. The incubation medium was distilled water, or distilled water supplemented

with HCl or various metal salts. When indicated, the medium was buffered with M/15 Sorensen buffer pH 7.0. The incubations were performed at room temperature for 45 min. During this period, the tubes were shaken several times. The pigment granules were sedimented by centrifugation at 35,000 *g* for 10 min in an MSE 25 High Speed centrifuge. The concentrations of chlorpromazine, chloroquine, paraquat, and Ni^{2+} remaining in the supernatants were then measured. Identically treated samples in which the pigment suspensions were replaced by distilled water served as reference samples. Spectrophotometric measurements were made in a Hitachi Perkin–Elmer 124 spectrophotometer. Chlorpromazine was measured at 307 nm, chloroquine at 256 nm, and paraquat at 257 nm. Measurements by liquid scintillation counting were made in a Packard Tricarb model 2405 liquid scintillation spectrometer. Quenching was corrected by the use of an external standard. Ni^{2+} was always quantified by liquid scintillation counting. The organic substances were quantified by liquid scintillation counting only at the low concentrations. A comparison of quantitations performed with spectrophotometry and with liquid scintillation counting showed a good agreement. The pH of the incubation solutions was determined by means of a PHM62 Standard pH-meter with a glass-electrode (Radiometer, Copenhagen, Denmark).

Potentiometric titrations. Potentiometric titrations were performed essentially as described by Albert [15]. The principle of the method is that when a mixture of two substances which form a complex is titrated with alkali, and H^+-ions are liberated, the curve obtained will strike a path which is independent of those of the components, while when no complex is formed the curve obtained reproduces the component curves.

Potentiometric titrations were performed only with paraquat. Paraquat is a bisquaternary amine and hence the degree of ionization cannot be changed by variation in pH, which makes it suitable for the titrations. On the other hand, chlorpromazine and chloroquine will form non-ionized water-insoluble bases as the pH increases, which make the results of the titrations difficult to interpret.

In the experiments, solutions to be used in the titrations were prepared by mixing (a) 1.0 ml pigment granule (10 mg) suspension in distilled water with 10 ml 10^{-2} M HCl, (b) 1.0 ml pigment granule (10 mg) suspension in distilled water with 10 ml 10^{-2} M HCl containing $2 \cdot 10^{-2}$ M paraquat and (c) 1.0 ml distilled water with 10 ml 10^{-2} M HCl containing $2 \cdot 10^{-2}$ M paraquat. To these solutions was added $5 \cdot 10^{-2}$ M NaOH in small portions. The pH was recorded after each addition.

Analysis of melanin-binding. To determine if more than one class of binding sites on the melanin is implicated and to derive association constants, the binding of chlorpromazine, chloroquine, paraquat, and Ni^{2+} was analyzed by the method developed by Scatchard and his associates [16–18].

In our investigation, the value n for the number of binding sites cannot be considered to be an integer, since the molecular weight of melanin is unknown. Furthermore the molar ratio $\bar{\nu}$ is used for the ratio between the number of μmoles of bound ions or molecules and the dry weight of melanin in milligrams. In the experiments, 5 mg pigment was used for each determination. However, unpublished experiments have shown that the melanin-content of the pigment granules can be approximated to 50 per cent (w/w). Therefore the calculations of $\bar{\nu}$ have been based on a melanin content of 2.5 mg/incubation.

RESULTS

Initially, the effect of various amounts of Na^+-ions on the binding of chlorpromazine and chloroquine to melanin was studied in a buffered and in an unbuffered medium (Fig. 2). It was found that Na^+ inhibited the binding of both chlorpromazine and chloroquine to melanin. The degree of inhibition increased with the concentration of Na^+ in the medium. The melanin-binding was somewhat lower in the aqueous than in the buffered media. The buffered media had a pH 7.0 and contained Na^+ and K^+ in concentrations of, respectively, about 10^{-2} M and $4 \cdot 10^{-3}$ M. The pH-values of the aqueous media were somewhat lower than the buffered media (pH 5.9–6.7). As will be seen below, H^+-ions will influence the binding of chlorpromazine and chloroquine to melanin. This probably explains the lower melanin-binding in the aqueous media. The concentrations of Na^+ and K^+ in buffered media were too low to exert any marked interference. Phenothiazines may form micelles in aqueous solutions and the presence of sodium chloride will facilitate the micelle formation [19]. At the concentration where the micelle formation begins (the critical micelle concentration) there is a sudden decrease in the pH of the solution [19]. We determined the critical micelle concentration for chlorpromazine in 1 M sodium chloride solution (the highest NaCl concentration used). It was found to be about 10^{-3} M of chlorpromazine, which is approximately 3 times higher than the concentration used in the melanin incubations ($3.57 \cdot 10^{-4}$ M). Thus,

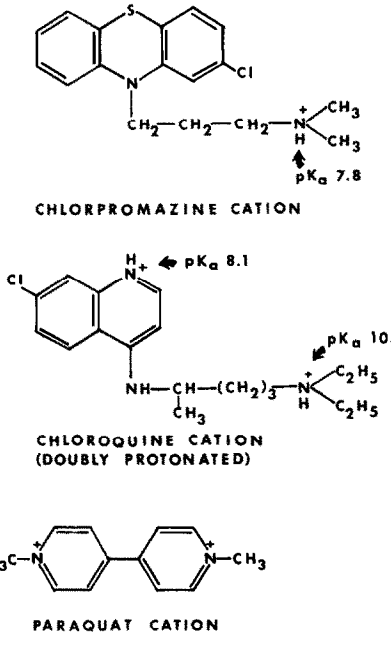

Fig. 1. Structural formulae of the studied substances.

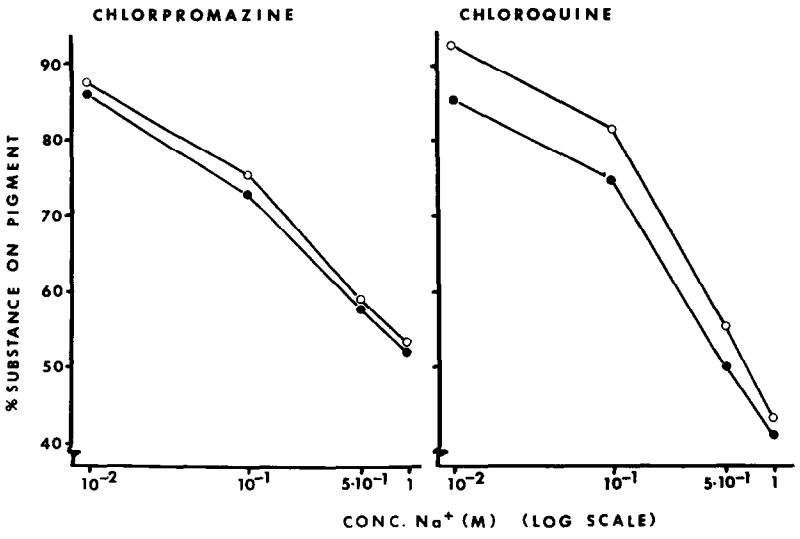

Fig. 2. Effect of Na^+ on the binding of chlorpromazine and chloroquine to melanin. 2.5 μmoles of chlorpromazine or chloroquine were added to pigment granule (10 mg) suspensions in distilled water or in phosphate buffer (pH 7.0), containing various amounts of NaCl. After 45 min incubation, the binding of substance to pigment was determined. (○——○) buffered medium; (●——●) aqueous medium.

the melanin-binding of chlorpromazine at the increased sodium chloride concentration is not influenced by micelle formation.

The ability of other metal ions to inhibit the binding of chlorpromazine, chloroquine and paraquat to melanin was then studied (Table 1). It was found that the metal ions inhibited the binding and that—with one exception—their effectiveness increased with increasing valencies. The exception was Pb^{2+}, which was more effective than La^{3+} in inhibiting the melanin-binding of chlorpromazine. It has previously been shown that Pb^{2+} has a very high melanin affinity [13]. Some variations were found in the pH-values of the incubation solutions (Table 1). However, as will be seen below, these variations are too small to have any relation to the inhibiting capacities of the metal ions. To exclude the possibility that anions might interfere with the melanin-binding, experiments were performed in which chlorpromazine was incubated in media with various anionic compositions (Table 2). It was obvious that the influence on the melanin-binding of chlorpromazine was caused by the cations in the incubation media and not by the anions, which seemed to be without effect. In an additional experiment, the binding of chlorpromazine to pigment granules which had been pretreated with $Pb(NO_3)_2$ was studied (Table 3). It was found that the pretreated pigment granules had a markedly reduced capacity to bind chlorpromazine.

In further experiments, various concentrations of HCl were present in the incubation solutions together with chlorpromazine, chloroquine or Ni^{2+} (Fig. 3). A marked inhibition of the melanin-binding was seen at H^+-ion concentrations of 10^{-4} M–10^{-2} M, which is indicative of a competition between the H^+-ions and chlorpromazine, chloroquine and Ni^{2+} for a receptor site on the melanin with the character of a weak acid. The binding of chloroquine was depressed from over 80 per cent to about 7 per cent. The binding of Ni^{2+} was

Table 1. The ability of metal ions to inhibit the binding of chlorpromazine, chloroquine and paraquat to melanin *

Metal ion	pH of 0.1 M metal ion solution	Per cent substance bound to pigment in a 0.1 M metal ion solution		
		Chlorpromazine	Chloroquine	Paraquat
—[+]	6.0‡	85.7	85.5	68.0
Na^+	5.7	71.0	72.7	39.7
K^+	5.5	67.8	68.6	34.8
Ca^{2+}	7.3	36.2	22.8	7.2
Ni^{2+}	5.5	30.7	16.4	5.9
Pb^{2+}	4.0	16.6	—§	0
La^{3+}	4.6	19.0	—§	0

* 2.5 μmoles substance were incubated in 7 ml pigment granule (10 mg) suspensions containing 0.1 M NaCl, KCl, $CaCl_2$, $NiCl_2$, $Pb[NO_3]_2$ or $LaCl_3$. After 45 min incubation, the binding of substance to pigment was determined.

[+] Control: no metal ion added.

‡ pH of distilled water.

§ Chloroquine is not soluble in 0.1 M $Pb(NO_3)_2$ or $LaCl_3$.

Table 2. Binding of chlorpromazine to melanin in media with various anionic compositions *

Salt	Concn salt (M)	Concn cation (M)	Concn anion (M)	pH of salt solution	% Chlorpromazine bound to pigment
—†	—†	—†	—†	6.0‡	85.7
KCl	0.1	0.1	0.1	5.7	68.2
KBr	0.1	0.1	0.1	6.3	69.3
KI	0.1	0.1	0.1	5.6	69.9
$CaCl_2$	0.1	0.1	0.2	7.3	36.2
$Ca(NO_3)_2$	0.1	0.1	0.2	5.7	34.3
$Pb(NO_3)_2$	0.1	0.1	0.2	4.0	16.6
NaCl	0.3	0.3	0.3	5.7	66.8
$LaCl_3$	0.1	0.1	0.3	4.6	19.0

* 2.5 μmoles chlorpromazine were incubated in 7 ml pigment granule (10 mg) suspensions containing the stated amount of salt. After 45 min incubation, the binding of chlorpromazine to pigment was determined.

† Control: no salt added.

‡ pH of distilled water.

completely depressed by H^+ and this has previously been shown to be the case also with paraquat [10]. In contrast the H^+-ions were less effective in inhibiting the binding of chlorpromazine. The lowest value was obtained at 10^{-1} M HCl with about 29 per cent chlorpromazine-binding. At higher H^+-ion concentrations there was an increase in the chlorpromazine-binding to over 40 per cent at 2 M HCl. The increased binding coincided with the appearance of a red reaction product of chlorpromazine. The absorption spectrum of this product showed absorption maxima at 530 nm and 255 nm. This has previously been shown to be a positive ion radical of chlorpromazine formed as the first oxidation product in concentrated solutions of HCl or H_2SO_4 [20]. The increased binding of chlorpromazine at high HCl-concentrations is also obtained with pigment from which the protein part has been removed by hydrolysis (i.e. free melanin) (unpublished observation).

The titration experiments showed that for paraquat alone there was a steep increase in the pH when about 2.0 ml $5 \cdot 10^{-2}$ M NaOH had been added (Fig. 4). The path of this curve indicates that no reaction has occurred between the paraquat and the added H^+- or OH^--ions. The pigment curve had a different path, which can be explained by assuming an initial binding of H^+-ions to the melanin with a gradual liberation as NaOH is added. The paraquat + pigment-curve had yet a different path. It can be explained by assuming a competition between paraquat and H^+-ions for binding-sites on the melanin. As the pH increases, paraquat will become bound to the melanin, and H^+-ions will then be liberated and dislocate the titration curve to the right.

The analysis of the binding by the method of Scatchard showed that the plot $\bar{v}/c$ versus $\bar{v}$ was curvilinear with an upward concavity in all cases (Fig. 5), indicating that more than one binding class must be implicated. In addition an upward convexity of the plot was present at the low concentrations of paraquat and Ni^{2+}. We interpret this as an inhibition of the binding caused by a competition with "endogenous" cations in the melanin polymer (see Discussion). The results presented in Fig. 5 show that the data must be analyzed in terms of more than one class of binding sites. The analysis of the data are shown in Table 4 (the data from the upward convex parts of the paraquat- and Ni^{2+}-curves are not included). It was found that the data for

Table 3. Binding of chlorpromazine to melanin which has been pretreated with $Pb(NO_3)_2$ *

	Dist. water	Pretreatment 0.05 M $Pb(NO_3)_2$	0.1 M $Pb(NO_3)_2$
Per cent chlorpromazine bound	85.7	40.3	30.9

* Ten mg pigment granules were incubated in 10 ml 0.05 M $Pb(NO_3)_2$, 10 ml 0.1 M $Pb(NO_3)_2$ or 10ml dist. water for 45 min. After that, the pigment granules were washed twice with dist. water. Incubations for 45 min with 2.5 μmoles chlorpromazine were then performed and the binding of the substance to the pigment was determined.

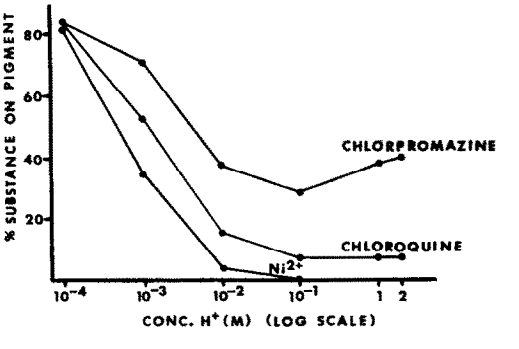

Fig. 3. Effect of H^+ on the binding of chlorpromazine, chloroquine and Ni^{2+} to melanin. 2.5 μmoles of chlorpromazine, chloroquine or $NiCl_2$ were added to pigment granule (10 mg) suspensions in distilled water containing various amounts of HCl. After 45 min incubation, the binding to the pigment was determined.

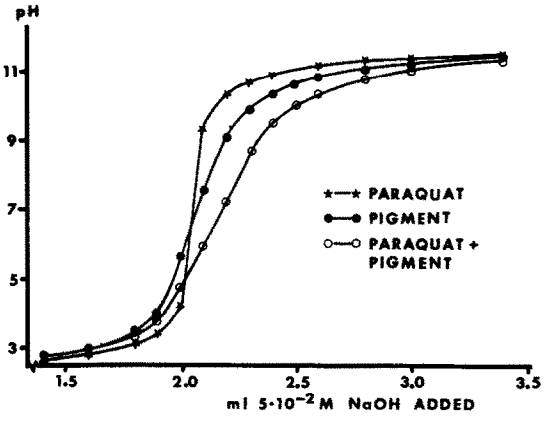

Fig. 4. Titration with NaOH of acidified solutions containing paraquat, pigment granules or paraquat + pigment granules. The solutions contained $2 \cdot 10^{-2}$ M paraquat, 10 mg pigment granules or both.

chlorpromazine and chloroquine could be best fitted by assumption of three classes of binding sites, while for paraquat and Ni^{2+} the best fit was obtained on the assumption of two classes of binding sites. The data for the chlorpromazine-binding indicate a small number of

Table 4. Binding parameters for the interaction of chlorpromazine, chloroquine, paraquat and Ni^{2+} with melanin

Apparent association constants (K; M^{-1}) and number of binding-sites (n; μmoles per mg melanin) analyzed by the method of Scatchard		
Chlorpromazine	$n_1 = 0.14$	$K_1 = 7.3 \cdot 10^6$
	$n_2 = 0.38$	$K_2 = 8.8 \cdot 10^4$
	$n_3 = 0.25$	$K_3 = 2.2 \cdot 10^3$
Chloroquine	$n_1 = 0.28$	$K_1 = 7.4 \cdot 10^5$
	$n_2 = 0.15$	$K_2 = 3.4 \cdot 10^4$
	$n_3 = 0.26$	$K_3 = 1.5 \cdot 10^3$
Paraquat	$n_1 = 0.31$	$K_1 = 6.0 \cdot 10^5$
	$n_2 = 0.34$	$K_2 = 2.6 \cdot 10^4$
Ni^{2+}	$n_1 = 0.31$	$K_1 = 5.2 \cdot 10^6$
	$n_2 = 0.34$	$K_2 = 2.7 \cdot 10^4$

binding sites (n_1) with a very high association constant (K_1). The number of strongly reacting sites (n_1) are more for chloroquine than for chlorpromazine, but K_1 for chloroquine is much lower than K_1 for chlorpromazine. The values n_2 and K_2 also differ for chlorpromazine and chloroquine—more binding-sites with a higher association constant being present for chlorpromazine than for chloroquine. The number of weakly

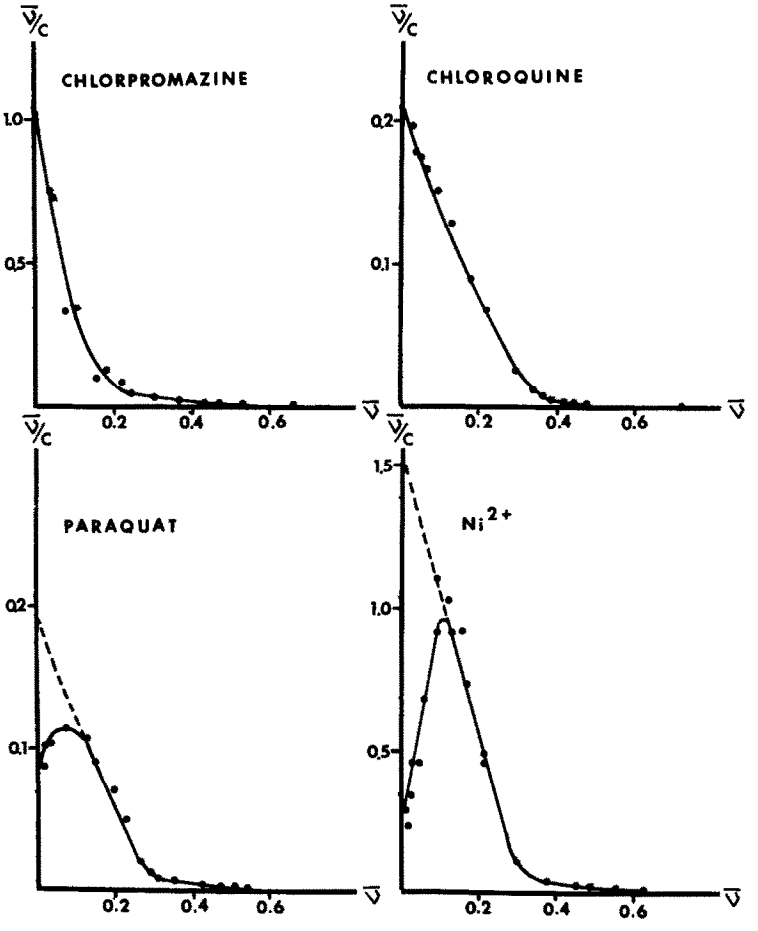

Fig. 5. Scatchard plots for the melanin-binding of chlorpromazine, chloroquine, paraquat and Ni^{2+}. $\bar{\nu}$: μmoles substance (or ion) bound per mg melanin; c: concentration (μ M) of the unbound fraction of substance (or ion). The dotted parts of the paraquat and Ni^{2+}-curves are extrapolations. The data in Table 4 comprise the properties of these extrapolated curves.

reacting sites (n_3) and the corresponding association constants (K_3) showed rather close values for chlorpromazine and chloroquine. For paraquat and Ni^{2+} the number of strongly reacting sites (n_1) and weakly reacting sites (n_2) attained identical values, indicating that the binding occurs to the same classes of sites on the melanin. In addition, the binding to the weakly reacting sites occurred with approximately the same association constant (K_2). On the other hand, the association constant for the strongly reacting sites (K_1) was much higher for Ni^{2+} than for paraquat. A comparison of the binding parameters for chlorpromazine and chloroquine with those for paraquat and Ni^{2+} reveals no obvious concordance. It can be noted that the total binding capacity (Σn_i) of the melanin for the studied compounds falls within a relatively narrow range (0.65–0.77 μmoles/mg).

DISCUSSION

In order to simulate the *in vivo* situation as closely as possible, we chose to perform the present study with pigment from beef eyes, i.e. with melanin attached to a protein-moiety. Unpublished experiments at our department have shown that "free" melanin, released through hydrolysis of the protein-moiety, has the same or even higher binding capacity than that of the pigment granules when the latter is calculated on basis of the melanin content. This indicates that the protein-moiety takes no part in the binding process to the pigment granules and it is therefore assumed that the data of the present study reflects the binding to the melanin-part of the pigment granule.

The results of the present study have shown a marked influence of the ionic environment on the ability of chlorpromazine, chloroquine and paraquat to bind to melanin. This indicates that electrostatic forces are important in the complex formation. At physiological pH in dilute aqueous solutions, chlorpromazine exists mainly as a mono-protonated cation ($pK_a \sim 7.8$ [21]) and chloroquine as a doubly protonated cation (pK_a values 8.1 and 10.2 [22]) (Fig. 1). Paraquat is a bisquaternary amine and thus is present as cation regardless of the pH. The binding of inorganic cations to melanin show characteristics which suggest that the interaction takes place by ion exchange [12–14]. Characteristics of weak acid cation exchangers are a high affinity for H^+ and an increasing affinity for cations with increasing valence [23]. H^+ was (although less pronounced for chlorpromazine—which will be discussed later) found in the present study to be an effective inhibitor of the melanin-binding, and the ability of the metal ions to inhibit the binding increased with increasing valence. The potentiometric titration which was performed with paraquat indicated that paraquat was able to compete with H^+-ions for the same binding-sites on the melanin.

Melanin is a polymer composed basically of indole-5,6-quinone units, which can occur in different stages of oxidation [24]. Several dopachrome-units and units of 5,6-dihydroxyindole carboxylic acids are present in the polymer [24]. Melanin therefore contains many free carboxyl groups in addition to phenolic and/or quinonoid groups. Presumably ionic attraction occurs between the positively charged substance molecules and the anionic carboxyl groups of the melanin.

The experiments with Ni^{2+} and the analysis of the binding by the method of Scatchard provide further information on the mechanism of the binding. The Scatchard analysis indicates that more than one binding class must be implicated in all cases. The binding probably occurs to the free carboxyl groups, but steric hindrances and physico-chemical conditions at the binding sites may make them differentially available for interaction. It is also possible that additional binding sites are provided by groups such as the phenolic OH, which are present in the melanin. The Scatchard analysis further showed a concordance between the number of weakly and strongly reacting binding sites for paraquat and Ni^{2+}, indicating that their binding occurs to the same classes of sites on the melanin. This, in turn, suggests that electrostatic forces are of major importance for the binding of paraquat to melanin. Ni^{2+}, which is a small cation, may be able to attain a close contact with the anionic sites, explaining why K_1 is higher for Ni^{2+} than for paraquat. Both for paraquat and Ni^{2+} there was also an upward convex part of the Scatchard plot $\bar{v}/c$ versus v at the low concentrations. The diminished melanin binding at the low concentrations of paraquat and Ni^{2+} may be caused by a competition with "endogenous" cations, which may be present in the melanin polymer. A positive cooperativity between the binding sites would also give a similar curve. This type of binding presupposes that conformational changes of the binding sites take place when occupancy increases. We consider it less likely that the electrostatic binding of paraquat or Ni^{2+} to anionic sites on the melanin polymer would induce such conformational changes. The upward convexity was not found at the low concentrations of chlorpromazine and chloroquine. A probable explanation is that non-electrostatic forces play an important role for the binding of these substances at the low concentrations. That non-electrostatic contributions must be added to form the binding sites for chlorpromazine and chloroquine is further indicated by the observation that—in contrast to paraquat and Ni^{2+}—it was not possible completely to depress the melanin-binding of chlorpromazine and chloroquine with H^+-ions. Neither were the number and classes of binding sites for chlorpromazine and chloroquine the same as for paraquat and Ni^{2+}. In addition, the fact that the association constants K_1 and K_2 were higher for the monovalent chlorpromazine-cation than for the divalent chloroquine-cation indicates that non-electrostatic forces are operative. It is possible that the non-electrostatic contribution is provided by van der Waals forces occurring at the conjunctions of the aromatic rings in the substances and the aromatic indole-nuclei of the melanin. Similar attractive forces have been proposed for the intercalative binding of aminoacridines and other substances to nucleic acids [25, 26]. The latter binding probably involves an ionic attraction between the positively charged substance-molecules and the anionic phosphate groups of the nucleic acids, supplemented by additional forces due to conjunctions of the aromatic rings of the substances and the purines and pyrimidines of the nucleic acids [25]. Thus, there may be a similar mode of binding of substances to melanin and to nucleic acids. For example chloroquine and acridine orange are both bound to melanin [2], and they also show the mentioned form of affinity for nucleic acids [25, 27].

In strong HCl or H_2SO_4 a red reaction product with absorption maxima at 530 and 255 nm is formed from chlorpromazine. This has been shown to be a positive ion radical of the substance formed by a single-electron-transfer process as the first oxidation step of the drug [20]. The positive charge of the ion radical is probably localized to the thiazine nucleus [28]. This product was formed in our experiments and in spite of a very high H^+-ion concentration, the melanin-affinity increased at the formation of the positive ion radical. It is possible that the increased melanin-affinity is a consequence of the introduction of the positive charge into the thiazine nucleus. The positive ion radical may be formed by enzymatic action at physiological conditions [29]. It has been shown that melanin may participate in oxidation reactions such as the oxidation of reduced nicotinamide adenine dinucleotide (NADH) [30, 31]. It has further been shown that phenothiazines inhibit the ability of melanin to catalyze the oxidation of NADH [30]. Baldry and Swan [9] propose that the oxidation of NADH may occur because quinone units in the melanin might act as electron acceptors and that phenothiazines might be able to block the quinonoid reaction centres. An interesting possibility is that melanin may be able to oxidize chlorpromazine to a positive ion radical. This may take place via an electron-transfer process to the quinone units, which then will become blocked for further oxidation reactions. As shown in the present study, the formed positive ion radical will have a very high melanin-affinity, which may partly explain the firm binding of chlorpromazine to melanin. Ion radicals are formed from other phenothiazine derivatives [20] and a similar interaction with melanin is then also conceivable. An interaction of the ion radical of chlorpromazine with DNA has also been shown [32].

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