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Interaction of Quinacrine Mustard with Mononucleotides and Polynucleotides

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1. The interaction between quinacrine mustard and mononucleotides and polynucleotides was investigated by fluorimetry and absorbance spectrophotometry. 2. The fluorescence spectrum of quinacrine mustard is independent of the ionic strength and pH. The dependence of the quinacrine mustard fluorescence intensity on ionic strength, pH and anions is described. 3. The fluorescence intensity of quinacrine mustard was enhanced with the mononucleotide adenylic acid and polynucleotides such as poly(rA), poly(rU) and poly(rA,rU). 4. Quenching of the fluorescence intensity of quinacrine mustard occurred with the mononucleotide guanylic acid and with poly(rG) and poly(rC,rG). 5. The mononucleotide cytidylic acid or poly(rC) showed no effect on the fluorescence intensity of quinacrine mustard. 6. The interaction between the dye and native DNA species was also dependent on the presence of base-specific binding sites in the DNA. The higher the (G+C) content was in the native DNA tested the higher was the quenching effect on the fluorescence intensity of quinacrine mustard. 7. No interaction was found between the dye and methylated DNA. The binding between quinacrine mustard and apurinic DNA was confirmed to be in the phosphate groups of the purines.

The acridine dyes are able to bind to nucleic acids in different ways. Proflavine, acriflavine and Acridine Orange are examples of basic polycyclic dyes without side chains but with aromatic groups (Albert, 1966). They are all capable of ionic binding to phosphate groups in nucleic acids and of intercalation between nucleic acid bases (Blake & Peacocke, 1968; Löber & Achtert, 1969; Thomes *et al.*, 1969; Caspersson *et al.*, 1969*a*).

The acridine dye quinacrine has a basic side chain (Albert, 1966) and represents a class of agents capable of ionic binding and intercalation as well as base-specific interaction with nucleic acids. The quinacrine interacts with bi-helical polynucleotides, such as poly-(dA-dT) and poly(dA),poly(dT), and considerably enhances the fluorescence intensity. Conversely, poly(dG) or poly(rG) both quenched the fluorescence intensity of quinacrine (Weisblum & de Haseth, 1972). Actinomycin D (Kleiman & Huang, 1972), chloroquine (Cohen & Yielding, 1965), nogalamycin (Ward *et al.*, 1965) and ethidium bromide (LePecq & Paoletti, 1967) are all examples of agents that are able to bind to bases in nucleic acids by non-covalent or covalent bindings with or without intercalation.

According to Caspersson *et al.* (1969*b*) the cationic acridine dye quinacrine mustard with a basic side chain binds to the 7-amino group in guanine. The dye is also capable of intercalation and ionic binding in the same way as quinacrine. Caspersson *et al.* (1969*a*) chose quinacrine mustard for staining cytological preparations because of the assumed guanine specificity of the dye. Nowadays, both quinacrine and quinacrine mustard are used as specific dyes for staining chromosomes *in situ*. Both dyes give a specific fluorescence banding pattern of the chromosomes, which makes it possible to characterize the individual chromosomes. The banding pattern of the chromosomes has not yet been biochemically explained. Ellison & Barr (1971) proposed that the quinacrine-bright area in the nucleus of *Samoiaia leonensis* was characterized by a high (A+T) content.

The aim of the present investigation with synthetic mononucleotides and polynucleotides has been to explain the variations in the intensity of quinacrine mustard fluorescence along metaphase chromosomes. Studies on the interaction between the dye and nucleic acid polymers in solution are reported.

Experimental

Materials

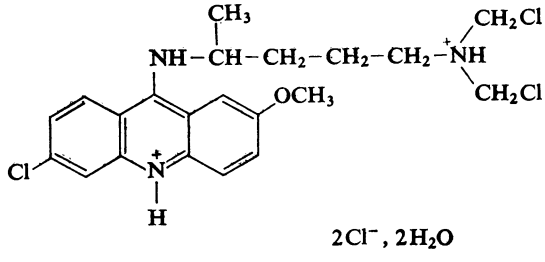
Mononucleotides. Mononucleotides were purchased from Fluka A.G., Buchs., Switzerland: cytidine 5′-monophosphoric acid; guanosine 5′-monophosphoric acid; thymidine 5′-monophosphoric acid,

disodium salt; cytidylic acid; adenosine 3'-(and 2'-)-monophosphoric acid; guanosine 3'-(and 2'-)monophosphoric acid; adenosine 5'-monophosphoric acid; deoxyadenosine 5'-monophosphoric acid; deoxycytidine 5'-monophosphoric acid; deoxyguanosine 5'-monophosphoric acid.

Polynucleotides. The potassium salt of polyadenylic acid was purchased from Research Products Division, Miles Laboratories Inc., Ind., U.S.A., and the other polynucleotides from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. The latter were the potassium salt of polycytidylic acid (5'-linked), the sodium salt of polyguanylic acid (5'-linked) and the potassium salt of polyuridylic acid (5'-linked). The phosphorus content of the polynucleotides was approx. 7.0%.

Species of DNA. DNA from adult mouse liver, from adult human blood and from *Saccharomyces cerevisiae* was isolated according to Marmur (1961). DNA from *Escherichia coli*, calf thymus and *Clostridium perfringens* was purchased from Sigma (London) Chemical Co. Ltd.

Quinacrine mustard. 2-Methoxy-6-chloro-9-[4-bis-(β-chloroethyl)-amino-1-methylbutylamino]-acridine with mol.wt. 514.28 ($2H_2O$) was a gift from the Sterling-Winthrop Research Institute, Rensselaer, N.Y. U.S.A. The structure of the double protonated cation is shown in (I) (Jones *et al.*, 1957).



(I)

Quinacrine mustard is highly fluorescent with maximal fluorescence in alkaline solution at pH 11–12, as is shown in Figs. 1 and 3. The fluorescence has two excitation peaks, one at 285nm and the other at 424nm, and a fluorescence peak at 514nm (uncorrected). The absorption spectrum is shown in Fig. 2, with peaks at 280, 345, 424 and 455nm. The molar extinction coefficient at 424nm was calculated to be 14.62×10^3. At 280nm the molar extinction coefficient was 8.6×10^4. The spectrum obeyed Beer's law between 325nm and 500nm, up to 2.0×10^{-4}mol of quinacrine mustard, and between 200nm and 320nm up to 0.6×10^{-4}mol of quinacrine mustard. Stock solutions of quinacrine mustard (approx. 2mg/ml) in water were stable for 2 weeks in the dark at 4°C and remained clear the whole time.

Buffer system. McIlvaine's buffer was made by mixing 0.1 M-citric acid and 0.2 M-disodium phosphate in various proportions to obtain different pH values (McIlvaine, 1921).

Methods

Fluorescence intensity and spectra. A fluorescence attachment ZFM4 to the Zeiss (PMQ II) spectrophotometer was used for determining the fluorescence intensity and spectra, with an excitation filter at 436nm. The spectrophotometer was standardized with a fluorescence standard cuvette F53, with excitation at 436nm and fluorescence peak at 530nm, and a no. 10 slit arrangement. The fluorescence spectra were not corrected for variation in the photocell at different wavelengths etc., but the fluorescence of quinacrine mustard was determined as a test. The nucleic acids, polynucleotides or mononucleotides do not interfere with the quinacrine mustard fluorescence at 514nm (Udenfriend, 1962). The fluorescence intensity of quinacrine mustard at 514nm was linear between 0.02×10^{-4} and 0.4×10^{-4}mol of quinacrine mustard. It was established that the alterations in fluorescence intensity that occurred after mixing the polymer and dye were complete within a few seconds and showed no further changes with time or additional mixing. Fluorescence intensity was measured with a constant amount in a volume of 3ml (32.5μmol of dye).

Measurement of DNA, mononucleotides and polynucleotides. Absorption measurements were recorded with a Zeiss (PMQII) spectrophotometer. The absorption of the polymers did not disturb the determinations of quinacrine mustard at 424nm. The concentrations of the polymers were determined at their respective absorption peaks. Phosphorus analysis was done as described by Tuan & Bonner (1969).

Results

Fluorescence and absorption spectra of quinacrine mustard

The fluorescence spectrum of quinacrine mustard is shown in Fig. 1, with excitation at 436nm and a fluorescence peak at 514nm. The fluorescence spectrum is independent of the ionic strength and pH of the solution, although the fluorescence intensity greatly depends on both the ionic strength and the pH, as shown in Fig. 3. Maximal fluorescence was obtained in alkaline solution at pH 11 in 0.05 M-Tris solution. Enhancement of intensity was found at pH 3 in McIlvaine's buffer. The absorption spectrum of quinacrine mustard in McIlvaine's buffer, pH 7, is shown in Fig. 2, the ratio of the absorption at 424nm and 365nm being 6.20. At pH values above 8 the peak at 280nm was shifted towards lower wavelengths and the absorbance at 280nm and 424nm was depressed, thus lowering the 424nm to 365nm ratio.

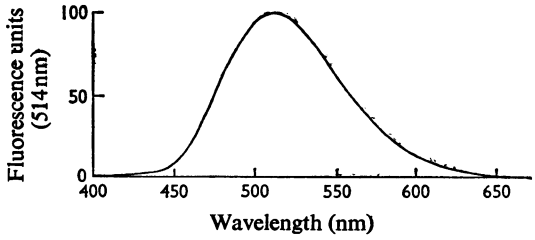


Fig. 1. *Fluorescence spectrum of quinacrine mustard*

The amount of quinacrine mustard was 32.5 μmol, in McIlvaine's buffer, pH 7, with excitation at 436 nm.

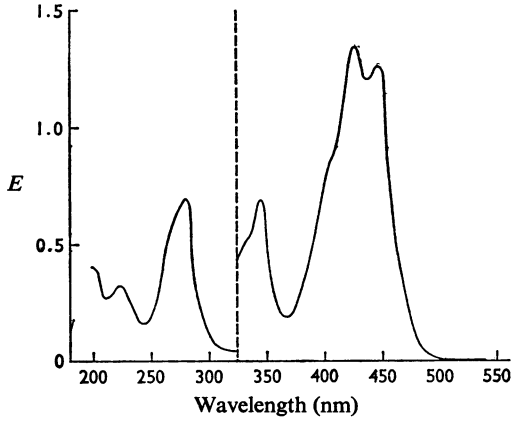


Fig. 2. *Absorption spectrum of quinacrine mustard*

The amount of quinacrine mustard was 156 μmol, in McIlvaine's buffer, pH 7, between 325 and 500 nm, and 15.6 μmol in the same buffer between 200 and 320 nm.

Effect of anions on the fluorescence intensity of quinacrine mustard

The anionic dependence of quinacrine mustard fluorescence is shown in Fig. 4 at 514 nm. The anions Cl^- and SO_4^{2-} both slightly enhanced the fluorescence intensity in the acetate buffer system. The enhancement was greater with sulphate at lower concentrations of the anion. There was slight quenching with the phosphate anion, which may in part be due to the high ionic strength and the high pH of the solution. Marked quenching was seen with the iodide anion (not shown in the figure).

Interaction between quinacrine mustard and mononucleotides

This interaction was pH-dependent. At pH values below 7 there was decreased interaction and at pH values above 7 there was increased interaction. The

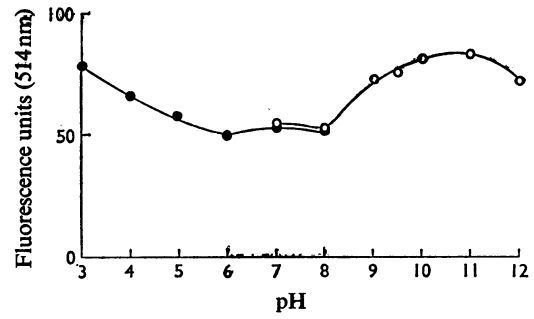


Fig. 3. *Effect of pH on the fluorescence intensity of quinacrine mustard*

The amount of quinacrine mustard was 32.5 μmol in McIlvaine's buffer (●) or in 0.05 M-Tris solution (○).

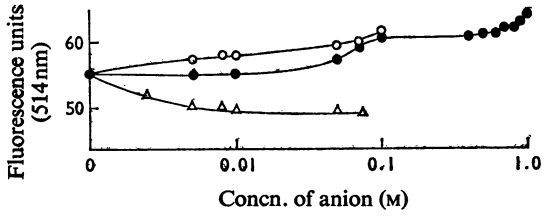


Fig. 4. *Effect of different anions on the fluorescence intensity of quinacrine mustard*

The amount of quinacrine mustard was 32.5 μmol in 17 mM-acetate buffer, pH 4.7, with added chloride (●) and sulphate (○) anions, or 32.5 μmol in sodium phosphate buffer, pH 7, with increasing amounts of phosphate (△).

mononucleotides have pK_a values 1.5 and 6.0 and are therefore fairly stable in alkali, in which they exist as dianions. They are hydrolysed by acid and also in the neutral solution in which the monoanion reacts (Ulbricht, 1964). The interaction between different mononucleotides and quinacrine mustard was measured fluorimetrically at 514 nm in McIlvaine's buffer at pH 7. The values obtained are reported in Table I. The greatest quenching was found with guanylic acids (rGuo-5′-*P* gave $-25.8\Delta\%$). The fluorescence data were expressed as the percentage change ($\Delta\%$) relative to the intensity of the fluorescence of quinacrine mustard alone. Cytidylic acids or dThd-5′-*P* had little or no effect on the fluorescence intensity of quinacrine mustard. A marked enhancement of intensity was found with rAdo-2′:3′-*P* ($+13.5\Delta\%$), whereas the other adenylic acids had almost no effect. In a mixture with two

Table 1. *Effect of mononucleotides on the fluorescence intensity of quinacrine mustard*

The concentration of mononucleotides was 2mg/ml and the amount of quinacrine mustard was 32.5μmol, in McIlvaine's buffer at pH7.

Mononucleotide	Change in fluorescence intensity of quinacrine mustard at 514nm (Δ%)
dAdo-5′-*P*	−3.2
dCyd-5′-*P*	0.0
dGuo-5′-*P*	−18.0
dThd-5′-*P*	−2.5
rAdo-5′-*P*	−5.5
rAdo-2′:3′-*P*	+13.5
rCyd-5′-*P*	0.0
rCyd-2′:3′-*P*	−2.3
rGuo-5′-*P*	−25.8
rGuo-2′:3′-*P*	−21.5
dAdo-5′-*P*+dThd-5′-*P*	−5.3
dCyd-5′-*P* + dGuo-5′-*P*	−9.6

mononucleotides: dCyd-5′-*P* plus dGuo-5′-*P*, the cytidylic acid altered the quenching effect of guanylic acid. Conversely the result from a mixture of dAdo-5′-*P* and dThd-5′-*P* was similar to that from either of the mononucleotides alone with quinacrine mustard.

Interaction between quinacrine mustard and polynucleotides

Quenching was seen with poly(rG) and poly(rC,rG) and the fluorescence intensity of quinacrine mustard was enhanced with poly(rA), poly(rU) and poly(rA,rU) (Fig. 5). No effect was found with poly(rC). The curve obtained with an increasing amount of poly(rC,rG) lies between the poly(rC) and poly(rG) curves, whereas the effect of poly(rA,rU) on the enhancement of fluorescence intensity was less than either fraction assayed alone with quinacrine mustard. These results suggest that fluorescence quenching is due to the guanine residue alone. The reverse curves obtained with constant amounts of polynucleotides and increasing amounts of quinacrine mustard are shown in Fig. 6. The greatest enhancement of the fluorescence intensity of quinacrine mustard was found with poly(rA) and the least with poly(rG). These curves agree with those given in Fig. 5. Poly(rG) and poly(rC,rG) altered the absorption spectrum of quinacrine mustard, which is shown in Fig. 7. These changes were of the same order as those produced by nucleic acids and are therefore considered specific. The failure of the other polynucleotides to bring about a significant change in the quinacrine mustard spectrum compared with the changes caused by poly(rG) and poly(rC,rG) shows that the change is due to the guanine residue. Actinomycin D has a similar guanine requirement (Kleiman & Huang, 1972). The slight changes in the absorption spectrum of quinacrine mustard caused by poly(rC) or poly(rU) were of the same order as those produced

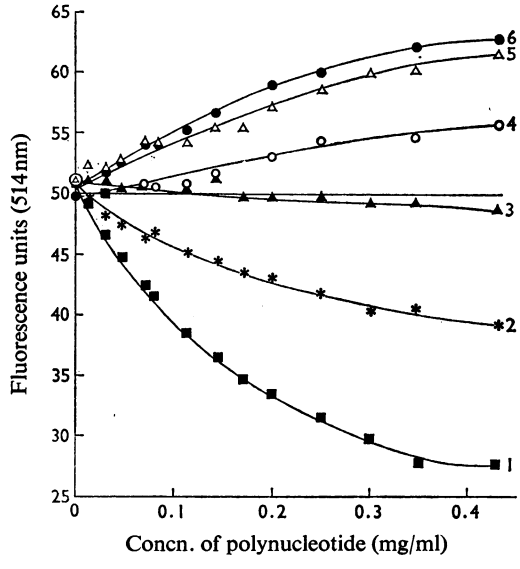


Fig. 5. *Effect of polynucleotides on the fluorescence intensity of quinacrine mustard*

A constant amount (26μmol) of quinacrine mustard was used with increasing concentrations of polynucleotides (mg/ml), in McIlvaine's buffer at pH7: ●, poly(rA); △, poly(rU); ○, poly(rA,rU); ▲, poly-r(C); *, poly(rC,rG); ■, poly(rG).

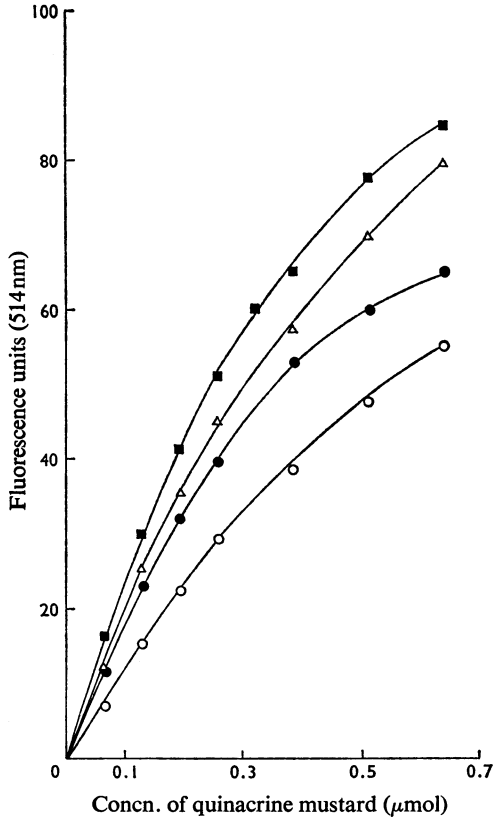


Fig. 6. *Fluorescence enhancement of quinacrine mustard bound to polynucleotides*

A constant concentration (0.125mg/ml) of poly(rA) (■), poly(rU) (△), poly(rC) (●) and poly(rG) (○) and increasing amounts of quinacrine mustard (μmol), in McIlvaine's buffer, pH7, were used.

by heparin (Stone & Bradley, 1967), and may therefore be considered as non-specific.

Comparison of different nucleic acids

The fact that a reaction occurred between quinacrine mustard and DNA was apparent since the mixing of DNA with solutions containing high concentrations of quinacrine mustard caused yellow fibres to precipitate. In quinacrine mustard–DNA mixtures quenching of the fluorescence intensity of quinacrine mustard is noted at 514nm.

The quenching of the fluorescence intensity of quinacrine mustard with an excess of different native DNA species is summarized in Table 2. DNA with the highest (G+C) content (*E. coli*) gave the strongest quenching of the fluorescence intensity of quinacrine

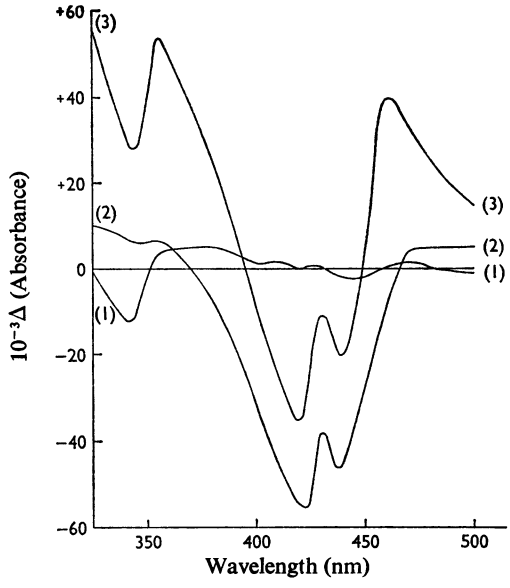


Fig. 7. *Difference spectra of quinacrine mustard produced by polynucleotides*

Quinacrine mustard (26μmol) and 0.43mg of polynucleotides/ml, in McIlvaine's buffer, pH7, were present: (1) poly(rC); (2) poly(rC,rG); (3) poly(rG).

mustard. Natural DNA species quench the fluorescence intensity of the dye apparently in relation to their (G+C) content. This finding is in line with the results reported by Weisblum & de Haseth (1972) for the interaction between quinacrine and nucleic acids.

Methylated DNA: S. cerevisiae

DNA was methylated according to Uhlenhopp & Krasna (1971) and gave an absorption peak at 262.5nm in NaCl–sodium citrate soln. (15mM-NaCl–1.5mM-sodium citrate, pH7.0). The phosphorus content after methylation was 12.0%. Fluorimetric titration with constant amounts of quinacrine mustard (32.5μmol) and increasing amounts of methylated DNA, from 0.02 to 1mg, was performed without any change in the fluorescence intensity of quinacrine mustard. Hence there was no interaction between quinacrine mustard and methylated DNA. These results suggest that quinacrine mustard reacts with guanine in DNA, because there was no reaction in methylated DNA, in which almost all amino groups in guanine are blocked (Ramstein *et al.*, 1971).

Apurinic acid

DNA from *S. cerevisiae* was prepared according to Tamm *et al.* (1952). This DNA had an absorption

Table 2. *Effect of various DNA species on the fluorescence intensity of quinacrine mustard*

The concentration of native DNA species was 0.05 mg/ml and the amount of quinacrine mustard was 32.5 μmol in 15 mM-NaCl–1.5 mM-sodium citrate at pH 7. Values for (G+C) content are taken from Sober (1968).

Source of native DNA	(G+C) content (%)	Change in fluorescence intensity of quinacrine mustard at 514 nm (Δ%)
E. coli	50	−32.4
Mouse liver	40	−19.1
Human	39.5	−22.9
Calf thymus	39	−21.8
C. perfringens	31	−13.1

peak at 265 nm and contained 11.7% phosphorus. Fluorimetric titration with a constant amount of quinacrine mustard (32.5 μmol) in NaCl–sodium citrate soln. and increasing amounts of apurinic DNA (from 0.025 to 1 mg) was performed. There was no change in the fluorescence intensity of quinacrine mustard before the addition of 0.3 mg of apurinic DNA, but at this point fluorescence intensity was noticeably enhanced, indicating an interaction between quinacrine mustard and the apurinic acid.

Discussion

O'Brien *et al.* (1966) showed that the interaction between quinacrine and nucleic acids does not depend on the base-specific sites in the nucleic acid. In contrast Weisblum & de Haseth (1972) suggested that the interaction was base-specific. The higher the (G+C) content of DNA, the more it quenched the fluorescence intensity of quinacrine in solution. Quenching was also seen with poly(dG) or poly(rG) in mixtures with quinacrine. The same type of base-specificity is seen for the quinacrine mustard–nucleic acid complexes. In this investigation strong quenching of the fluorescence intensity of quinacrine mustard was observed with mononucleotides where guanine was base component. The effect of either different cytidine mononucleotides or dThd-5′-*P* were rather slight. The mononucleotide rAdo-2′:3′-*P* gave an increase of the fluorescence intensity of the dye. However, when the phosphate was in the 5′-position in the ribonucleotide a decrease of the fluorescence intensity of the quinacrine mustard was shown. The same quenching was observed with the corresponding deoxymononucleotide. Thus the effect of mononucleotides on the fluorescence intensity of quinacrine mustard is dependent on the base in the mononucleotide and the position of the phosphate group, i.e. the molecular conformation of the mononucleotide. Quenching of the fluorescence intensity of quinacrine mustard was also seen with poly(rG) and poly(rG,rC) but not with the other polynucleotides tested. Poly(rA), poly(rU) and poly(rA,rU) all enhanced the fluorescence intensity of quinacrine mustard, and poly(rC) showed no effect.

The base-specificity of quinacrine mustard is also shown in the interaction between different DNA species and the dye. Native DNA species quench the fluorescence intensity of quinacrine mustard, depending on the ratio (A+T)/(G+C). A higher (G+C) content in the DNA tested gave a decrease in the fluorescence intensity of the dye.

There is no interaction between denatured methylated DNA and quinacrine mustard, because both guanine and phosphate groups are blocked (Ramstein *et al.*, 1971). This is also evidence in favour of the base-specificity of quinacrine mustard. In the interaction between apurinic DNA and quinacrine mustard, the dye interacts with the phosphate groups alone. According to O'Brien *et al.* (1966) chloroquine reacts with the 2-amino group in guanine, but Caspersson *et al.* (1969*a*) hold that quinacrine mustard reacts with the 7-amino group in the base. Brookes & Lawley (1961) found that the reaction of mono- and di-functional alkylating agents with nucleic acids was with the 7-amino group in guanine. The inhibited interaction between quinacrine mustard and methylated DNA, almost exclusively methylated at the 7-amino group in guanine (Ramstein *et al.*, 1971), also supports the view that quinacrine mustard binds to the 7-amino group in guanine.

As shown in this paper, the quinacrine mustard fluorescence intensity decreased and its spectral characteristics changed upon the addition of ions. This may be due to salt-induced dye aggregation (Azzi *et al.*, 1971). Aggregation of quinacrine mustard may also occur in solvents of low dielectric constant due to stronger interaction between dye molecules not separated by solvent molecules. When basic dyes interact with anions they tend to aggregate as a consequence of decreased electrostatic repulsion or facilitated hydrophobic interaction. The aggregating effects of the buffer solution on the dye molecules may

have to be taken into account when the quincrine mustard banding patterns of the chromosomes are discussed.

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