Interaction of Quinacrine Mustard with Calf Thymus Histone Fractions

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1. Interactions of histone fractions with quinacrine mustard were investigated by fluorimetry and spectrophotometry and the results were interpreted with the aid of thin-layer chromatography. 2. Characteristic differences were found between the various histone fractions of calf thymus. The conditions that favoured histone conformational changes and aggregation, also favoured interaction between histones and the dye; low concentrations of SO_4^{2-} brought about more interaction than did Cl^- ; urea, guanidinium and iodide ions were inhibitory to binding. 3. Changes in the physical state of all the quinacrine mustard–protein complexes occurred as a function of ionic strength and pH. The most salt-dependent interaction was found in the arginine-rich histone fraction. 4. The interaction of the calf thymus histone fractions with quinacrine mustard was compared with the interaction of bovine plasma albumin and protamine with quinacrine mustard. 5. The relationship between dye-binding and the aggregation of histone fractions was discussed.

The interactions of histone fractions with anionic dyes in tissue sections have been studied by Alfert & Geschwind (1953) and Swift (1964). Loeb (1964) studied the interaction of Eosin with histones and Shepherd & Noland (1965) and Laurence (1966) used 8-anilidonaphthalene-1-sulphonic acid for the estimation of proteins in solution. In the interaction of this anionic dye with calf thymus histone fractions Laurence (1966) found characteristic differences in the histone fractions, depending on the physical state of the histones.

The recent interest in the cationic acridine dye quinacrine mustard stems from the fact that it produces a characteristic banding pattern in mitotic chromosomes. It has proved a powerful tool in the identification of individual chromosomes (Caspersson *et al.*, 1969). Little is known about the mechanisms by which areas of brighter and duller fluorescence along the chromosomes are produced. Recent work has shown that binding to DNA species of different composition may play an important role (Weisblum & de Haseth, 1972; Pachmann & Rigler, 1972). However, it would seem possible that chromosomal proteins also affect the fluorescence patterns.

The present investigation is an attempt to elucidate this subject. The characteristics of the interaction between quinacrine mustard and different calf thymus histone fractions and other proteins are reported.

Experimental

Materials

Proteins. Histone fractions F1, F2(a), F2(b) and F3 from calf thymus were isolated according to Johns

(1964). The histones were dissolved in water as hydrochlorides and stored frozen at -25°C for approx. 1 month. Gel electrophoresis of these fractions was performed according to Comings (1967), and the histone fractions showed characteristic gel-electrophoretic patterns. Calf thymus histone fractions IIA, III and IV were purchased from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. Protamine and protamine sulphate were also purchased from Sigma (London) Chemical Co. Ltd. and crystalline bovine plasma albumin from Armour Pharmaceutical Co., Eastbourne, Sussex, U.K. The protein concentrations were determined by the method described by Lowry *et al.* (1951), with bovine plasma albumin as standard.

Quinacrine mustard. 2-Methoxy-6-chloro-9-[4-bis-(β -chloroethyl)-amino-1-methylbutylamino]-acridine was a gift from the Sterling–Winthrop Research Institute, Rensselaer, N.Y., U.S.A. Its mol.wt. is 514.28 ($2\text{H}_2\text{O}$). The fluorescence and absorption spectra and some other characteristics of quinacrine mustard were studied by Selander (1973). Stock solutions of quinacrine mustard (2 mg/ml) in water were stable for 2 weeks at 4°C in the dark.

Buffers. McIlvaine's buffer (0.1 M-citric acid – 0.2 M-disodium phosphate) was made as described by McIlvaine (1921), and the barbital buffer as described by Michaelis (1931).

Methods

Fluorescence and absorption measurements. Fluorescence attachment ZFM4 for a Zeiss (PMQ) spectrophotometer with an excitation filter of 436 nm was

used. The fluorimeter was standardized with the fluorescence standard cuvette F53. The proteins do not interfere with the fluorescence of quinacrine mustard at 514 nm (Teale, 1960). The fluorescence data of complexes and mixtures were expressed as the percentage change ($\Delta\%$) relative to the intensity of the fluorescence of quinacrine mustard alone. Absorption was determined with a Zeiss spectrophotometer (model PMQ II). At 424 nm the proteins do not interfere with the absorption of quinacrine mustard. All results were read at room temperature after the mixtures had stood overnight (18 h) at 20–25°C in the dark.

Thin-layer chromatography. The chromatography was made on cellulose MN 300 polyethyleneimine-impregnated thin-layer plates purchased from Macherey-Nagel and Co., Düren, Germany. The solvent was 65% propan-2-ol in 2M-hydrochloric acid (Adkisson *et al.*, 1971).

Interpretation of binding data. The equation of Scatchard (1949) was used to evaluate the results. For fluorimetric estimations where r is less than n the equation may be rewritten $i/(i_\infty - 1) = knT_p$, according to Laurence (1966), where i is the fluorescence intensity of the quinacrine mustard–histone complex, i_∞ is the intensity of the quinacrine mustard–histone complex at high histone concentration when all the available quinacrine mustard is bound, T_p is the concentration (M) of histone, k is the association constant (M^{-1}) and n the number of sites on the histone available for absorption of quinacrine mustard.

Results

Effect of pH and buffer on the fluorescence intensity of quinacrine mustard

Fig. 1 shows the effect of pH and two different buffer systems on the fluorescence intensity of quinacrine mustard. The maximum intensity of quinacrine mustard is obtained in alkaline solutions at pH 11 (Selander, 1973). A marked enhancement of the intensity of quinacrine mustard occurs at pH values over 8 and a minimum is found between pH 7.0 and 7.5 in both buffer systems. No significant differences between the two buffer systems were found between pH 3 and 6.

Effect of urea

At constant concentrations of quinacrine mustard and histone fraction F2(a), and at constant NaCl concentration with 0.1M- Na_2SO_4 as activator, the effect of urea concentrations up to 4M was investigated. The results are shown in Fig. 2. 1M-urea gave the greatest enhancement of the fluorescence intensity of the quinacrine mustard–histone F2(a) mixture (+36.5 $\Delta\%$) because this concentration of urea was the most successful in quenching the intensity of quin-

acrine mustard alone. There was a decrease in the fluorescence intensity of quinacrine mustard between 0 and 1M-urea, and a marked enhancement between 1M- and 4M-urea. The pH varied between 4.9 and 5.3. If the quinacrine mustard fluorescence curve reflected only the effect of pH it would have a negative slope (cf. Fig. 1). Hence urea itself has an effect on the fluorescence intensity of both free quinacrine mustard and quinacrine mustard–protein mixtures.

Dialysis

Results from experiments involving dialysis of solutions containing excesses of quinacrine mustard and histone fraction F2(a) in Tris solution are presented in Fig. 3. In this figure, the fluorescence intensity at 514 nm of the contents of the dialysis tubing is plotted against the number of hours of dialysis preceding the sampling. As noted in the figure, unbound quinacrine mustard was not completely dialysable, and so the fluorescence intensity did not fall to 0. However, these low values may depend on errors in the estimation of the intensity at very low concentrations of the dye. Absorption of quinacrine mustard by the Viscose cellophane tubing and the loss of protein to the external solution were small. Determinations of the concentration of quinacrine mustard and proteins after dialysis had been completed indicated that 5 ml of histone fraction F2(a) bound 0.010 mg of quinacrine mustard whereas the 2.5 mg of histone fraction F2(a) bound 0.005 mg of quinacrine mustard. This

Fig. 1. Fluorescence intensity of quinacrine mustard in two different buffers

Fluorescence intensity at 514 nm is shown for quinacrine mustard (0.067 mg/ml) in 0.2M-sodium phosphate buffer (●) and in barbital buffer (○).

Fig. 2. Effect of urea on the fluorescence intensity of free quinacrine mustard and of quinacrine mustard-protein mixture

Various concentrations (M) of urea, in 17 mM-acetate buffer and histone fraction F2(a) (0.663 mg/ml) (●) were added to a solution with quinacrine mustard (0.0133 mg/ml) (○). Δ, pH. The fluorescence intensity was measured at 514 nm.

result confirms a fixed stoichiometric compound and gave $2.4 \times 10^4 \text{ M}^{-1}$ as the value of nk for histone fraction F2(a) [the mol.wt. for fraction F2(a) was taken as 20000]. We wish to emphasize that this is not equilibrium dialysis, so possible dissociation of bound quinacrine mustard as the free concentration of quinacrine mustard approaches 0 would cause the fluorescence intensity at 514 nm to become very low. This could result in overestimation of the protein/dye ratio. However, negligible fall of the slope in the latter part of the curves in Fig. 3 indicates that the reaction dye + protein = dye-protein strongly favours the binding reaction with a very slight tendency to dissociation.

Thin-layer chromatography

When proteins were allowed to react with quinacrine mustard and the mixtures were chromatographed in propan-2-ol-HCl, a bright fluorescent spot (in u.v. light) was seen at R_F approx. 0.5. In this solvent system, free quinacrine mustard moved rapidly, having an R_F value >0.9 , while the control protein (unassociated with quinacrine mustard) remained at the origin, as shown with the ninhydrin spray reagent. This result shows that dye-protein complexes are indeed formed in solution.

Effect of time and sodium chloride concentration

The fluorescence intensity of quinacrine mustard-protein solutions depends on the time that elapsed

Fig. 3. Dialysis of a protein solution with excess of quinacrine mustard

The concentration of quinacrine mustard (Δ) was 0.01 mg/ml and that of the histone fraction F2(a) was 1 mg/ml (●) or 0.5 mg/ml (○). Dialysis was performed in a 0.05 M-Tris solution, pH 7.5, with a total volume of 5 ml in the bag. The fluorescence intensity of the solutions in the dialysis bags was measured at 514 nm.

after mixing histones and dye and does not become stable at room temperature for approx. 18 h. An increased concentration of NaCl in solution enhanced the fluorescence intensity of the quinacrine mustard-protein mixture (Fig. 4). These results were interpreted to indicate a change in the physical state of the protein in the presence of salt. In buffer solutions without added NaCl the fluorescence intensity decreased between 1% and 6%, and the presence of 0.3 M-NaCl increased the intensity from 1% to 5%, 20 h after the mixing of reagents. For the three histone fractions investigated, the fluorescence intensities were from 5% to 10% higher in the presence of NaCl (Fig. 4, B, D and F) than in the absence of the salt (curves A, C and E). The greatest effect of NaCl was found on the quinacrine mustard-histone fraction F3 mixture (curves E and F), i.e. on the arginine-rich histone fraction. By contrast, the effect of NaCl on the lysine-rich histone fraction (quinacrine mustard-histone fraction F1 mixture, curves A and B) was considerably weaker. Table 1 summarizes the effect of NaCl on the fluorescence intensity of quinacrine mustard-protein mixtures. The increase in intensity varied between 3% and 14%. In the acetate buffer used (pH 4.7) little or no aggregation of the histones is to be expected (Phillips, 1971). In the absence of NaCl the intensity is most enhanced in the quinacrine mustard-histone fraction IIA mixture (+5.6Δ%), whereas the greatest effect of protein on the fluorescence intensity of quinacrine mustard in the presence of 0.3 M-NaCl is found in the arginine-rich histone fraction IV (+9.2Δ%).

The effect of the increased concentration of NaCl on the interaction between histone fraction F2(a) and

Fig. 4. Time-course of interaction of quinacrine mustard with different histone fractions

At the time of measurement, the concentrations of the histone fractions were 0.667 mg/ml and that of quinacrine mustard was 0.017 mg/ml, in 17 mM-acetate buffer, pH 4.7. ●, Fraction F1; ○, fraction F1 with 0.3 M-NaCl in the solution; ■, fraction F2(a); *, fraction F2(a) with 0.3 M-NaCl in the solution; ▲, fraction F3; △, fraction F3 with 0.3 M-NaCl in the solution. The results are given as $\Delta\%$.

III with quinacrine mustard is shown in Fig. 5. The fluorescence intensity of the quinacrine mustard-histone fraction F2(a) mixture was greatly enhanced between 0.05 and 0.7 M-NaCl, whereas the effect on the quinacrine mustard-histone fraction III mixture was negligible. At very high concentrations of NaCl the fluorescence intensity of the mixtures began to fall.

Effect of other salts

Fig. 6 shows that phosphate was the most effective anion in producing enhancement, since it was active at much lower concentrations than either Cl^- or SO_4^{2-} . Between 0.01 and 0.1 M- SO_4^{2-} was more effective than Cl^- . The effect of the guanidinium ion was insignificant. The great effect obtained with phosphate could also depend on the higher pH (7) of the solution at which the histone fraction F2(a) tends to aggregate (Phillips, 1971). Sodium iodide was less effective (not shown in Fig. 6). At higher iodide concentrations the quinacrine mustard-histone fraction F2(a) complex tended to precipitate.

Effect of pH and ionic strength

The fluorescence intensities of quinacrine mustard-histone fraction F2(a) mixtures in solution vary with

Fig. 5. Effect of sodium chloride concentration on the fluorescence intensity of quinacrine mustard-protein mixtures

The concentration of histone fractions F2(a) (●) and III (○) was 0.667 mg/ml, and that of quinacrine mustard was 0.033 mg/ml, in 17 mM-acetate buffer, pH 4.7.

pH and I as shown in Table 2. The greatest effect (+27.8 $\Delta\%$) was obtained in a solution with high pH (6.9) and I (2.4). No change was found in hydrochloric acid at pH 2.2.

The effect of pH in two different buffer systems is shown in Fig. 7. With the quinacrine mustard-histone fraction F2(a) mixture, maximal effect was obtained between pH 7.5 and 8.0 in the phosphate buffer and at pH 8.5 in the barbital buffer. At pH 9.5 the enhancement of the fluorescence intensity of the quinacrine mustard-bovine albumin in the barbital buffer was approx. three times higher than that for the quinacrine mustard-histone fraction F2(a) mixture. At pH below 8 the differences between quinacrine mustard-histone fraction F2(a) and quinacrine mustard-bovine albumin were insignificant. Similar pH effects were also demonstrated with the other histone fractions. The barbital buffer has a high concentration of NaCl (Michaelis, 1931) but I is much higher in the phosphate buffer (0.2 M). Thus the differences in fluorescence intensity of quinacrine mustard-histone fraction F2(a) and quinacrine mustard-bovine albumin mixtures depend mostly on pH.

The effects of different buffer systems on the fluorescence intensity of quinacrine mustard-protein mixtures are given in Table 3. In the barbital buffer (pH 8, I 0.31) the greatest increasing effect was obtained in the quinacrine mustard-histone fraction

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

The concentration of the proteins was 0.667 mg/ml, that of quinacrine mustard was 0.017 mg/ml and the acetate buffer (pH4.7) was 17 mM.

Protein fraction	Concn. of NaCl (M) ...	Fluorescence at 514 nm (Δ%)	
		0	0.3
F1		-2.7	+2.2
F2(a)		-1.4	+4.6
F2(b)		-5.0	+5.2
F3		-6.6	+4.0
IIA		-6.4	+7.7
III		+5.6	+8.8
IV		0.0	+9.2
Protamine		-5.4	+6.0
Bovine plasma albumin		-11.5	-2.5

Fig. 6. *Effect of anions on the fluorescence intensity of quinacrine mustard-histone fraction F2(a) mixture*
The concentration of histone fraction F2(a) was 0.667 mg/ml and that of quinacrine mustard was 0.033 mg/ml, in 17 mM-acetate buffer, pH4.7, except that the acetate buffer was omitted when sodium phosphate was used. ●, Sodium phosphate; △, sodium sulphate; ○, sodium chloride; ▲, guanidinium chloride.

Fig. 7. *Fluorescence intensity of quinacrine mustard-protein mixtures in two different buffers*

F2(a) mixture (+66.7Δ%) and the greatest quenching effect in the quinacrine mustard-histone fraction III mixture (-13.0Δ%). In the sodium phosphate buffer (pH 7.6, 12.40) only enhancement of the intensity was found, the greatest increase occurring in the quinacrine mustard-histone fraction IIA mixture (+167.0Δ%). The effect of McIlvaine's buffer (pH 7.0, 12.33) was not as marked, although both enhancement and quenching of the fluorescence intensity was found with this buffer. The enhancement was greatest (+11.7Δ%) in the quinacrine

The change in the fluorescence intensity (Δ%) of quinacrine mustard was read at 514 nm in solution with 0.067 mg of quinacrine mustard/ml and 0.667 mg of protein/ml. ●, Quinacrine mustard-histone fraction F2(a); ○, quinacrine mustard-bovine albumin in barbital buffer; △, quinacrine mustard-histone fraction F2(a) in 0.2M-sodium phosphate buffer.

Table 2. *Effect of pH and ionic strength on the fluorescence intensity of a quinacrine mustard-histone mixture*

Histone fraction F2(a) (0.663 mg/ml) was mixed with quinacrine mustard at 0.067 mg/ml.

Buffer solution	pH	<i>I</i>	Fluorescence at 514 nm ($\Delta\%$)
0.0063 M-Hydrochloric acid	2.2	0.0063	0.0
0.017 M-Acetate	4.7	0.017	+9.3
0.2 M-Sodium phosphate	6.9	2.4	+27.8

Table 3. *Effect of various buffer systems on the fluorescence intensity of quinacrine mustard-protein mixtures*

Protein (0.662 mg/ml) was mixed with quinacrine mustard at 0.017 mg/ml.

Protein fraction	Fluorescence at 514 nm ($\Delta\%$)		
	Barbital buffer	Sodium phosphate buffer	McIlvaine's buffer
F2(a)	+66.7	+62.2	-1.9
F2(b)	+4.5	+61.2	+6.2
F3	+3.0	+81.5	+5.8
IIA	+43.3	+167.0	+1.3
III	-13.0	+17.4	-7.0
IV	0.0	+7.4	-5.2
Protamine	-6.0	+11.8	-2.6
Protamine sulphate	-12.2	+4.7	-1.3
Bovine plasma albumin	+62.3	+64.4	+11.7

Table 4. *Effect of various protein mixtures on the fluorescence intensity of quinacrine mustard*

Proteins were mixed with quinacrine mustard (0.017 mg/ml) in three different buffer systems: 1, 0.2 M-sodium phosphate, pH 6.9; 2, 0.05 M-Tris solution, pH 7.5; 3, barbital, pH 6.5.

Protein fraction	Concn. (mg/ml)	Buffer	Fluorescence at 514 nm ($\Delta\%$)
F2(a)	0.067	1	+12.0
IV	0.067	1	+6.1
F2(a) + IV			+10.1
III	0.5	2	+1.9
IV	0.5	2	+3.1
III + IV			+2.4
F2(b)	0.1	3	+4.1
IIA	0.1	3	+7.8
F2(b) + IIA			+11.6
F2(b)	0.1	3	+4.1
Bovine plasma albumin	0.1	3	+19.2
F2(b) + bovine albumin			+19.9

mustard-bovine albumin mixture and the quenching was greatest ($-7.0\Delta\%$) in the quinacrine mustard-histone fraction III mixture. The hybrids, i.e. the histone fractions F2(a), F2(b) and IIA, showed the

greatest changes in fluorescence intensity when pH or *I* or both were changed in the solution. In these three buffer systems pH was high enough to cause self-aggregation of the histone fractions used

(Phillips, 1971). pH 7.6 and 12.40 (sodium phosphate buffer) were the most successful in obtaining enhancement of the fluorescence intensity of quinacrine mustard-protein mixtures.

Interaction of quinacrine mustard with mixtures of histone fractions

The fluorescence intensity of single histone fractions and of mixtures of two fractions were compared (Table 4). Since three different buffer systems were used, the results from the different mixing experiments are not directly comparable. However, the effect of a single protein on the fluorescence intensity of quinacrine mustard can be compared with that of a mixture of two proteins.

The results indicate that when the arginine-rich fraction IV is mixed with another histone fraction [fraction F2(a) or III] the fluorescence intensity of the mixture is approximately intermediate between that of the two fractions when tested alone. The mixture of two hybrid histone fractions [F2(b) and IIA] results in a stronger fluorescence enhancement than that produced by either fraction alone. The values obtained indicate an additive effect. The strong enhancement (+19.2Δ%) produced by bovine albumin was little changed (+19.9Δ%) when an equal amount of histone fraction F2(b) was added.

Scatchard plot

The value of nk for fraction F2(a) in the barbital buffer (pH 8.5) obtained from fluorimetric titration and interpretation of the binding data according to the equation of Scatchard (1949) was $5.7 \times 10^4 \text{ M}^{-1}$. This value is twice that obtained from dialysis experiments. However, as indicated above, the value of nk was obtained from non-equilibrium dialysis. On the other hand, the estimations were made in different buffer systems; their different pH and I values must yield divergent results.

Discussion

The results show that the binding of quinacrine mustard to different histone fractions varies. There is a correlation between quinacrine mustard binding and the preparative method used, because different preparation methods result in histone fractions of different molecular compositions (Phillips, 1971). The quinacrine mustard binding also depends on the physical condition of the histone molecules in solution. The conditions which increase the fluorescence intensity of the quinacrine mustard-protein mixture are those which also favour conformational changes of the histones. The arginine-rich fractions aggregate easily in solution, especially in a solution with a high

salt concentration (Phillips, 1971), which leads to a high fluorescence intensity of the mixture. The differences in the fluorescence intensity of different arginine-rich fractions may simply indicate differences in the molecular composition of the fractions. According to Laurence (1966), histone fraction F3 corresponds in amino acid composition to the easily aggregating β -histone. Mauritzen & Stedman (1959) found that other histone fractions inhibited the aggregation of β -histones. The effect of lysine-rich histone fractions F1 and III on the fluorescence intensity of quinacrine mustard varied, and that of the salt concentration in the solution was slight. Fraction F1 is the only histone fraction that does not aggregate in solution between pH 2 and 9 (Phillips, 1971). Sulphate and phosphate anions, which both increase the aggregation of histones in solution, also cause an enhancement of the fluorescence intensity. The high activity of SO_4^{2-} agrees with the observation of Ui (1956) that SO_4^{2-} causes histone aggregation more readily than Cl^- . Urea or guanidinium ions at high concentrations reverse the aggregation of histones (Ui, 1956) and parallel our observations that these agents inhibit the fluorescence intensity. Generally speaking, the aggregation or conformational change of histones is increased by an increase of I or anionic valence in the buffer solution, a rise in temperature, increased histone concentration and by very high or low pH values (Phillips, 1971). This investigation has shown that by changing some of these parameters a comparable change will occur in the fluorescence intensity.

The relationship between aggregation and dye-binding may not be the only important factor, because the α -helical content of histones may grow when the salt concentration of the solution is increased (Bradbury *et al.*, 1965). The increased α -helical content leads to a more organized structure of the peptide chains, thus favouring conformational changes of histone.

Non-polar amino acids in the histones favour interaction with non-polar parts of the quinacrine mustard molecule. Histone fractions F2(a), IIA, F3 and IV contain more non-polar amino acids than histone fractions F2(b), F1 and III (Johns, 1964). Thus the fluorescence intensity of quinacrine mustard mixed with histone fractions F2(a), IIA, F3 or IV is higher than with other histone fractions, owing to an increased binding of quinacrine mustard to the non-polar molecules.

The value of nk for histone fraction F2(a) was calculated to be $2.4 \times 10^4 \text{ M}^{-1}$ (non-equilibrium dialysis) and $5.7 \times 10^4 \text{ M}^{-1}$ (fluorimetric titration). These values differ from the value given by Laurence (1966) for fraction F2(a) mixed with 8-anilinonaphthalene-1-sulphonic acid. However, the molecular structures of these two dyes are quite different, and both the extraction method and the buffer system used by

Laurence (1966) were different from ours. Thus different nk values are not unexpected.

Salt may also cause aggregation of acridines, and aggregation may also occur in a solution of low I , depending on the greater interaction between the dye molecules (Azzi *et al.*, 1971). These phenomena must also be noticed when the changes in the fluorescence intensity of dye-protein mixtures are discussed.

It is difficult to relate the findings here to the possible effects of proteins on the fluorescence of quinacrine mustard in cytological chromosome preparations. In addition to histones, chromosomes contain acidic and possibly some other proteins. As is shown in this paper, other proteins, e.g. bovine albumin, also react with quinacrine mustard.

Typical banding patterns occur in chromosomes stained with quinacrine mustard even after the cells have been treated with agents interfering with protein structure. Such is the case after treatment with, e.g., HCl and NaOH (de la Chapelle *et al.*, 1971), trypsin and pepsin (Adkisson *et al.*, 1971). It is not possible to assess to what quantitative and qualitative extent such agents effect chromosomal proteins *in situ*.

Different histone fractions showed remarkable differences in the fluorescence intensity of quinacrine mustard. This could mean that the specific fluorescence patterns *in situ* could be, at least in part, due to the histone moiety of chromosomes. It appears logical to propose that the occurrence of specific band patterns on chromosomes after staining with quinacrine mustard is to a considerable extent influenced by the composition and physical state of the DNA itself (Weisblum & de Haseth, 1972; Pachmann & Rigler, 1972).

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References

- Adkisson, K. P., Perreault, W. J. & Gay, H. (1971) *Chromosoma* **34**, 190–205
- Alfert, N. & Geschwind, I. I. (1953) *Proc. Nat. Acad. Sci. U.S.* **39**, 991–993
- Azzi, A., Fabbro, A., Santato, M. & Gherardini, P. L. (1971) *Eur. J. Biochem.* **21**, 404–410
- Bradbury, E. M., Crane-Robinson, C., Phillips, D. M. P., Johns, E. W. & Murray, K. (1965) *Nature (London)* **205**, 1315–1316
- Caspersson, T., Zech, L., Modest, E. J., Foley, G. E., Wagh, U. & Simonsson, E. (1969) *Exp. Cell Res.* **58**, 141–152
- Comings, D. E. (1967) *J. Cell Biol.* **35**, 699–708
- de la Chapelle, A., Schroder, J. & Selander, R.-K. (1971) *Hereditas* **69**, 149–153
- Johns, E. W. (1964) *Biochem. J.* **92**, 55–59
- Laurence, D. J. R. (1966) *Biochem. J.* **99**, 419–426
- Loeb, J. (1964) *C. R. Acad. Sci.* **258**, 5087–5088
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mauritzen, C. M. & Stedman, E. F. R. S. (1959) *Proc. Roy. Soc. Ser. B* **150**, 299–305
- McIlvaine, T. C. (1921) *J. Biol. Chem.* **49**, 183–186
- Michaelis, L. (1931) *Biochem. Z.* **234**, 139–141
- Pachmann, U. & Rigler, R. (1972) *Exp. Cell Res.* **72**, 602–608
- Phillips, D. M. P. (1971) *Histones and Nucleohistones*, Plenum Press, London
- Scatchard, G. (1949) *Ann. N. Y. Acad. Sci.* **51**, 660–672
- Selander, R.-K. (1973) *Biochem. J.* **131**, 749–755
- Shepherd, G. R. & Noland, N. J. (1965) *Anal. Biochem.* **11**, 443–448
- Swift, H. (1964) in *The Nucleohistones* (Bonner, J. & Ts'o, P., eds.), p. 169, Holden-Day Inc., San Francisco
- Teale, F. W. J. (1960) *Biochem. J.* **76**, 381–388
- Ui, N. (1956) *Biochim. Biophys. Acta* **22**, 205–206
- Weisblum, B. & de Haseth, P. L. (1972) *Proc. Nat. Acad. Sci. U.S.* **69**, 629–632