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The Physical State of Nucleohistone under Physiological Ionic Strength. The Effect of Interaction with Free Nucleic Acids*

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ABSTRACT: Nucleohistone aggregates in 0.05–0.30 M NaCl. The extent of aggregation depends upon ionic strength and increases with nucleohistone concentration and molecular size. At a given ionic strength aggregation is greater at pH 5 than at pH 8. Aggregated nucleohistone regains its original properties when redissolved in low ionic strength buffer. We propose that nucleohistone aggregates because its negative charge is effectively neutralized, thus leading to decreased hydration and increased interaction between nucleohistone molecules. Free deoxyribonucleic acid and ribonucleic acid inhibit nucleohistone precipitation by complexing lysine-rich histones, thus leaving the nucleohistone molecules more negatively charged.

Chromosomal material isolated from higher organisms is a disperse gel in solutions of low ionic strengths (<0.1 M) but is precipitated from solution at ionic strengths in the range 0.15–0.40 M (Oth and Desreux, 1957). This is true even if the nucleoprotein is of low (<30S) sedimentation coefficient.

The monovalent cation concentration in animal cell nuclei is in the region of 0.2 M (Langendorf *et al.*, 1961). A low level of divalent cations is also present. Chromatin should therefore be present *in vivo* largely in the precipitated state. We have studied the properties of calf thymus nucleohistone in solutions 0.15 M in NaCl. The properties of isolated nucleohistone under these conditions are expected to be similar to those of nucleoprotein *in vivo*.

Materials and Methods

Materials. Calf thymus nucleohistone was prepared by the method previously described (Chalkley and Jensen, 1968). At least 90% of the nuclear DNA of thymus cells is recovered complexed with histone and nonhistone protein in the mass ratios DNA, 1.0; histone, 1.0; and nonhistone, 0.30.

Our preparations contain a continuous distribution of nucleohistone molecules which range in sedimentation coefficient from 25 to about 200 S in 0.01 M Tris (pH 8.0). As previously reported, we normally isolate two fractions with $s_{20,w}$ of 30 and 130 S, respectively. The $s_{20,w}$ of DNA isolated from each of the two fractions is 14 S. We have shown that 130S nucleohistone consists of units of 30S nucleohistone linked together by protein. Therefore, its chemical properties are very similar to those of 30S nucleohistone.

DNA was isolated from calf thymus nucleohistone by a modified Marmur procedure (Marmur, 1961), followed by two phenol treatments and subsequent ether extraction of phenol. M. Dahmus kindly donated the tRNA which was isolated from ascites tumor cells by phenol extraction and purified by methylated albumin Kieselguhr column chromatography. rRNA was isolated from *Escherichia coli* strain MRE600 and was a gift of J. Sedat. All nucleic acid solutions were extensively dialyzed against 0.01 M Tris (pH 8.0) before use. Melting profiles were determined on samples dialyzed against 2.5×10^{-4} M EDTA (pH 8), immediately prior to study.

Histones were extracted into 0.4 N H_2SO_4 for 30 min. The precipitate of DNA plus nonhistone protein was removed by centrifuging at 31,000*g* for 20 min. Four volumes of 95% ethanol were added to the supernatant and the solution was held at −20° for 24 hr. The precipitated histone was collected by centrifugation at 23,500*g* for 20 min and washed four times with 95% ethanol. Eth-

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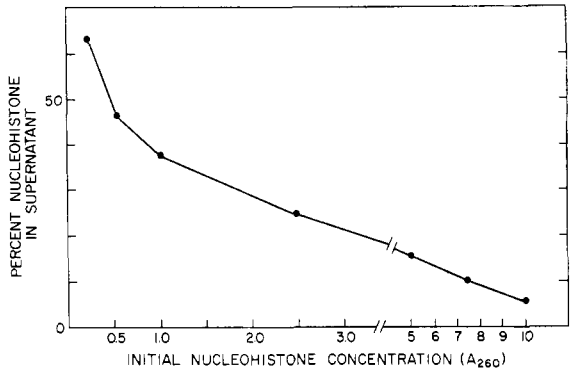


FIGURE 1: Concentration dependence of nucleohistone precipitation in 0.15 M NaCl. Precipitation was carried out as described in Materials and Methods, and absorbance of the supernatant at 260 mμ was measured. The abscissa is the A_{260} of the solution before addition of one-third volume of 0.6 M NaCl.

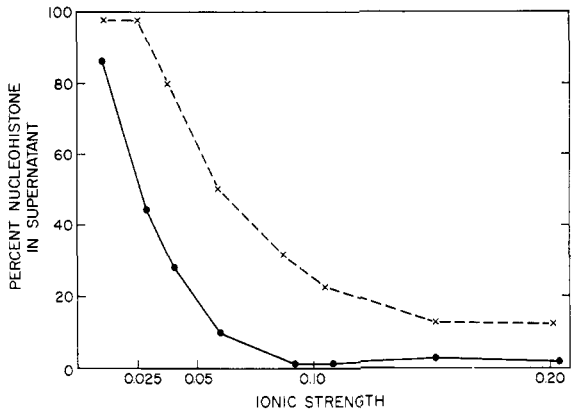


FIGURE 2: Dependence of nucleohistone precipitation upon NaCl concentration at pH 5 (—●—●—) and pH 8 (--×--×--). Solutions were dialyzed against 0.01 M acetate buffer (pH 5) or 0.01 M Tris buffer (pH 8) and precipitated by adding one-third volume of concentrated NaCl solution to the nucleohistone solution (A_{260} 10) as in Materials and Methods.

anol was evaporated from the solid histone sulfate at room temperature *in vacuo*.

Methods. Thermal melting profiles were measured using a Gilford Model 2000 spectrophotometer with a jacketed cuvet compartment and a linear temperature programmer. The rate of temperature increase was 0.5°/min.

Free-zone electrophoresis was performed as described by Olivera *et al.* (1964) in 0.01 M NaCl–0.01 M Tris (pH 7.5), using their apparatus.

Acrylamide gel electrophoresis of isolated histones was performed by the method of Bonner *et al.* (1968).

Protein and DNA analyses were performed as described previously (Chalkley and Jensen, 1968).

As the ionic strength of calf thymus nucleohistone solution is increased, its sedimentation velocity increases to the point of precipitation in the range 0.15–0.4 M NaCl (see Results). In order to obtain a standard and reproducible measure of precipitation, we have arbitrarily defined precipitated nucleohistone as that material sedimented from 1 to 2 ml of solution in 20 min at 23,500*g*. A typical precipitation assay was performed by slowly adding 0.33 ml of 0.6 M NaCl–0.01 M Tris (pH 8) to 1.0 ml of nucleohistone solution in 0.01 M Tris (pH 8) with vigorous agitation (Vortex mixer). After 10 min, the solution was centrifuged in a Servall SS-34 rotor at 14,000 rpm for 20 min. The supernatant was pipetted for analysis. All manipulations were accomplished on solutions kept in ice.

Results

Precipitation of Nucleohistone from 0.15 M NaCl. The extent of precipitation of nucleohistone from 0.15 M NaCl depends upon the size of the nucleohistone molecules and on nucleohistone concentration. The concentration dependence of precipitation is shown in Figure 1. The size dependence of precipitation is shown by the fact that at a concentration of 75 μg/ml, nucleohistone of $s_{20,w}$ = 30 S is precipitated to the extent of only 25% while nucleohistone of $s_{20,w}$ = 130 S is 55% precipitated.

The mass ratio of protein to DNA in nucleohistone also influences its precipitability from 0.15 M NaCl. Nucleohistone of $s_{20,w}$ = 30 S was very slightly dissociated by treating it in 0.3 M NaCl. DNA and 92% of the protein cosedimented into a sucrose underlayer which was then dialyzed against 0.01 M Tris (pH 8). An aliquot of A_{260} 10 was tested for precipitation from 0.15 M NaCl, and it precipitated to the extent of only 50%. The original native nucleohistone was 95% precipitated from 0.15 M NaCl.

The constancy with which separate preparations precipitate is a good indication that the starting material is reproducible in the amount of histone complexed to DNA.

Precipitation of nucleohistone from 0.15 M NaCl might be due to either of two causes. The protein–DNA complex is negatively charged, and thus simple neutralization of charge might allow hydrophobic interactions between separate nucleohistone molecules to become more important than nucleohistone–water interactions. The result would be to aggregate molecules which would then fall out of solution. The second possibility is that some of the histone–DNA bonds (which are to a large extent electrostatic) become weakened in 0.15 M NaCl, so that neighboring DNA molecules become linked through histone bridges to form a large aggregated network.

To distinguish between these possibilities we have made use of the fact that at pH 8.0 the carboxyl groups of histone molecules are less protonated than at pH 5.0, a pH higher than that needed to protonate DNA (Walker, 1965). Increasing protonation of these carboxyl groups neutralizes the over-all negative charge on the nucleohistone complex, thus permitting charge neutralization at a lower ionic strength, without weakening (and perhaps strengthening) the bonds between histones and their respective DNA molecules.

The data of Figure 2 show the dependence of nucleohistone precipitation upon increasing NaCl concentration at pH 8, at which neither histone carboxyl groups

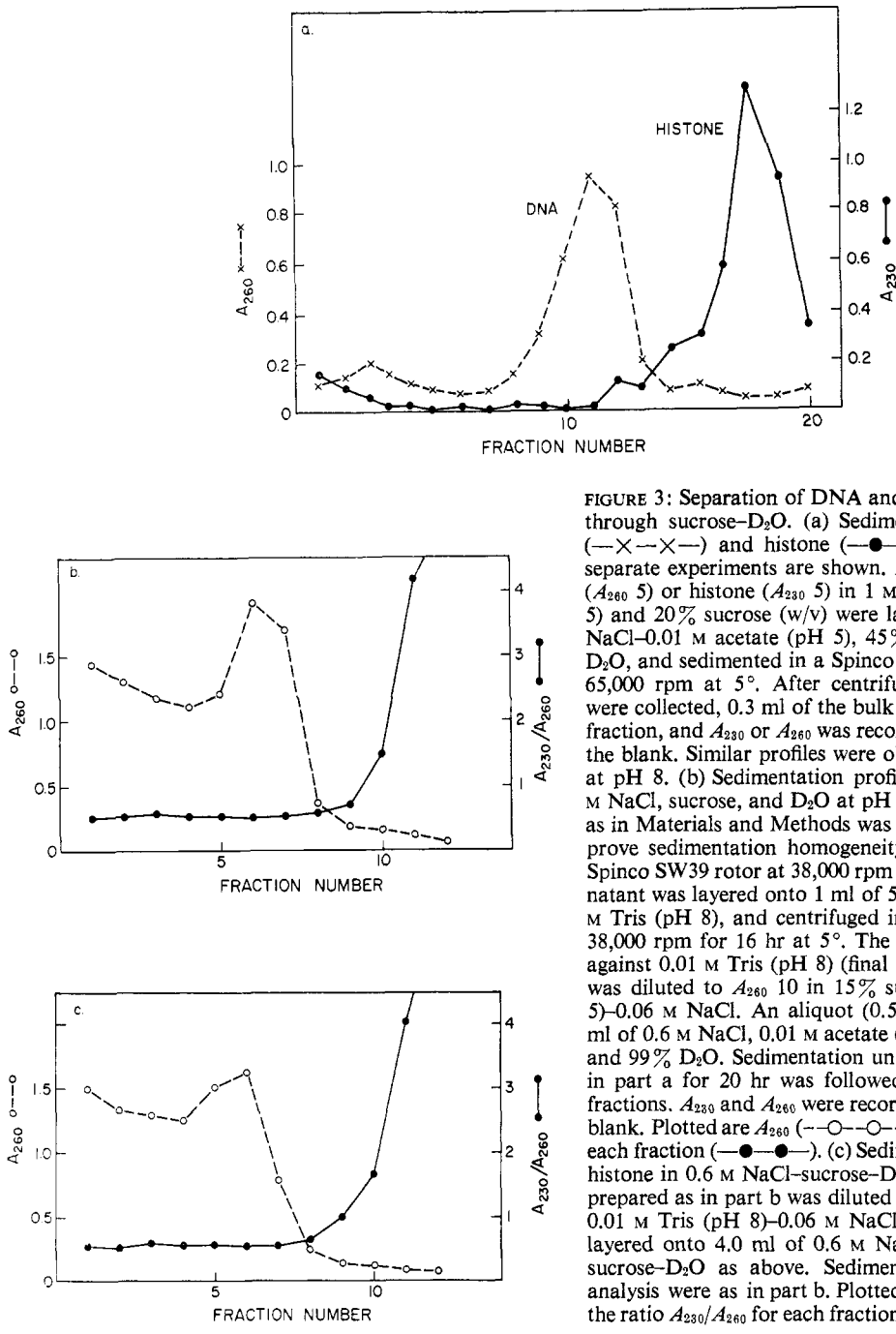


FIGURE 3: Separation of DNA and histone by sedimentation through sucrose–D_2O. (a) Sedimentation profiles of DNA (—×—×—) and histone (—●—●—). The results of two separate experiments are shown. Portions (0.5 ml) of DNA (A_{260} 5) or histone (A_{230} 5) in 1 M NaCl–0.01 M acetate (pH 5) and 20% sucrose (w/v) were layered onto 4.0 ml of 1 M NaCl–0.01 M acetate (pH 5), 45% sucrose (w/v), and 99% D_2O, and sedimented in a Spinco SW65 rotor for 14.5 hr at 65,000 rpm at 5°. After centrifugation, 20-drop fractions were collected, 0.3 ml of the bulk solvent was added to each fraction, and A_{230} or A_{260} was recorded using bulk solvent for the blank. Similar profiles were obtained in 0.6 M NaCl and at pH 8. (b) Sedimentation profile of nucleohistone in 0.6 M NaCl, sucrose, and D_2O at pH 5. Nucleohistone prepared as in Materials and Methods was fractionated further to improve sedimentation homogeneity. It was centrifuged in a Spinco SW39 rotor at 38,000 rpm for 2.5 hr at 5°. The supernatant was layered onto 1 ml of 50% sucrose (w/v) and 0.01 M Tris (pH 8), and centrifuged in a Spinco SW39 rotor at 38,000 rpm for 16 hr at 5°. The bottom 1 ml was dialyzed against 0.01 M Tris (pH 8) (final A_{260} 35). A portion of this was diluted to A_{260} 10 in 15% sucrose–0.01 M acetate (pH 5)–0.06 M NaCl. An aliquot (0.5 ml) was layered onto 4.0 ml of 0.6 M NaCl, 0.01 M acetate (pH 5), 45% sucrose (w/v), and 99% D_2O. Sedimentation under the same conditions as in part a for 20 hr was followed by collection of 20-drop fractions. A_{230} and A_{260} were recorded using bulk solvent as a blank. Plotted are A_{260} (–○–○–○–) and the ratio A_{230}/A_{260} for each fraction (—●—●—). (c) Sedimentation profile of nucleohistone in 0.6 M NaCl–sucrose–D_2O at pH 8. Nucleohistone prepared as in part b was diluted to A_{260} 10 in 15% sucrose–0.01 M Tris (pH 8)–0.06 M NaCl. An aliquot (0.5 ml) was layered onto 4.0 ml of 0.6 M NaCl–0.01 M Tris (pH 8) in sucrose–D_2O as above. Sedimentation, fractionation, and analysis were as in part b. Plotted are A_{260} (–○–○–○–) and the ratio A_{230}/A_{260} for each fraction (—●—●—).

nor DNA is protonated, and at pH 5, where about half of the carboxyl groups are protonated (Walker, 1965). The greater degree of precipitation at pH 5 is a strong indication that aggregation caused by charge neutralization is responsible for precipitation.

Although we do not expect that protonation of the histone carboxyl groups should weaken the histone–DNA bonds, we have checked this directly. The extent of dissociation at pH 5 and 8 constitutes a direct comparison of the strength of binding of histone to DNA at each pH. Our technique for separating the dissociated components from the still-associated material utilizes sedimentation velocity in 99% D_2O, 45% (w/v) sucrose solutions. The high density of these solutions ($\rho \approx 1.28$ g/cc) minimizes any aggregation effects on sedimentation of the dissociated histones. This is necessary since in solutions of high salt concentration some of the isolated histones aggregate to form rapidly sedimenting particles (Cruft *et al.*, 1957). Since the density of our sedimentation medium is close to that of protein (*ca.* 1.3 g/cc), even aggregated proteins do not sediment rapidly because of the buoyancy effect.

Figure 3a shows that under the conditions used, acid-extracted histone sediments more slowly than does free DNA isolated from nucleohistone. Native nucleohistone sediments to the bottom in the same time period. Figure 3b,c shows sedimentation profiles of nucleohistone at pH 5 and 8 in 0.6 M NaCl. The ratio of A_{230}/A_{260} is used

as a measure of the amount of protein still associated with DNA. The average A_{230}/A_{260} in the region of DNA sedimentation is 0.55 ± 0.01 at pH 5 and 0.54 ± 0.01 at pH 8. Dissociation in 1 M NaCl results in an average A_{230}/A_{260} of 0.50 ± 0.02 at both pH 8 and 5. This corresponds to a dissociation of approximately 70% of the protein. It is clear that there is no more dissociation of histone from DNA at pH 5 than at pH 8.

The results of the above experiments support the hypothesis that precipitation of calf thymus nucleoprotein from 0.15 M NaCl is most likely due to simple neutralization of negative charges. This hypothesis is further supported by the fact that precipitation from 0.15 M NaCl does not irreversibly change the sedimentation characteristics of the precipitated material, for if the precipitate is redissolved in 0.01 M Tris (pH 8), its sedimentation profile is identical with that of the original material.

Inhibition of Precipitation in 0.15 M NaCl by Free Nucleic Acids. The presence of free DNA or RNA inhibits the precipitation of calf thymus nucleohistone from 0.15 M NaCl as shown in Table I. Neither ribo- nor deoxyribonucleoside triphosphates affect precipitation, unless assembled into a polynucleotide of moderately large size.

TABLE I: Inhibition of Nucleohistone Precipitation.[a]

Material Added	% Pptd
No addition	85
d-NTP[b]	75
r-NTP[b,c]	75
Degraded t-RNA	75
tRNA	35
28S-RNA[c]	14
Calf thymus DNA	14
Pea bud DNA	14

[a] The materials were mixed in an equimolar ratio 10 min before sedimentation. The nucleohistone in the mixture had a final A_{260} of 5. Precipitation was performed as described in Materials and Methods. [b] d-NTP refers to an equimolar mixture of the deoxyribose triphosphates of cystosine, thymine, adenine, and guanine, and r-NTP to an equimolar mixture of the ribose triphosphates of cystosine, adenine, guanine, and uridine. [c] In these experiments the material added to the nucleohistone was radioactive and precipitation was measured both by absorbance methods and by following the distribution of radioactivity between pellet and supernatant. In neither case did the RNA nor RNA precursors appear in the precipitate.

Interaction between Nucleohistone and Nucleic Acids at Low Ionic Strength. Even in solutions of low ionic strength, added nucleic acids interact with nucleohistone. Equimolar amounts of calf thymus DNA and calf thymus nucleohistone were mixed in 0.01 M Tris-HCl (pH 8) and sedimented through a 5–20% (w/v) sucrose gradient. The sedimentation profile (Figure 4c) is identical with the sum of those of the separate components (Figure 4a,b), and DNA is clearly separated from nucleohistone. The fractions indicated as A and B in the figure were isolated, and aliquots of each were subjected to thermal denaturation or to electrophoresis. The data of Table II show that material from fraction A melts significantly differently from free DNA, while material from fraction B melts differently from nucleohistone. The data suggest that a small amount of histone has shifted from the nucleohistone to the free DNA. Free zone electrophoresis of the separated fractions confirms this hypothesis as is shown by the data of Table III.

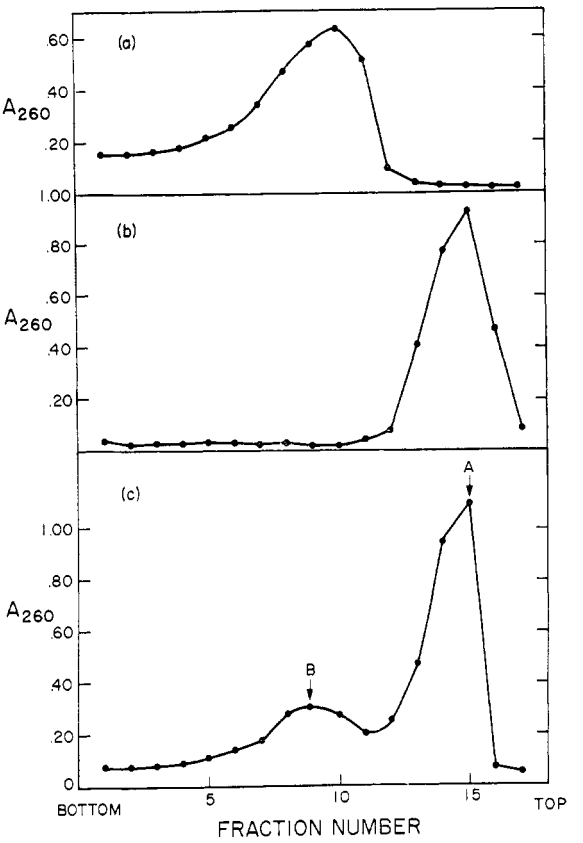


FIGURE 4: Sucrose gradient sedimentation of nucleohistone and DNA in 0.01 M Tris (pH 8). Aliquots (0.5 ml) of (a) nucleohistone (A_{260} 6.7), (b) DNA (A_{260} 3.4), and (c) an equimolar mixture (A_{260} 3.4 each) of DNA and nucleohistone in 0.01 M Tris (pH 8) were layered onto 4.5 ml of a linear sucrose gradient 5–20% (w/v) in 0.01 M Tris (pH 8) and centrifuged in a Spinco SW39 rotor at 38,000 rpm for 5 hr at 5°. To each of the collected 20-drop fractions 0.30 ml of 0.01 M Tris was added and A_{260} was recorded.

Still further shifts of histone are also possible. Thus, an aliquot of fraction B above was again treated with an equimolar concentration of free DNA. The mixture was again examined using free zone electrophoresis. The mobilities of the two fractions were again different from those of the starting materials, although the differences were smaller than for the first treatment. We conclude that for a fraction of the histone complexed in nucleo-

TABLE II: Thermal Denaturation of Nucleohistone–Nucleic Acid Mixtures.

Sample Denatured	T_0	T_{10}	T_m	T_{100}
DNA	33	38	44	54
Nucleohistone	57	62	75	87
Sucrose gradient fraction B	42	48	61.5	80
Sucrose gradient fraction A	35	39	47	73

All samples were thermally denatured in 2.5×10^{-4} M EDTA, pH 8. T_0 and T_{100} are the temperatures at which the absorbance change began and concluded, respectively. T_{10} and T_m are the temperatures at which the hyperchromic change is 10% and 50% complete.

TABLE III: Free-Zone Electrophoresis.[a]

Sample	Mobility $\times 10^4$ ($cm^2\ V^{-1}\ sec^{-1}$)
DNA	2.18
Nucleohistone	1.28
DNA + nucleohistone	1.53 + 1.84[b]
Sucrose gradient fraction A	1.83
Sucrose gradient fraction B	1.54
Sucrose gradient fraction B + DNA	1.66 + 2.06[b]
DNA + nucleohistone (0.15 M NaCl)	1.68 − 1.78[c]

[a] Prior to electrophoresis, all samples were dialyzed against 0.01 M Tris (pH 8) and sucrose was added to a final concentration of 2% (w/v). Free zone electrophoresis was performed as described by Olivera *et al.* (1964). The sample labeled (0.15 M NaCl) was prepared by addition of a small volume of concentrated NaCl to an equimolar mixture of DNA and nucleohistone, followed by dialysis *vs.* 0.01 M Tris (pH 8). [b] Two clearly separated peaks were observed. [c] One broad unresolved peak.

histone, an equilibrium is established between nucleohistone and free DNA.

To determine which classes of histone molecules participate in such equilibria, the histone of the separated fractions were characterized. After sucrose gradient separation of an equimolar mixture, as in Figure 4, the histones of each fraction were isolated and analyzed by acrylamide gel electrophoresis. Microdensitometer traces of the gels are shown in Figure 5. It is clear that lysine-rich histones (histones Ia,b in the nomenclature of Murray, 1964) are preferentially exchanged between nucleohistone and added free DNA.

Free zone electrophoresis of a mixture of rRNA and nucleohistone demonstrates that histone also shifts from nucleohistone to RNA in 0.01 M NaCl. Figure 6 shows a preparative electrophoresis of an equimolar mixture of [^{3}H]rRNA and unlabeled nucleohistone. That an interaction has occurred is shown by the decrease in mobility of RNA from 1.76×10^{-4} to 1.54×10^{-4} $cm^2\ V^{-1}\ sec^{-1}$. In order to obtain significant interaction it was necessary to use rRNA, and separation of nucleohistone and rRNA by sedimentation techniques was not possible. As a result a histone analysis of the isolated components of such a mixture was not attempted.

Interaction between Nucleohistone and Nucleic Acids in 0.15 M NaCl. An increase in ionic strength increases the shift of histones from nucleohistone to added nucleic acid. Thus sucrose gradient sedimentation of a mixture of nucleohistone and DNA in 0.15 M NaCl yielded a single broad band in the region of 25 S. The melting profile of a mixture exhibited a broad transition intermediate between those of nucleohistone and of DNA. Free zone electrophoresis of the mixture (after dialysis into 0.01 M Tris, pH 8) gave one broad band of mobility $1.68–1.78 \times 10^{-4}$ $cm^2\ V^{-1}\ sec^{-1}$. The results obtained can be explained in terms either of the formation of a complex of nucleohistone with free DNA or by an increased shift of histone from nucleohistone to DNA. To distinguish between these two possibilities, we have utilized a fraction of calf thymus nucleohistone of sedimentation coefficient 130 S. Sedimentation of mixtures of 130S nucleohistone and ^{32}P-labeled DNA from ascites tumor was performed both in 0.01 M Tris (pH 8) and in 0.15 M NaCl–0.01 M Tris (pH 8). The data of Figure 7 show that in 0.01 M Tris the sedimentation profile of the mixture is identical with the sum of the profiles of the components and that in addition each sediments as though but little changed. When a mixture of [^{32}P]DNA and nucleohistone was sedimented through 0.15 M NaCl–0.01 M Tris (pH 8) the two components were again clearly separated from one another, the specific activity of the DNA was unchanged, and approximately 50% of the nucleohistone was found in the lower part of the gradient (Figure 7d); the rest of the nucleoprotein had fully sedimented. Obviously no real complex between nucleohistone and DNA was formed. It is noteworthy that when free DNA was present in 0.15 M NaCl, more nucleohistone appeared in the gradient (not in the pellet) than in the case of nucleohistone alone in 0.15 M NaCl–0.01 M Tris (Figure 7c) where about 90% was fully sedimented from the gradient. Clearly the extent of aggregation had been reduced by the addition of free DNA, and it is presumably this factor which contributes to the measured reduction in gross precipitation.

An identical experiment was performed on a mixture of [^{32}P]RNA and 130S nucleohistone. The data of Figure 8 show that 90% of the [^{32}P]RNA was recovered in the gradient above and separate from the nucleohistone peak. Our interpretation of the results is therefore identical with that for the DNA experiment. RNA does not form a real complex with nucleohistone, but simply binds some histones which were previously involved in the nucleohistone complex.

Discussion

Whether nucleohistone exists in the soluble or in the

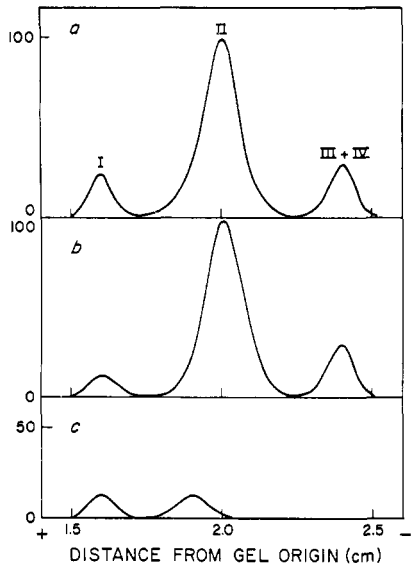


FIGURE 5: Microdensitometer traces of acrylamide gels after electrophoresis of (a) whole histone, (b) histone isolated from sucrose gradient fraction B of Figure 4c, and (c) histone isolated from sucrose gradient fraction A of Figure 4c. Histones were extracted as in Materials and Methods and electrophoresis gels were run in parallel as described by Bonner *et al.* (1968).

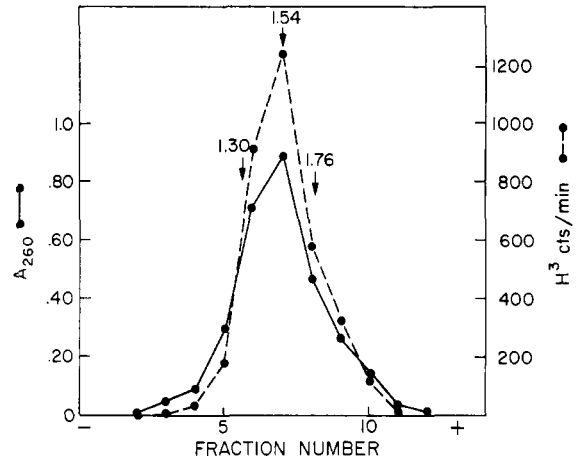


FIGURE 6: Preparative free zone electrophoresis of a mixture of [^{3}H]RNA and nucleohistone. Equal quantities of RNA and nucleohistone were mixed in 0.01 M Tris (pH 8) and free zone electrophoresis was performed as in Materials and Methods. After approximately 5 hr, the solution was collected in 0.5-ml fractions and A_{260} (—●—●—) and radioactivity (--O--O--) of each fraction were measured. The mobility (in $cm^2\ V^{-1}\ sec^{-1}$) is marked at three points on the plot. It was calculated from the rate of migration of that portion of the material.

aggregated state is largely dependent upon the effective charge on the nucleohistone molecules. Thus when the solvated, soluble form with its over-all negative charge is electrostatically neutralized in 0.15 M NaCl, solvation is reduced and the molecules interact to give the aggregated form.

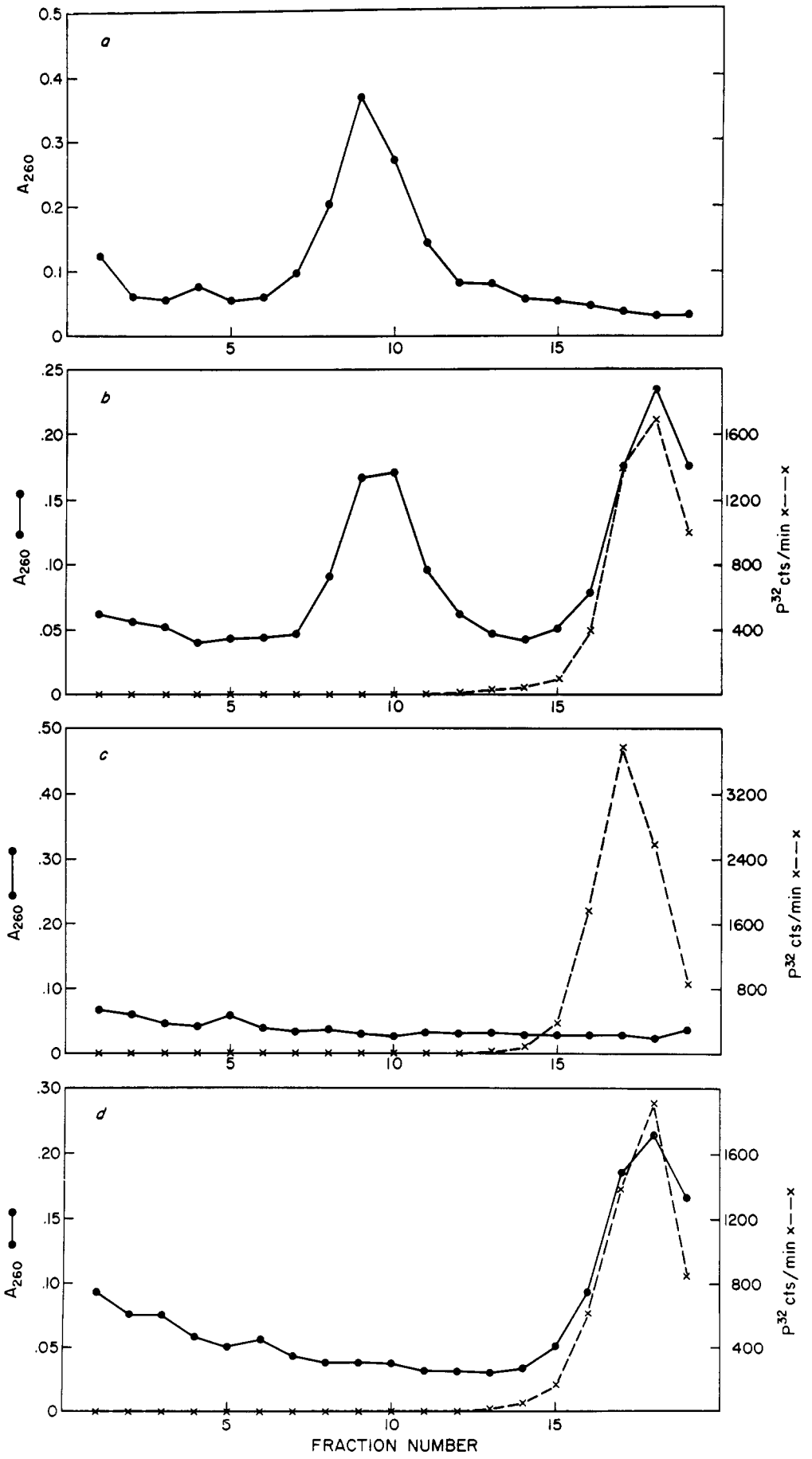
This information is germane to our understanding of the processes involved in isolation of soluble nucleohistone from native nuclear material. In the nucleus of higher organisms the bulk of the chromatin is extensively aggregated (Frenster *et al.*, 1963). During the isolation of nucleohistone, the ionic environment is changed from cellular (aggregating) conditions to a low ionic strength medium in which nucleohistone is soluble. Solubilization apparently is a result of the removal of cations which neutralize the charge on nucleoprotein *in vivo*. Thus the isolated nucleohistone should retain much of the chemistry of the native chromosomal material, although of course it has been mechanically sheared during the isolation procedures. That nucleohistone may retain some of its native biological function following solubilization has been suggested by Bonner *et al.* (1963) and by Paul and Gilmour (1966). Just as the soluble state of isolated nucleohistone can be regarded as closely related to native chromatin, the aggregated state of isolated nucleohistone serves as a useful model for the "condensed" chromatin visible in electron micrographs of calf thymus lymphocytes.

We have shown further that free nucleic acid decreases the amount of precipitation of nucleohistone from 0.15 M NaCl. The mechanism by which this solubilization occurs has been shown to be due to a shift of lysine-rich histone molecules from nucleohistone to the free nucleic acid. The over-all negative charge of the nucleohistone is therefore increased, and more effective electrostatic shielding is needed to precipitate the nucleohistone.

Mirsky and coworkers have shown that very little RNA synthesis occurs in thymus lymphocytes in the regions of "condensed" chromatin (Littau *et al.*, 1964; Allfrey and Mirsky, 1964). It is possible that DNA which contains information not required by the cell may be made unavailable for transcription in this manner. Conversely, areas of diffuse chromatin are thought to be regions of active RNA synthesis. Essentially nothing is known of the biochemical events which might trigger a transition from the condensed to the diffuse state. The discovery that DNA or RNA of moderately large size *drastically* reduces nucleohistone aggregation *in vitro* affords a possible mechanism for such an event.

It is also possible that local high concentrations of free DNA might arise in the immediate vicinity of a replication point. If this is so, we would predict that the newly synthesized DNA might rapidly become associated with lysine-rich histone from adjacent chromosomal material. This might serve not only to protect DNA from nucleolytic action, but also help to orient the DNA for the high level of organization required for its compact structure in the nucleus.

It is true that the concentrations of free nucleic acids which cause solubilization *in vitro* are rather high. We have no knowledge as to whether similar concentrations occur *in vivo*. However, such concentrations are often obtained in cell-free synthesis of RNA catalyzed by purified *E. coli* RNA polymerase. When nucleohistone is used as a template in this system, it is entirely possible to synthesize RNA to a concentration equal to or greater than that of the template. In that case we would certainly expect some of the histones to be shifted from template to product. What effect this could have requires further study.


a
b
c
d
A_{260}
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FRACTION NUMBER

FIGURE 7: Sucrose gradient sedimentation of DNA and nucleohistone. Nucleohistone of $s_{20,w} \approx 130$ S was isolated by sucrose gradient sedimentation as described by Chalkley and Jensen (1968). (a) Sedimentation profile of nucleohistone in 0.01 M Tris (pH 8). An aliquot (0.5 ml) of nucleohistone (A_{260} 1.5) in 0.01 M Tris (pH 8) was layered onto 4.5 ml of a linear sucrose gradient 5–20% (w/v) (0.01 M Tris, pH 8), and centrifuged in a Spinco SW39 rotor at 38,000 rpm for 50 min at 5°. Fractions (19 drops) were collected and A_{260} of each fraction was measured. (b) Sedimentation profile of a mixture of nucleohistone and [^{32}P]DNA in 0.01 M Tris (pH 8). Equal amounts of nucleohistone and [^{32}P]DNA were mixed in 0.01 M Tris (pH 8) such that $A_{260} = 0.75$ for each. An aliquot (0.5 ml) was layered onto a linear sucrose gradient, sedimented, and fractionated as in part a. Both A_{260} and counts per minute were measured for each fraction. (c) Sedimentation profiles of nucleohistone (—●—●—) and [^{32}P]DNA (--×--×--) in 0.15 M NaCl. Shown are profiles from two separate sedimentations. Procedures were as in part a. In this case all solutions were in 0.15 M NaCl–0.01 M Tris (pH 8). (d) Sedimentation of a mixture of nucleohistone and [^{32}P]DNA in 0.15 N NaCl. Equal amounts of nucleohistone and [^{32}P]DNA were mixed in 0.01 M Tris, after which one-third volume of 0.6 M NaCl was added. The final A_{260} of each component was 0.75. An aliquot (0.5 ml) was layered onto a linear sucrose gradient 5–20% (w/v) (0.15 M NaCl–0.01 M Tris, pH 8), centrifuged, fractionated, and analyzed as in parts a–c.

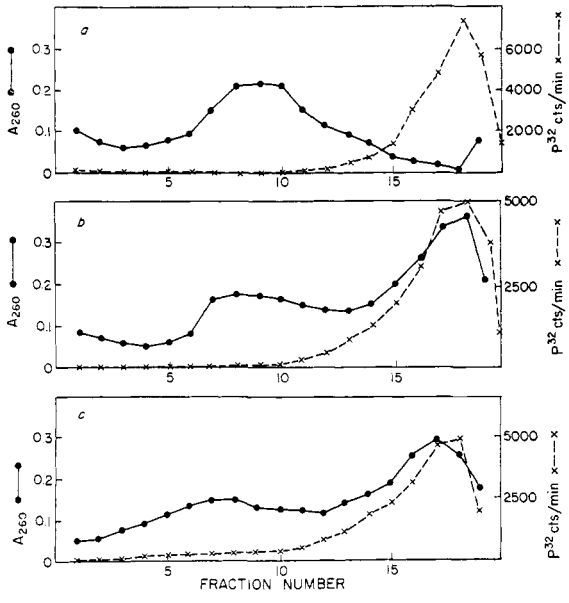

FIGURE 8: Sucrose gradient sedimentation of rRNA and nucleohistone. (a) Sedimentation profiles of nucleohistone (—●—●—) and [^{32}P]RNA (---×---×--). Shown are profiles from two separate experiments. Portions (0.5 ml) of RNA (A_{260} 1) or nucleohistone (A_{260} 1) in 0.01 M Tris were layered, centrifuged, fractionated, and analyzed as in Figure 7a. Only the counts per minute are plotted for RNA but A_{260} exactly parallels radioactivity. (b) Sedimentation profile of a mixture of nucleohistone and RNA in 0.01 M Tris (pH 8). Equal amounts of nucleohistone and [^{32}P]RNA were mixed in 0.01 M Tris (pH 8) such that $A_{260} = 1$ for each. An aliquot (0.5 ml) was layered onto a linear sucrose gradient, sedimented, fractionated, and assayed as in Figure 7a. (c) Sedimentation profile of a mixture of nucleohistone and [^{32}P]RNA in 0.15 M NaCl. Equal amounts of nucleohistone and [^{32}P]RNA were mixed in 0.01 M Tris (pH 8) after which one-third volume of 0.6 M NaCl was added. The final A_{260} of each component was 1. An aliquot (0.5 ml) was layered onto a linear 5–20% sucrose gradient (w/v) (0.15 M NaCl–0.01 M Tris, pH 8), centrifuged, fractionated, and analyzed as in Figure 7a.

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