Metal Constituents of Chromatin. Interaction of Mercury *in Vivo*†

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ABSTRACT: Purified liver chromatin isolated from mice challenged with nonlethal levels of mercury chloride (10^{-3} M) in drinking water for 1 month (experimental) and from animals given deionized water (control) was characterized as native according to several criteria. Analysis by flameless atomic absorption of gel-filtered chromosomal material from control revealed 0.05 ng of mercury/μg of DNA, corresponding to approximately one mercury atom per 10,000 nucleotide bases. However, chromatin isolated from challenged animals showed a tenfold enhancement of firmly associated metal not removed by gel filtration. The parameters of thermal denaturation, luminescence, and viscosity were used to further characterize the materials. First-derivative plots of the melting profile for experimental samples revealed a 4° thermal stabilization of the band melting at 68° in control and a slight destabilization of the 56° shoulder; both samples displayed a maximum at 88°. Accompanying the change in thermal stability was a decrease in intrinsic viscosity from 1.8 dl/g in the control to 1.1 dl/g in experimental samples. The intensity of phosphorescence emission for the two materials differed only slightly, yet an analysis of lifetimes showed quenching in the experimental sample where decay time was 1.7 sec in comparison to 3.3 for the control. The data suggest that structural alterations are most likely elicited by mercury as a consequence of mercury–protein binding. There is no evidence in the present study to support direct mercury–DNA interactions.

The conformation of DNA in chromatin has been the subject of numerous investigations (Shih and Lake, 1972; Ansevin *et al.*, 1971; Chalkley and Jensen, 1968; Slayter *et al.*, 1972). Stabilization of the supercoiled structure which may form when DNA is complexed with proteins in chromatin is believed to be due in part to histone binding (Simpson, 1972), specifically histone IV interactions (Wagner and Spelsberg, 1971). Recently, the crucial role of Ca^{2+} in maintaining the structure of Ca^{2+} nucleohistone IV suggests the possibility of similar functions for other divalent metals (Wagner and Vandergrift, 1972).

Mercury is a unique element because of its ability to bind to SH groups in proteins (Dorne and Hirth, 1970) and to form complexes with nitrogen atoms of the heterocyclic bases of DNA (Yamane and Davidson, 1961; Gruenwedel and Davidson, 1966; Izatt *et al.*, 1971), in addition to its ability to bind to oxygen (Carrabine and Sundaralingam, 1971). In particular, the metal binds to A + T rich DNAs more strongly than G + C rich DNAs and perhaps binds to N-3 of thymidine selectively (Nandi *et al.*, 1965). Studies on interactions with tomato bushy stunt virus indicate that mercury binds primarily to the ribonucleic acid inside the virus capsid (Dorne and Hirth, 1970) with a smaller amount binding to SH groups in the protein coat. In the previous investigations the nucleic acid and/or protein has been isolated and treated with mercury *in vitro*.

A systematic examination of nucleic acid isolated from a variety of tissues (Wacker and Vallee, 1959) revealed 13 metals firmly bound to the nucleic acid components; mercury was not included among these metals, possibly because of analytical difficulties previously associated with the determination of the low concentrations encountered in an *in vivo* study. However, in the present investigation, we have been able to establish that mercury must also be included among the naturally occurring metal constituents of chromatin and, furthermore, that the element appears to accumulate in nuclear material following prolonged exposures. These observations warranted further study; thus, we have used the techniques of phosphorescence, spectroscopy, viscosity, and thermal denaturation to characterize chromatin isolated from experimental animals treated with overdoses of mercury and from control animals to ascertain the possibility of structural alterations in the chromosomal material elicited by mercury binding *in vivo*.

Materials and Methods

Materials. Male Swiss-Webster strain white mice were obtained within 4 weeks of birth from commercial sources and separated into cages containing six mice each. All mice received *ad libitum* Purina Lab Chow throughout the study. Control animals were given deionized drinking water and experimental animals received deionized drinking water containing 1×10^{-8} M $HgCl_2$. After 4 weeks animals were etherized, livers were removed and quick-frozen in an acetone–Dry Ice bath, blood was collected by cardiac puncture, and serum was aspirated after centrifugation. Both whole livers and serum were stored at −5°. A typical experiment consisted of 30 mice: 15 control and 15 experimental.

Isolation of Mouse Liver Nuclei. A modification of the Chauveau isolation technique (Chauveau *et al.*, 1956) was used to prepare mouse liver nuclei and, subsequently, chromatin. Frozen livers were thawed, blotted with filter paper, weighed, minced with scissors, and homogenized at 4° in a Potter-Elvehjem homogenizer with 10–15 strokes of a motor driven pestle in 4 vol of 0.35 M sucrose containing 3 mM $CaCl_2$. After filtering through four layers of cheesecloth the

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homogenate was centrifuged at 600*g* in a Sorvall RC-2 centrifuge at 1° for 10 min. The supernatant was discarded and the pellet was homogenized in 5 vol of 0.25 M sucrose–3 mM $CaCl_2$ in a Dounce homogenizer with 4–6 strokes of a tight-fitting pestle and again centrifuged at 600*g*. The pellet was rehomogenized in a Potter-Elvehjem homogenizer in 10 vol of 2.2 M sucrose–3 mM $CaCl_2$ and centrifuged in a Beckman L2-75B centrifuge at 50,000*g* for 1 hr at 1° using an SW 27 swinging bucket rotor. The nuclear pellet was then resuspended in 4 vol of 0.25 M sucrose–3 mM $CaCl_2$ by gentle homogenization in a Dounce homogenizer using a loose fitting pestle and the suspension was centrifuged at 600*g*. The whole cell/intact nuclei ratio was determined using a Levy counting chamber under a phase-contrast microscope.

Cytoplasmic Contamination. Liver from control and experimental animals was subjected to the cell lysis and homogenation treatment described above. The supernatant (total cytoplasmic fraction) and crude nuclear pellet were separated, each was divided into two portions, and the supernatant was used as a substrate for incubating the nuclear pellet as follows: one-half the nuclear pellet from control was placed in one-half of the experimental supernatant while the remainder of the control pellet was returned to control supernatant; one portion of the experimental nuclei was placed in control supernatant and the remaining experimental pellet was replaced in experimental supernatant. All four samples were incubated at 4° for 1 hr (essentially twice the time that nuclei are exposed to the cytoplasmic fraction in a routine isolation experiment). Nuclei were then isolated by the techniques described above and each of the four samples suspended in 10 ml of 1.0 × SSC.[1] A 2-ml aliquot was used for DNA analysis and the remaining portion was centrifuged and the nuclei were removed, and the sample was then suspended in concentrated nitric acid–sulfuric acid and digested at 95° for 2 hr prior to flameless atomic absorption analysis.

Preparation of Chromatin from Isolated Nuclei. The purified nuclear pellet was resuspended with gentle strokes in 0.14 M NaCl using a Dounce homogenizer with a loose-fitting pestle and centrifuged at 3000*g*. This step was repeated twice: first the pellet was resuspended in 1:10 SSC and centrifuged at 10,000*g*, and finally the pellet was resuspended in 1:100 SSC and centrifuged again at 10,000*g*. The recovered opalescent pellet constitutes purified chromatin which was stored at −5° in 2 vol of 1:100 SSC. Immediately before use, samples were thawed and gently rehomogenized; soluble chromatin thus prepared exhibited a ratio, $A_{320}:A_{258}$, less than 0.1, usually about 0.03.

Further Purification of Chromatin. Gel filtration of chromatin was performed in a water-jacketed column (1.6 cm × 50 cm) packed with Sephadex G-25 coarse (Pharmacia) and equilibrated with 1:100 SSC. The temperature was maintained at 5 ± 0.1° by a Lauda K-2/4 circulator. The sample, usually 1 ml, was applied on the column and a flow rate of 5 ml/min was established. Sample dilution across the column was measured by comparing ultraviolet optical densities of samples before and after gel filtration. Blue Dextran 2000 was eluted from the column at least once prior to every set of samples to check for changes in void volume or dilution. The column was washed with approximately 1 l. of 1:100 SSC following elution of a sample.

Quantitation of DNA in Isolated Chromatin. The total DNA in isolated chromatin was determined by a diphenylamine colorimetric assay (Dische, 1930). Standards were made from purified, highly polymerized salmon sperm DNA (Sigma Corp.) dissolved in 1 N perchloric acid. DNA standards were stable for several weeks at 4°; the diphenylamine reagent was prepared on the day of use.

All ultraviolet spectra were obtained using a Cary Model 15 recording spectrophotometer and matched sets of silica or quartz cells of 1-cm path length.

Quantitation of Mercury. Using flameless atomic absorption, levels of mercury down to 0.5 ppb[1] in solution can be detected (Manning, 1970). A Varian Techtron Type AA5 atomic absorption spectrometer was used with a 51-RO spectrophotometer grating monochromator, a Techtron D1-30 digital indicator, and a Techtron DC-31 digital corrector. The light source was a Jarrel-Ash Type 45493 hollow cathode tube specific for mercury with a main resonance line at wavelength 2536.5 Å, with a slit width of 100 μ and noise suppression set at 3 or 4. When a recorder was used (Sargent, Model 160), the scale selector was set at 10 mV and the chart speed was 0.5 in./min. A flow cell was constructed by sealing quartz windows on a piece of Pyrex tubing 10 cm × 2 cm with hose connectors near each end. The cell was aligned in the light beam by attachment to the burner assembly of the instrument. The burner adjustments were then used to move the cell vertically and horizontally to give maximum transmission of light.

All parts of the apparatus were washed in concentrated nitric acid before assembly. Since certain materials (*e.g.*, cyclic organic compounds and water vapor) may volatilize in the sample along with the mercury giving enhanced background signals, no organic reagents were used in cleaning or drying the apparatus and a drying tube containing anhydrous granular calcium chloride (4 mesh) was placed between the reaction flask and the flow cell. Samples were digested by refluxing for 2 hr in a concentrated nitric acid–sulfuric acid mixture. (In one experiment (Table II, III) samples were not refluxed.) The sample (0.5 ml) was placed in a digestion flask to which was introduced 15 ml of nitric acid (15.7 N) and 5 ml of sulfuric acid (17.8 N). A Graham reflux condensor was placed on the flask and samples were refluxed with a flask heating mantle while water circulated through the condensors at 5°. Following digestion, the samples were cooled and diluted to 50 ml giving a final 1:100 dilution of sample. Internal mercury standards were added to samples to test recovery.

All reagents including the chelexed water were tested to establish a background level of mercury for samples. To check for contamination of chromatin by mercury during isolation, purification, or digestion, glass beads were subjected to the entire isolation procedure including storage at −5°. All samples were stored in plastic containers (Nalgene) for less than 48 hr before mercury determinations were made.

Mercury standards were prepared from a 1000-ppm stock and diluted with 1 N hydrochloric acid (Jarrell-Ash, 1970; Lindsfedt, 1970). Standards were prepared immediately prior to use and a standard curve was plotted for each digestion batch. Immediately prior to determinations of mercury, the reducing agent, 10% (w/v) $SnCl_2$ in concentrated hydrochloric acid, was prepared.

Thermal denaturation was done using a Gilford 2400 recording spectrophotometer with a reference compensator and equipped with a four-cell automatic sample changer employing Gilford thermospacers heated by the circulation of ethylene glycol at a rate of about 2 l./min from a Lauda K-4/R10 circulator. Circulator temperature was increased at a rate of about 0.5°/min. Gel-filtered samples were diluted in 1:100

[1] Abbreviations used are: SSC, standard saline-citrate (0.14 M NaCl–0.014 M sodium citrate adjusted to pH 7.0); ppb, parts per billion.

TABLE I: Chemical and Physical Properties of Chromatin.

Sample	DNA[a] (μg/ml)	Mercury Content: μg of Hg/μg of DNA $\times 10^3$	Mercury Content: Nucleotides[e] per Hg atom	Uv Absorption: 230:258	Uv Absorption: 258:280	Thermal Denaturations (deg)[b]: T_m	Max$_1$	Max$_2$	Max$_3$	H (%)	Intrinsic[g] Viscosity, η (dl/g)
Control											
Stock	180.0	0.47	1,276	0.87	1.65						
Gel filtered[c]		0.05	10,870			71	Sh[f]	68	88	33.5	
Glass filtered[d]	128.5										1.8
(salmon sperm DNA)						(59)				(39)	
Experimental											
Stock	166.0	1.35	446	0.87	1.58						
Gel filtered[c]		0.66	912			72	56	72	88	34.5	
Glass filtered[d]	119.0										1.1

[a] Diphenylamine assay. [b] Max refers to maximum inflection in thermal denaturation profile based on first derivatives; H = hyperchromicity. [c] Sephadex G-25. [d] Sintered glass filtered prior to viscosity determinations. [e] DNA–phosphorus in chromatin was estimated from absorbance at 258 nm using an extinction coefficient of 7400/mol of phosphorus. [f] Shoulder. [g] Slopes for lines for control and experimental samples were 0.62 and 0.79, respectively.

SSC to give an initial absorbance of 0.3–0.4 Å (13.5–18 μg/ml of DNA) at 30° and degassed under line vacuum for 10 min.

Thermal denaturation was monitored by the absorption change at 258 nm and hyperchromicity relative to absorption at room temperature was calculated degree by degree. The final absorbance at wavelength 320 nm was measured. The data were calculated with a Dec System 10 digital computer (48 K core memory) and results were plotted by a Houston Instruments plotter. The computer program normalized the absorbance value to hyperchromicity.

Phosphorescence spectra were obtained with a Perkin-Elmer Model MPF-2A fluorescence spectrophotometer equipped with a liquid nitrogen dewar of the cold finger type. Fluorescence light and scattered light were eliminated by the use of a rotating shutter device driven by a variable speed motor. Lifetimes were determined from an analysis of the photographed signal appearing on a No. 564 storage oscilloscope screen (Tektronix, Inc., Portland, Ore.). The mean lifetime of phosphorescence is the time, $T_{1/e} - T_0$, where T_0 is the initial phosphorescence intensity and $T_{1/e}$ is the amplitude of the signal reduced to 36.78% ($1/e$) of its maximum value.

All samples were made up in 50% spectral grade glycerol and were degassed by repeated freezing (liquid nitrogen and thawing under vacuum). Phosphorescence of solvent was negligible under these conditions of the experiment.

Viscosity. A couette type viscometer (Beckman Instruments) of the Zimm and Crothers design was used. Temperature was maintained at 20 ± 0.01° by a Lauda K-2/R circulator. A least-squares fit line was calculated for data given in Table I.

Chemicals. A pure sample of the protein tropomyosin was a gift of John Murray. Other chemicals were reagent grade. Deionized chelexed (100) water was used in cleaning glassware and in the preparation of solutions.

Results

Chromatin isolated from both control and experimental animals exhibited similar solubility properties. Nonsolubilized materials were sedimented from solutions in 0.01 M NaCl; the supernatant was dialyzed 76 hr against three changes of solvent. The chromatin was assumed to be solubilized in 0.01 M NaCl since this dialysate showed little or no absorbance at wavelength 258 nm. Dissociation of chromatin proteins occurring with increasing ionic strength was monitored at NaCl concentrations of 0.001, 0.01, 0.025, 0.5, and 1.0 M by the absorbance ratio of 230:258 which decreased from 0.87 in 0.001 M to 0.63 in 1.0 M, reaching a value of 0.67 in 0.5 M NaCl, comparing favorably with chromatin isolated under comparable conditions (Henson and Walker, 1971; Jensen and Chalkley, 1968).

Other chemical and physical properties of the chromatin samples are given in Table I where again similarities exist in DNA content and in protein composition, as estimated from absorbance at 230:258 nm (Chalkley and Jensen, 1968). The mercury content, however, is greatly elevated in material isolated from animals subjected to constant exposure to the metal. Although present at readily detectable levels in control, there is a threefold increase in mercury in experimental stock chromatin. Mercury levels in serum and liver are likewise elevated (Table II). In spite of the precautions taken to prevent contamination, nonbound mercury isolated along with the chromatin is contributed partly by that present in nucleoplasm or cytoplasm (Table III). The crude control nuclear pellet incubated in the experimental cytoplasmic fraction prior to isolation of nuclei showed only a 1.4-fold increase in metal bound to nuclei. In contrast there was a 24–29-fold enrichment in experimental nuclei exposed to either control or experimental cytoplasmic fractions. This indicates that experimental nuclei had accumulated about 20 times more mercury from *in vivo* processes than could be attributed to contamination as a consequence of the isolation procedure. Furthermore, it must be noted that one-half of the mercury remained associated with chromatin after gel filtration in experimental samples in contrast to one-tenth in control samples. Comparing gel-filtered control with gel-filtered experimental samples, there is a tenfold increase in specific bound metal, suggesting a strong tendency for mercury to interact with and perhaps to accumulate in the chromatin fraction *in vivo*.

Thermal denaturation profiles are shown in Figure 1 from which derivative plots were constructed in Figure 2. While the

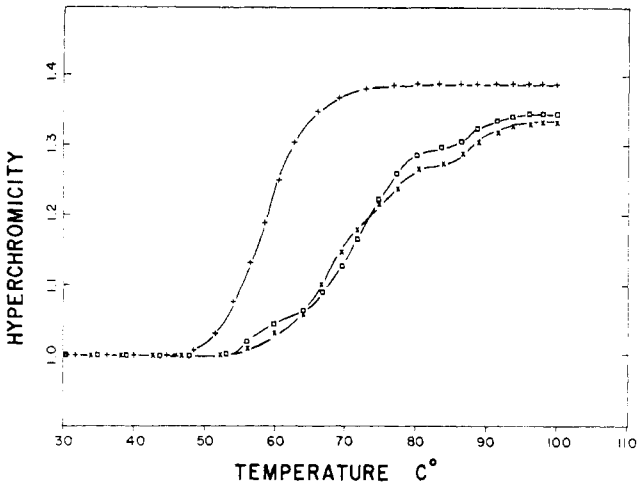

FIGURE 1: Thermal denaturation of DNA (+), control chromatin (×), and experimental chromatin (□). Melting was performed on gel-filtered samples diluted in 1:100 SSC (pH 7.0) to give an initial absorption of 0.3–0.4 Å.

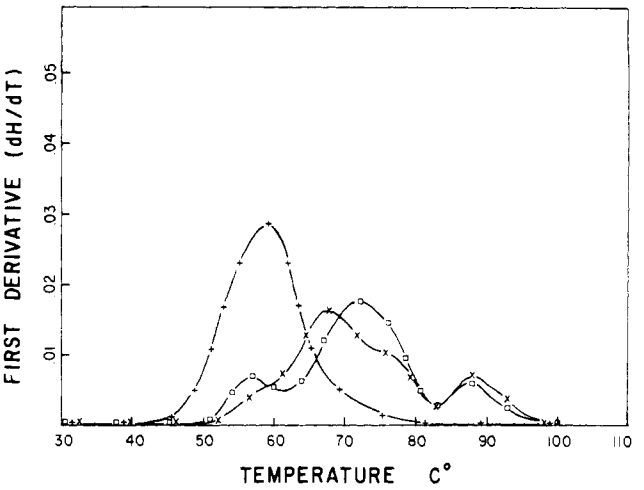

FIGURE 2: Derivative plots constructed from the thermal denaturation profiles given in Figure 1: (+) DNA; (×) control chromatin; (□) experimental chromatin.

midpoint transition temperatures (T_m) are essentially identical, the denaturation profiles are distinctly different as follows. (1) A slight shoulder at 56° in the control sample is resolved into a band in high mercury chromatin which is very close to the pure DNA band; it could represent melting of uncomplexed DNA or regions bound by nonhistone protein (Ansevin *et al.*, 1971; Li and Bonner, 1971; Shih and Lake, 1972). (2) The major melting transition is displaced from 68° in the control to 72° in the experimental sample and the 75° shoulder in the control is masked. This may correspond to the transitions reported for histone stabilization of DNA histone structures (Shih and Lake, 1972) and for formaldehyde chromatin stabilization (Ansevin *et al.*, 1971) also attributed to histone binding. (3) A third band appearing in both samples in our experiments has not previously been reported by the other investigators, possibly because of differences in solvents, manner of preparation, and intrinsic differences between sources. We have omitted the use of EDTA in our experiments in order to avoid the removal of mercury. Thus, the 88° band could reflect interactions with divalent metals other than mercury which are normally removed by EDTA. In the thermal denaturation studies, light scatter measurements at wavelength 320 nm were initially 0.03 of the absorbance at 258 nm. The light scatter at 100° was slightly higher for experimental (0.10) than for control samples (0.07).

Luminescence spectra are shown in Figure 3 and the emission parameters are summarized in Table IV. The phosphorescence of chromatin samples is suggestive of a composite emission spectrum obtained from proteins containing aromatic residues. Spectra of two such proteins are included in Figure 3 for comparisons: bovine serum albumin containing five tyrosine, seven phenylalanine, and one tryptophan (Truong *et al.*, 1967) and tropomyosin containing aromatic residues of tyrosine exclusively. The emission of DNA also shown in Figure 3 is known to have a weaker phosphorescence with an unstructured spectrum at wavelength 450 nm (Eisinger and Lamola, 1971). Heterogeneities in protein emission spectra arise from variations in local environments of the individual aromatic residues (Purkey and Galley, 1970). Ordinarily, phenylalanine emission cannot be detected if the other two residues are present because of low absorbance and low quantum yield (Weinryb and Steiner, 1970). Furthermore, the contribution of the tryptophan group dominates the emission,

TABLE II: Mercury Levels in Serum and Liver Tissue.

Sample	μg of Hg/ml (ppm)
Expt I[a]	
Serum	
Control	0.085
Exptl	0.825
Total liver homogenate	
Control	0.105
Exptl	1.160
Expt II[b]	
Serum	
Control	0.080
Exptl	0.340
Liver nuclei	μg of Hg/mg of DNA
Control	0.009
Exptl	0.227

[a] Samples were digested by refluxing at 95° for 2 hr in a concentrated nitric acid–sulfuric acid mixture. [b] Samples were digested for 2 hr at 95° in a concentrated nitric acid–sulfuric acid mixture without refluxing.

TABLE III: Mercury Uptake in Nuclei Exposed to the Cytoplasmic Fraction *in Vitro*.[a]

Incubation Treatment Nuclei–Cytoplasmic Fraction	Mercury Uptake ppb of Hg/μg of DNA	Enrichment Ratio[c]
Control–control	9.5	1.0
Control–experimental[b]	13.5	1.4
Experimental[b]–control	278.0	29
Experimental[b]–experimental[b]	227.0	24

[a] Samples were digested for 2 hr at 95° in concentrated nitric acid–sulfuric acid mixture without refluxing. [b] Animals given 1×10^{-3} M $HgCl_2$ for 1 month. [c] Enrichment ratio: sample/control nuclei–control cytoplasmic fraction.

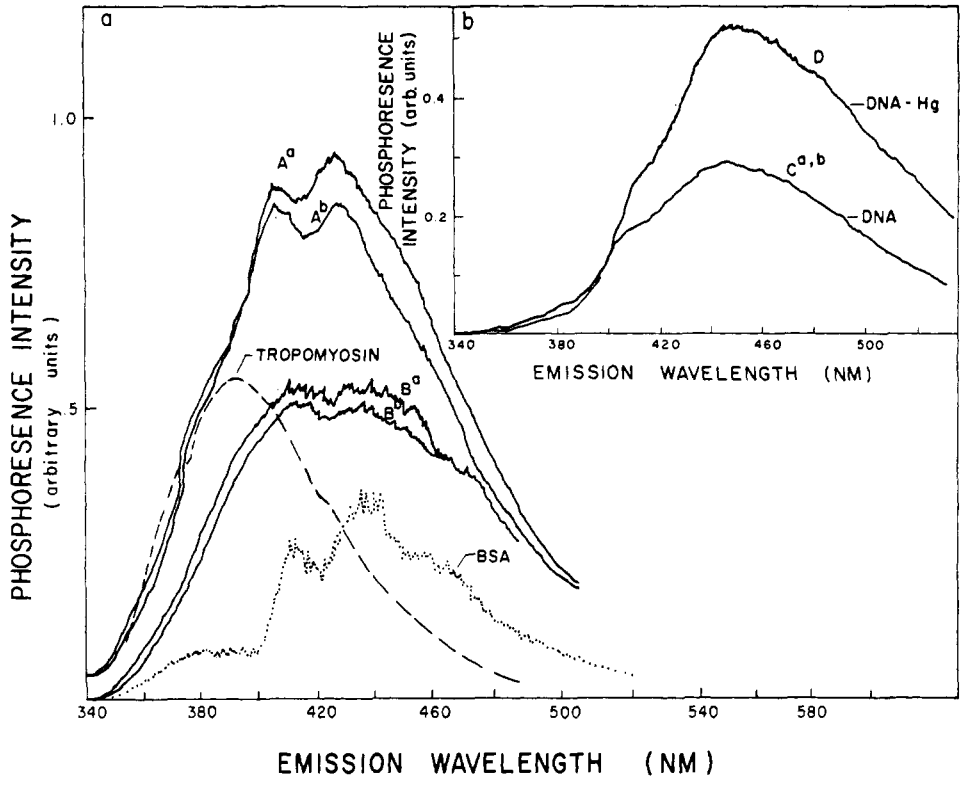

FIGURE 3: (a) Phosphorescence emission of control (A^a) and experimental (A^b) chromatin in 1:100 SSC (pH 7.0), made up 1:1 (v/v) with glycerol at 77°K. The two samples were then treated with NaCl to bring the concentration to 0.5 M NaCl; dissociated control (B^a) and dissociated experimental (B^b) chromatin. Emission of two proteins is shown for comparisons: tropomyosin (---) and bovine serum albumin (· · ·). (b) Emission of DNA (C^a) in 5 mM $NaNO_3$ (pH 6.8) made up 1:1 (v/v) in glycerol at 77°K. C^b is the same solution with mercury added to bring DNA/Hg to 500. The top curve (D) is the emission of DNA–Hg where the DNA/Hg ratio is 0.5.

even if tyrosine is present in substantial quantities (Weber and Teale, 1965). Both control and experimental spectra reflect a considerable contribution from tyrosine emission in native material along with tryptophan emission, with a possible contribution from DNA. Tyrosine emission disappears following the partial dissociation of proteins.

The lifetime found for control chromatin at 440 nm, pH 7.0, was significantly less than that reported for tryptophan (6 sec) and approaches the lifetime reported for tyrosine (Weinryb and Steiner, 1970). However, phosphorescence decay in chromatin monitored at 440 nm could be partly influenced and shortened by the DNA component which had a lifetime of 0.3 sec (Eisinger and Lamola, 1971).

While the phosphorescence spectrum of experimental chromatin is quite similar to control, there is nevertheless a shift in relative peak heights with a decrease in intensity at 430 nm and a decrease in lifetime by a factor of 2 (Table IV). These results could be due to the direct interaction of mercury with tryptophan, resulting in "heavy atom" quenching (Weinryb and Steiner, 1970). Alternatively mercury may induce conformational changes which result in an altered tryptophan environment. Evidence for such a conformational change is supported by viscosity measurements (Table I) where the intrinsic viscosity decreased from 1.8 to 1.1 indicating a small structural alteration.

Interactions between mercury and DNA probably have little or no influence on the observed emission properties of experimental chromatin. At nucleotide mercury levels compatible with the mercury content of experimental chromatin (DNA/Hg = 500) there was no observed effect on either intensity or decay time; however, where DNA/Hg is low (0.5) there is an enhancement of intensity and a marked decrease in decay time (Table IV and Figure 3). Following partial protein dissociation after treatment with 0.5 M NaCl, the emission properties of both chromatin samples are essentially identical. That the luminescence of chromatin is characterized by the protein–DNA ratio and overall conformation is reflected in data (Table IV) obtained from a partial fractionation of control chromatin. There is a shift in emission intensity maximum and a decrease in decay times as the ratio of DNA to protein increases. Thus, the quenching effect produced by mercury is eliminated in dissociated chromatin; further, the DNA contribution becomes evident as the DNA to protein ratio increases and is reflected in the shift in maximum intensity and shortening of decay time.

Discussion

Isolated chromatin is believed to retain much of the chemistry of native chromosomal material and some of its native biological function (Jensen and Chalkley, 1968). The chromatin prepared for this study was considered pure and in the native state on the basis of: (1) behavior in various sodium chloride concentrations; (2) light scattering measurements at wavelength 320 nm; (3) DNA content as determined by diphenylamine assay and ultraviolet absorption at wavelength 258 nm; (4) protein content as estimated from the ultraviolet absorption ratio at wavelengths 230 and 258 nm. An analysis of physical and chemical properties of the materials obtained from the two sources was considered to be germane to a better understanding of the structural details of chromatin.

The thermal denaturation experiments indicate that melting properties of experimental chromatin are altered most likely as a consequence of mercury–protein interactions. The small

TABLE IV: Emission Properties of Chromatin and DNA at 77°K.

Sample	pH	Solvent	Emission[f] λ_{max} (nm)	Lifetime[g] τ (sec)[d]
Native DNA	6.8	(1:1 v/v) glycerol–5 mM $NaNO_3$	450	0.30
DNA–Hg[a]			450	0.35
DNA–Hg[b]			470	0.07
Native chromatin	7.0	1:100 SSC		
Control			410, 430	3.3
Exptl			410, 430	1.7
Dissociated chromatin	7.0	0.5 M NaCl		
Control			c	1.0
Exptl			c	0.9
Partially fractionated control chromatin[e]	8.0	0.01 M Tris		
DNA/protein (mg/ml)				
1.0			430	1.3
1.5			435	1.0
3.0			440	0.6

[a] DNA/Hg = 500. [b] DNA/Hg = 0.5. [c] Broad band, see Figure 3a. [d] Estimated precision ±0.1 sec. [e] According to the method of Yasmineh and Yunis (1970). [f] Excitation wavelength was 265 nm. [g] Monitored at wavelength 440 nm.

intrinsic viscosity differences indicate some change in native chromatin structure and hydrodynamic behavior in favor of a less rigid, more compact particle. Alternatively, it must be noted that partial protein (histone) dissociation elicited by mercury could cause aggregation, thus introducing light scattering artifacts. However, one must stress that chromatin was passed through Sephadex G-25 prior to melting to remove nonbound mercury and samples were sintered-glass filtered prior to viscosity determinations to reduce the possibility of aggregate formation.

Luminescence experiments are also consistent with protein–mercury binding and provide no evidence for specific DNA–mercury interactions. When DNA phosphorescence measurements were made in the absence and presence of mercury and using mercury concentrations equivalent to that present in the experimental chromatin, no difference could be detected in either the emission intensity or in the decay time. This is in contrast to reports on effects observed by optical rotatory dispersion (Luck and Zimmer, 1971) where a marked deformation on secondary structure was observed at 1 mercury atom/500 DNA nucleotides. DNA phosphorescence is readily observed at 77°K and the DNA triplet is reported to be virtually identical with the thymine triplet (Eisenger and Lamola, 1971). Assuming that mercury binds selectively to thymine, the metal, known to be an effective quencher, should produce pronounced effects on DNA phosphorescence; this indeed was observed when the ratio of nucleotide to metal was suitable (Figure 3b). It is possible that mercury could bind to DNA in chromatin in ways that are different from the reactions of free DNA with mercury. Thus, the phosphorescence studies do not disprove mercury binding to DNA. Chromatin phosphorescence can be explained qualitatively on the basis of emissions from aromatic residues of the protein component, and both the emission spectra and the decay times are compatible with proteins containing a significant number of tyrosine residues; tryptophan appears to be present but does not dominate the emission spectrum for native materials.

A report appearing after our studies had been completed (Chanda and Cherian, 1973) revealed that a nonhistone component, firmly bound to DNA, incorporates 35–45 times more ^{203}Hg *in vivo* than any other nuclear protein fraction. This work gives firm support to our findings.

In summary, we proposed in the present investigation to examine the mercury content of chromatin. Material isolated and characterized as pure and native according to several criteria contained one mercury atom/10,000 nucleotides in the absence of external exposure (control). Equally important is the observation that material obtained from animals challenged with a constant level of mercury showed a 20-fold enrichment of metal in nuclei and a 10-fold enhancement of the metal in chromatin which could not be removed by gel filtration. Trace amounts of nonbound mercury isolated along with chromatin were probably contributed by cytoplasm, yet the data indicated that a much larger portion was present as an intrinsic constituent of chromatin and that the metal had a strong tendency to interact with and to induce structural alterations in the nuclear material. It was known from previous experiments that the sublethal levels of mercury used in the present study were seemingly nontoxic levels in that animals lived as long as controls and appeared healthy throughout the study (Bryan and Hayes, 1972); yet serum proteins monitored by electrophoresis (Bryan and Bright, 1973) which were markedly altered after short term ingestion of the metal appeared normal after prolonged exposure. The mechanisms which bring about such physiological adaptations in mammals are not known. Thus, the present study raises numerous questions concerning possible structural and regulatory roles for mercury in native chromosomal material.

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Interaction between Steroids and a Uterine Progestogen Specific Binding Macromolecule†

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ABSTRACT: Forty-five steroidal compounds, derivatives of both progesterone and testosterone, have been studied for their ability to bind to the rabbit uterine progestogen receptor. Introduction of a hydroxyl group in the 17α, 16α, 6α, 11α, or 14α position precipitously reduces the affinity of progesterone for the macromolecule, whereas introduction in the 11β position has no effect. If the polarity of the hydroxyl groups is altered by acylation, binding affinity is not depressed. When an alkyl group is added at C-6α or C-16α, binding affinity increases, whereas a β-alkyl group at C-16 prevents binding. Although the C-3 ketone is not required for binding affinity, introduction of a bulky thioketal group at C-3 completely prevents binding. Reduction of the $\Delta^{4,5}$ double bond of progesterone to yield a compound with A/B trans juncture has no effect on binding affinity, but the isomer with a nonplanar A/B cis juncture possesses lower binding affinity. Removal of C-19 from progesterone results in a slightly greater binding affinity for the uterine macromolecule. The two modifications of testosterone which most dramatically increase binding affinity are removal of the C-10 methyl group and introduction of an alkyl group in the 17α position. In the latter case, binding affinity increases as the electron-donating power of the group is increased. The *d* isomer of norgestrel binds, whereas the *l* isomer does not bind to the uterine progestogen receptor.

It has been reported that a protein which specifically binds progesterone and progestational drugs exists in the high-speed supernatant fraction of mammalian uteri (Milgrom *et al.*, 1970; Rao and Wiest, 1971; McGuire and DeDella, 1971; McGuire and Bariso, 1971, 1972). This macromolecule, termed a receptor, appears to be target tissue specific (McGuire and Bariso, 1972) and a positive correlation exists between the protein's affinity for steroids *in vitro* and the progestational activity of those steroids *in vivo* (McGuire *et al.*[1]). Although the physiological role of this binding protein is unknown, the

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