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Publication Date

1971-08-01

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August 1971

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AN INVESTIGATION OF SPECIFIC INTERACTIONS OF
DNA AND LYSINE-RICH (F1) HISTONE PREPARATIONS[*]

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ABSTRACT: The interaction of F1 histones and DNA was studied by means of circular dichroism spectroscopy and enzymatic assays with DNAase I and RNA polymerase. F1 histones prepared by TCA extraction from a given/species were more effective in protecting DNA of that species from DNAase hydrolysis, than a similar preparation from a foreign species. Analogous results have been obtained with RNA polymerase assays. Changes in the circular dichroism spectra of DNA-histone complexes were attained at lower histone: DNA ratios when both histone and DNA were from the same species than if heterologous mixtures were used. In contrast, when chromatographically purified F1 histones were used in these experiments, no evidence for species specificity was obtained. We conclude that some component present in the TCA extracts and absent from the purified fractions imparts species-specific properties on the nucleoprotein complex either by acting alone or in concert with the F1 histones.

Introduction

The genetic material of higher organisms exists as a complex of nucleic acid (DNA and RNA) and chromosomal proteins (histone and non-histone). When isolated from interphase nuclei, this complex (chromatin) is less active than deproteinized DNA, isolated from the same chromatin, in supporting DNA-dependent RNA synthesis (Huang and Bonner, 1962). The template activity of chromatin may be raised to that of DNA by the selective removal of chromatin-bound histones by techniques which leave non-histone proteins bound to DNA (Marushige and Bonner, 1966). Evidence for the regulatory role of histones comes from the finding that the template activity of deproteinized DNA may be reduced by reannealing DNA with histones (Barr and Butler, 1963); furthermore, histones alter the base composition and nearest neighbor frequency of the transcribed RNA (Salha et al., 1966). Bonner et al. (1963) have elegantly demonstrated that, by removal of histones from pea bud chromatin, a pea cotelydon-specific RNA could be synthesized. The RNA so produced was found to be capable of directing the synthesis of a cotelydon-specific globulin in an in vitro protein synthesizing system.

If histones do indeed serve some regulatory role, and do not act solely as structural elements in the architecture of chromosomes, one might expect to see variations in the pattern of distribution of the classes and numbers of histones throughout nature. Several groups (Crampton et al., 1957; Fambrough and Bonner, 1966; Stellwagen and Cole, 1968) have failed to detect such variations, while Panyim et al. (1971) have recently reported differences in the electrophoretic

patterns of vertebrate histones. Evidence that histones do not act as
genetic regulators, but rather as generalized repressors, comes from
the finding that poly-L-lysine can act as an inhibitor of DNA-directed
RNA synthesis (Shih and Bonner, 1970). Huang and Bonner (1965) have
advanced the view that histones act as specific genetic regulators only
when they are complexed with a special class of RNA (chromosomal RNA).
This RNA was reported to be of wide occurrence and organ-specific
(Bonner and Widholm, 1967; Benjamin et al., 1966; Huang, 1967; Shih and
Bonner, 1969). Bekhor et al. (1969) have shown that chromosomal RNA is
required for the sequence-specific interaction of DNA and chromosomal
proteins. Recently, however, the unique nature of this RNA has come
under question (Heyden and Zachau, 1971).

The conservation of the primary structure of the arginine-rich
F2al histones throughout evolution (De Lange et al., 1969) refutes
the hypothesis that this histone component acts as an organ- or species-
specific regulator of gene activity. Such a role, however, could be
played by the lysine-rich F1 histone fraction. Rall (1970) has found
that in the N-terminal third of three individual lysine-rich histones,
one from calf thymus and two from rabbit thymus, there are between
seven and fourteen amino acid replacements. Furthermore, the F1 his-
tones of different vertebrate species vary considerably in electro-
phoretic mobility (Panyim, et al., 1971).

In light of the similarity of F2al histones from calf and pea and
the pronounced differences between F1 histones of calf and rabbit, it
appeared interesting to test whether F1 histones of a given species
might bind preferentially to DNA from that species. Over the past

few years, a variety of methods have been used to probe the nature of the DNA-histone interaction: Bonner and his coworkers have pioneered the use of the RNA polymerase system with chromatin and reconstituted nucleohistones (e.g., Bonner et al., 1968); Clark and Felsenfeld (1971) have studied the action of nucleases on chromatin; many groups have investigated DNA-protein interactions by means of thermal denaturation studies (Olins and Olins, 1971) and by means of circular dichroism (Simpson and Sober, 1970; Fasman et al., 1970; Shih and Fasman, 1971; Olins and Olins, 1971). We have used several of these methods in a search for species specificity in the interaction of the lysine-rich F1 histones with DNA.

Materials and Methods

Preparation of lysine-rich histones. Thymus glands from calf were obtained from a local slaughter house and transported to the laboratory in wet ice. Rabbit thymus glands were obtained from the Pel-Freeze Biologicals Co., Rogers, Ark., and shipped to the laboratory on solid CO_2. Tissue was stored at -20° until needed. Lysine-rich histones from these tissues were prepared by the method of de Nooij and Westenbrink (1962) as modified by Kinkade and Cole (1966). Thymus glands were homogenized in 10% (w/v) trichloroacetic acid (TCA) and insoluble material was removed by centrifugation for 10 min at 1100 X g. The supernatant solution was made 20% (w/v) in TCA by the addition of 100% (w/v) TCA, and was stirred for 5 min. The precipitate was collected by centrifugation at 1100 X g for 10 min, and washed once with acidified acetone (475 ml acetone plus 0.25 ml concentrated HCl), and then repeatedly with neutral acetone. The final products were frozen at -20° and then dried under reduced pressure over anhydrous P_2O_5 at room temperature. This preparation will be referred to as the acetone powder.

Column chromatography of the lysine-rich histones was carried out on Amberlite IRC-50 (Bio-Rad Laboratory, Richmond, Calif.) on a column measuring 2.5 X 36.5 cm, which had been prepared by the method of Kinkade and Cole (1966). 100 mg of acetone powder was dissolved in 12 ml of 0.1 M sodium phosphate buffer, pH 6.8, containing 7% (w/v) guanidine-HCl, by stirring overnight at room temperature. A small amount of insoluble material was removed by centrifugation, and the supernatant was applied to the column. The chromatogram was developed by elution with a linear gradient between 7 and 14% guanidine-HCl, in 0.1 M sodium phosphate buffer, pH 6.8. A flow rate of 9 to 10 ml per h was maintained with a peristaltic pump. Fractions were collected every 30 min, and protein content was determined by turbidimetry. Each fraction (0.2 ml) was incubated for 30 min with 2.8 ml 18% (w/v) TCA. The absorbancies were then measured at 400 nm with a Zeiss PM QII spectrophotometer, using cells of 1 cm path length. Guanidium chloride concentrations were determined by refractometry.

Amino acid composition analysis was performed on a Beckman/Spinco analyzer, according to the method of Spackman, Moore and Stein (1958). The compositions presented are uncorrected for loss during 22-h hydrolysis at 110°.

Protein determinations were performed routinely by the method of Lowry et al. (1951) using F1 histones purified by Amberlite chromatography as the standard.

Deoxyribonucleic acid was isolated from rabbit thymus and rat liver after the tissue had been treated in the following manner: frozen tissue was cut into small pieces and pressed through a sieve to remove connective tissue. This material was then homogenized in 200 ml of 0.32 M sucrose

with a teflon homogenizer. The homogenate was centrifuged at 1000 X g

for 10 min at -5°; the supernatant was discarded, and the pellet (crude

nuclei) was carried through the procedure of Marmur (1961) for the iso-

lation of DNA. DNA prepared in this manner showed an A_{320}/A_{260} value of

0.03 and an A_{260}/A_{230} value of at least 2.0.

DNA was also obtained from commercial sources. Highly polymerized

DNA from calf thymus was the product of the Sigma Chemical Co., St. Louis,

Mo. (lot no. 60C-4800). DNA from salmon sperm was obtained from Calbiochem,

Los Angeles, Calif. (lot no. 40039). Polydeoxyadenylate-thymidylate (poly

d(AT)) was a product of Miles Laboratories, Elkhart, Ind. (lot no. 27).

DNA from phage T2 was a gift of Dr. M. Chamberlin, Dept. of Molecular

Biology, University of California, Berkeley.

DNA concentrations were determined either spectrophotometrically,

using an ε_{260} value of 6800, or by the diphenylamine procedure of Burton

(1956), using 2-deoxy-D-ribose as the standard. Protein contamination of

the various DNA samples was determined to be less than 0.5%.

RNA concentrations were determined by the orcinol reaction, as modified

by Hatcher and Goldstein (1969) (without cadmium salt precipitation),

using yeast RNA as the standard.

Sucrose gradient sedimentations were run on the DNA preparations to

test for homogenity with respect to molecular weight. A small volume

(about 100 μl) of the DNA preparation to be tested was layered on a 5 ml

gradiant of 5 - 20% (w/v) sucrose in 0.01 M Tris-HCl, pH 7, in a centri-

fuge tube. Centrifugation was carried out in a Beckman Model L-2 Ultra-

centrifuge with a no. 39 rotor at 39,000 rpm at 0° for 2 h. At the end

of a run, the tubes were punctured and 0.2 ml fractions were collected

with the aid of an apparatus previously described by Flamm et al. (1969).
To each fraction was added 0.8 ml of water, and the absorbancies were
read at 260 nm.

Complexes of DNA and lysine-rich histones were formed by three
different methods: (1) Complexes were prepared by the urea-salt gradient
dialysis procedure of Shih and Bonner (1970). Histone and DNA were mixed
in 1.8 M NaCl, 0.01 M Tris-HCl, pH 8.0, and dialyzed against a step gra-
dient of 0.5, 0.3, and 0.14 M NaCl in 5 M urea, 0.01 M Tris, pH 8.0.
Each step was for a minimum of 3 h at 4°. A final dialysis against 0.14 M
NaCl, 0.01 M Tris-HCl, pH 8.0, for the removal of urea was carried out
for 16 h. For enzymatic assays, the complexes were dialyzed against 1.5
mM NaCl, 0.01 M Tris-HCl, pH 7, for at least 24 h. Generally, 2 ml of
sample was dialyzed against 2 l of buffer at all stages of dialysis.

(2) The second method was based on the addition of excess histone
to DNA in moderate salt concentration (0.23 M NaCl) and the removal of
unbound histone from complexed DNA by means of chromatography on Bio-Gel
P-30 (Bio-Rad Laboratories, control no. 56433). Histone was in a 2.5
fold excess of DNA (by weight). Histone-DNA solution (3 ml) was applied
to a column measuring 2.4 X 20 cm, and was eluted with 0.01 M Tris-HCl,
pH 7.5. Fractions of 3 ml (180 drops) were collected, and the A_{260} of
each fraction was determined. A portion (0.25 ml) of each fraction was
taken for protein determination.

(3) The final method was direct addition of DNA to histone solutions
in low ionic strength buffer accompanied by vigorous agitation. Generally,
100-200 μl of DNA solution (about 1 mg/ml) was pipetted slowly into a

histone solution of desired concentration (10-200 µg/ml) while the histone solution was being agitated on a Vortex mixer. Solutions of complexes formed in this manner are more turbid than those formed by either of the two methods above.

Ultraviolet absorption spectra were obtained with a Cary Model 14 recording spectrophotometer and with a Cary Model 14R spectrophotometer equipped with the Cary Model 1462 scattered transmission attachment. The path length for all absorption spectra was 1 cm. Spectra taken with the Model 14R were obtained with a fluorscat cell (Dorman and Hearst, 1971). All spectra were taken at room temperature.

Circular dichroism spectra were taken with a Cary Model 60 recording spectropolarimeter equipped with a Cary Model 6001 CD attachment. Spectra were recorded at room temperature with the sample cell (1 cm path length) in a nitrogen atmosphere. CD spectra are reported in terms of the difference in the extinction coefficients for light and right circularly polarized light, respectively $[\epsilon_l - \epsilon_r]$ is defined as

$$\frac{\theta^0}{33050 \; lc} \; , \qquad \text{where}$$

θ^0 is the measured ellipticity in degrees, l is the path length of the sample in cm, and c is the concentration of the sample in moles per cm^3. All spectra presented herein are reported in terms of molar concentration of DNA nucleotide residues.

Deoxyribonuclease assays were performed using three separate assay techniques. The spectrophotometric assay method of Kunitz (1950) was the procedure used most frequently in this work. DNAse I (Worthington Biochemical Corp., Freehold, N.J., lot no. D9AJ) was used at a final concentration of 0.25 µg/ml, unless otherwise stated. The assay buffer

was 0.02 M Tris-HCl, pH 6.7, containing 8.0 mM $MgCl_2$ and 2.0 mM $CaCl_2$. Change in absorbance at 260 nm was followed on the Cary Model 14.

We have also measured the hydrolysis of DNA by the nuclease with a pH-stat as described by Gottesfeld et al. (1971). The third assay technique was based on the measurement of TCA-soluble material after hydrolysis of the DNA by the nuclease. To a DNA or DNA-histone complex solution (approximately 100 µg/ml in 3 ml of 0.02 M Tris-HCl, pH 6.7, 8.0 mM $MgCl_2$, 2.0 mM $CaCl_2$) was added 20 µl of DNAase I (100 µg/ml) and hydrolysis was allowed to proceed for 5 min. To this solution was added 1 ml of cold 10% (w/v) TCA, and the solution stored in the cold for 15 min to allow a precipitate to form. At the end of this time, the solution was filtered through a Millipore (0.3 µ pore size) filter, and the A_{260} of the filtrate was measured on the Cary 14.

RNA polymerase assays were carried out in a final volume of 0.25 ml in a reaction mixture previously described by Marushige and Bonner (1965). $[G^{14}C]$Uridine-5'-triphosphate (10 µC/ml) was the labelled nucleotide (Schwarz/Mann, Orangeburg, New York). RNA polymerase (E. coli B) was purchased from Miles Laboratories (lot no. 31-1-642). A sample of RNA polymerase, known to contain the σ factor, was the generous gift of Dr. M. Chamberlin. Incubation was carried at at 37° for 30 min, and the reaction stopped by the addition of 3 ml of cold 10% (w/v) TCA. After incubation at 4° for 15 min to allow the precipitate to form, the acid-insoluble material was collected on a Millipore filter (0.3 µ pore size). The filter was washed twice with 3 ml cold 10% TCA, and was sucked dry for 1-2 min before it was dissolved in 18 ml of a toluene-dioxane fluid previously described by Kinard (1957). Radioactivity was counted in a Packard Tri-Carb Liquid Scintillation Spectrometer.

Results and Discussion

Inhibition of DNAase I by F1 histones. Complexes of DNA and the very lysine-rich F1 histones (acetone powder and chromatographic fractions) prepared by the direct addition method (see Material and Methods) were resistant to the action of pancreatic deoxyribonuclease. We have measured the hydrolysis of DNA by DNAase, and the inhibition of this hydrolysis by F1 histones, using three separate assay techniques: hyperchromic effect, pH-stat and TCA-soluble material. Figure 1 illustrates the protection of calf thymus DNA by calf thymus F1 histones (acetone powder) as assayed separately by the hyperchromic effect and TCA-soluble material assay procedures. Fifty percent inhibition was attained, as determined by the hyperchromic effect procedure, at a histone: DNA ratio of 0.6 (w/w); with the assay based on TCA-soluble material this ratio was 0.75. The results of the pH-stat assay procedure are in general agreement with those of the hyperchromic effect assay procedure. Oth et al. (1958) have reported that the measurement of the hydrolysis of DNA by DNAase using four separate assay techniques (liberation of acid-soluble material, changes in viscosity, pH, and ultraviolet absorption) gave different results for the time course of hydrolysis for each assay technique. Since, however, each assay procedure measures changes in a different physical property of the DNA substrate on DNAase hydrolysis, slightly different results with each of the assay techniques was not unexpected. It is therefore not surprising that we have obtained slightly different values for the ratio of histone:DNA at 50% inhibition of hydrolysis using three different assay techniques.

While the binding of F1 histones to DNA causes a hyperchromic shift, which is at least partly due to an increase in light scattering, this does not invalidate the use of the spectrophotometric assay procedure. The similarity of the results obtained with the hyperchromic effect assay procedure and those of the pH-stat and the acid-soluble material assay procedures rules out the possibility that this initial hyperchromic shift on the binding of histones to DNA masks any subsequent hyperchromic shift on the addition of DNAase (thereby causing an apparent inhibition of DNAase hydrolysis). Due to the ease of the hyperchromic effect assay technique, we usually used this procedure to explore the interaction between DNA and the lysine-rich F1 histones.

That inhibition arose from the action of the histones on the DNA, and not by direct action of the histones on the enzyme, was demonstrated by an experiment on the order of addition as reported in Table I. This series of assays was performed using calf thymus DNA and calf thymus F1 histones (acetone powder). Preincubation of DNA with histones completely inhibited hydrolysis on the subsequent addition of DNAase (assay 2, compared with control, assay 1). However, preincubation of DNAase with histones had no effect on the rate of hydrolysis when DNA was added later (assay 3). This indicated that inhibition arose from the interaction of histones and DNA; furthermore, the DNA-histone complexes were not formed fast enough to protect the DNA from hydrolysis by the nuclease. If an aliquot of DNA was added to a cuvette containing DNAase and DNA which had been hydrolyzed by the enzyme, hydrolysis of the added DNA was observed (assay 5). It should be noted that the rate of hydrolysis of the second aliquot of DNA was lower than that of the first because (i) the concentration of DNA was 25 μg/ml in the second hydrolysis, versus 62 μg/ml

in the first; (ii) DNAase I is inhibited by the products of DNAase hydroly-sis (Privat de Garilhe, 1967).

When a second aliquot of DNA was added to a cuvette containing DNAase and an enzyme-resistant DNA-histone complex, the second aliquot of DNA was hydrolyzed by the nuclease (assay 4). This hydrolysis was faster than that in assay 5 because in assay 4 there was no accumulation of hydrolysis products in the first incubation. We conclude from these experiments that DNAase I is enzymatically active after exposure either to F1 histones or to DNA-F1 histone complexes.

Lineweaver-Burk plots showed that inhibition of DNAase I by F1 histones was competitive. It is interesting to compare this result with that of Shih and Bonner (1970), who found the inhibition of RNA polymerase by poly-L-lysine to be non-competitive. Since RNA polymerase binds to its DNA template and then transcribes the base sequence of the DNA into RNA in a continuous manner, non-competitive inhibition of this enzyme by poly-L-lysine is quite reasonable. In contrast, such a mode of action would not be expected for the hydrolysis of DNA by DNAase I, and, in fact, would not be consistent with our present data. We therefore suggest a single hit and release mechanism for DNAase I: each time a phosphodiester bond is to be broken, the nuclease must rebind to the DNA. Competitive inhi-bition by F1 histones is consistent with this mode of action.

Inhibition of transcription by F1 histones. The inhibitory action of histones on the transcription of DNA by RNA polymerase is well docu-mented (Barr and Butler, 1963; Huang and Bonner, 1962; Allfrey, et al., 1963).   . Using RNA polymerase from E. coli B, it was observed that inhibition by rabbit thymus F1 histones (acetone powder) of poly d(AT) transcription parallels the inhibition of DNAase I (Figure 2a); similarly,

TABLE I: Effect of order of addition on the DNAase hydrolysis of DNA-histone mixtures.

Assays based on the hyperchromic effect were performed on nucleohistone complexes formed by direct addition (see Materials and Methods) by the hyperchromic effect assay procedure. Final concentrations of calf thymus DNA and DNAase were 62 µg/ml and 1.0 µg/ml, respectively. The ratio of calf thymus F1 histone (acetone powder) to DNA in all assays was 1.5 (w/w). In assays 4 and 5, the second aliquot of DNA was 50 µg in 1.0 ml added directly to the cuvette and mixed immediately. In assay 5, hydrolysis of the first aliquot of DNA was complete after 10 min, as determined by no further increase in $A_{260\ nm}$.

Mode of assay	Initial Rate of Hydrolysis (ΔA_{260nm}/min)
1. DNA + DNAase	0.27^a
2. DNA + histone incubated 10 min; DNAase then added	0.00
3. Histone + DNAase incubated 10 min; DNA then added	0.28
4. DNA + histone incubated 10 min; DNAase then added	0.00
after 10 min, a second aliquot of DNA added	0.12
5. DNA + DNAase;	0.25
after 10 min, a second aliquot of DNA added	0.09

[a] Precision of assays is ± 0.01 (ΔA_{260nm} unit/min), average deviation.

the inhibition by rabbit thymus Fl histones (acetone powder) of rabbit thymus DNA transcription roughly parallels the inhibition of DNAase I (Figure 2b). While these experiments were not replicated enough for precise comparisons (due to the limited availability of RNA polymerase) the results clearly support the conclusions reached from the DNAase assays.

Circular dichroism of DNA-Fl histone complexes. Fasman et al. (1970) have reported the CD spectra of DNA-Fl histone complexes formed by gradient dialysis in the absence of urea. They found that the relative magnitudes of the nucleic acid CD bands in the complexes were dependent both upon the histone:DNA ratio in the complex, and upon the absolute concentration of complex when the spectra were taken. Furthermore, the CD spectrum of the complex was highly dependent on the salt concentration in the final step of the gradient; the CD spectrum of the complex formed by dialysis to 0.01 M NaCl was identical to the sum of the spectra of histones and uncomplexed DNA taken individually (Olins, 1969), and was different from that of the DNA-histone complex formed by dialysis to 0.14 M NaF (Fasman et al., 1970).

We have prepared complexes by adding excess histone to DNA in 0.23 M NaCl, and isolated the complexed DNA by chromatography on Bio-Gel P-30. Figure 3 illustrates one such chromatogram for calf thymus DNA and calf thymus Fl histones (acetone powder). The ratio of histone to DNA in this complex was 2.2 (w/w) for fraction number 11. It was demonstrated by means of thermal denaturation that complexes were indeed formed by the method of dialysis to 0.01 M NaCl (Olins, 1969; Olins and Olins, 1971), or by the Bio-Gel P-30 method (Gottesfeld and Schwab, 1971). Figure 4a shows the CD spectra of calf thymus DNA-calf thymus Fl histone complexes formed by direct addition, and Figure 4b shows spectra of the similar

complexes formed in moderate salt and isolated by Bio-Gel chromatography. These data suggest that the CD changes accompanying DNA-F1 histone complex formation, although resembling the transformation of DNA into the "C" form (Tunis-Schneider and Maestre, 1970), were actually due to the aggregation of the DNA-histone complexes. It is well known that native nucleohistones are least soluble at 0.14 M NaCl (Zubay and Doty, 1959). These CD changes might have been partly the result of light scattering (Glaser and Singer, 1971). A comparison of the UV spectra of calf thymus DNA-F1 histone complex and uncomplexed calf thymus DNA, taken with a Cary 14 spectrophotometer, and with a Cary 14R spectrophotometer equipped with a scattered transmission attachment and fluorscat cell (Dorman and Hearst, 1971), demonstrated that these complexes do scatter light (Figure 5). Although it is not yet known whether the CD of these DNA-F1 histone complexes arises from the differential scattering of left and right circularly polarized light (Dorman and Maestre, 1971), investigation of the CD spectral changes upon complex formation nevertheless is a useful even though empirical technique in exploring species specificity of DNA-histone interactions.

Experiments with Acetone Powders of F1 Histones.

Species-specific interaction of DNA and F1 histones as detected by DNAase and RNA polymerase inhibition. Both calf and rabbit thymus F1 histones (acetone powders) protected their homologous DNAs more effectively than any foreign DNA tested from attack by DNAase I. Figure 6 shows the inhibition of DNAase hydrolysis of calf and rabbit thymus DNAs, salmon sperm DNA, and poly d(AT), by calf and rabbit thymus F1 histones (acetone powders). This species-specific inhibition is also seen if the

results of assays with different histones and the same DNA are plotted

coordinately: Figure 7 demonstrates the protection of calf and rabbit

thymus DNAs from DNAase hydrolysis by calf and rabbit thymus F1 histones

(acetone powders). All complexes assayed for species specificity were

formed by the direct addition method.

It is interesting to note that calf thymus F1 histones (acetone

powder) protected poly d(AT) from DNAase hydrolysis almost as effectively

as they protected rabbit thymus DNA (Figure 6a); similarly, rabbit thymus

F1 histones protected poly d(AT) from DNAase hydrolysis just as efficiently

as they protected calf thymus DNA (Figure 6b). Barr and Butler (1963)

have noted that F1 histones precipitate (AT)-rich DNA preferentially

to (GC)-rich DNA. Our present data on the inhibition of DNAase hydrolysis

of poly d(AT) by calf and rabbit histones might be a reflection of this

base composition preference. The fact that F1 histones preferentially

protect homologous DNA from nuclease action indicates that there is more

than a base composition preference involved in the interaction of histones

and DNA.

The data for salmon sperm DNA (Figure 6) show that small amounts

of histone activate the nuclease. Figure 8 presents a more detailed

curve for the protection of salmon sperm DNA by calf thymus F1 histones.

Activation might result from the chelation by the histones of inhibiting

metals present in the salmon sperm DNA preparation, or, alternatively,

addition of the first amounts of histone might cause a conformational

change in the DNA which renders it more susceptible to the action of the

nuclease. We suspected such a conformational change might be detected

by means of CD. However, the spectrum of a salmon sperm DNA-calf thymus

F1 histone complex which gave maximal activation of DNAase was almost

identical to the CD spectrum of uncomplexed salmon sperm DNA. Since it

is not unlikely that commercial sperm DNAs contain significant amounts
of metal ions, we attempted to purify our salmon sperm DNA by gel
filtration on Bio-Gel P-30. The results obtained with DNA purified in
this manner were identical with our previous results.   Unless metal
ions are so tightly bound that they are not eliminated on Bio-Gel, the
initial binding of F1 histones to salmon sperm DNA probably either
promotes the binding of the nuclease to the DNA, or causes a minor
conformational change in the DNA which renders it more susceptible
to hydrolysis, thus causing activation of the nuclease.

RNA polymerase and DNAase assays showed a similar pattern of species
specificity. Figure 9a shows the inhibition by rabbit and calf thymus
F1 histones (acetone powders) of transcription of rabbit thymus DNA.
Fifty percent inhibition was attained at histone:DNA ratios of 0.65
and 0.9 (w/w) with rabbit and calf thymus F1 histones, respectively.
Rabbit thymus F1 histones (acetone powder) restricted the transcription
of rabbit thymus DNA more efficiently than it restricted the transcrip-
tion of either calf thymus DNA or poly d(AT) (Figure 9b).

Species specificity as detected by circular dichroism. CD spectra
of complexes between calf thymus F1 histones (acetone powder) and a variety
of DNAs (complexes formed by the direct addition method) have yielded the
most striking evidence for the species-specific interaction of DNA and
histones. CD spectra for calf thymus DNA-histone complexes have already
been presented (Figure 4a); Figures 10 - 12 depict the CD spectra of
complexes of calf thymus F1 histones   ·: with DNA from rat liver,
salmon sperm, and T2 phage, respectively.

The binding of calf thymus F1 histones to rat liver DNA caused a
change in the CD of the nucleic acid similar to that observed with the

binding of calf thymus F2al histone (glycine and arginine-rich) to either calf thymus DNA (Shih and Fasman, 1971) or to T7 DNA (Olins and Olins, 1971). The 275 nm and 220 nm positive bands of rat liver DNA were increased in magnitude, and the 245 nm negative band decreased, upon complexing with F1 histones from calf. At a histone:DNA ratio of 1.75 (moles of amino acid residues/moles of nucleotide residues) the 275 nm positive band reached a maximum. The spectrum of rat liver DNA at this histone to DNA ratio was similar to either DNA in the "A" form (Tunis-Schneider and Maestre, 1970) or native RNA (Yang and Samejima, 1969). On further addition of F1 histones, the positive band decreased in magnitude, and shifted to longer wavelengths until, at a histone:DNA ratio of 3.0 (moles amino acids/moles nucleotides), the spectrum was similar to DNA in the "C" form (Tunis-Schneider and Maestre, 1970) or to a calf thymus DNA-calf thymus F1 histone complex at a histone:DNA ratio of 0.75 (moles amino acids/moles nucleotides). This pattern of transformation of the CD spectrum of DNA first to that of DNA in the "A" form, and then to that of DNA in the "C" form, has only been observed with rat liver DNA-calf thymus F1 histone complexes.

Addition of even very large amounts of calf thymus F1 histones to salmon sperm DNA caused only small changes in the CD spectrum of the DNA (Figure 11). Complexes of salmon sperm DNA and calf thymus F1 histone were nevertheless formed since the DNA became resistant to DNAase hydrolysis at the ratios of histone:DNA shown on these spectra.

F1 histones from calf thymus caused only a slight change in the CD spectrum of T2 DNA (Figure 12). These data, however, should be

viewed with caution since the cytosine residues of T2 DNA are glucosylated, and the presence of glucose residues in the major groove of DNA might interfere with the formation of T2 DNA-histone complexes. Olins (1969) has reported that F1 histones interfere with the glucosylation of unglucosylated T2 DNA by glucosyl transferase when complexed with this DNA.

Analysis of the histone preparations. F1 histones isolated from calf and rabbit, and purified by the procedure of Kinkade and Cole (1966), gave elution patterns on Amberlite IRC-50 cation exchange columns very similar to those reported by Bustin and Cole (1968) for the same tissues, and their amino acid compositions as presented in Tables II and III are in good agreement with those presented previously. Unlike the earlier preparations of Busin and Cole (1968) and Kinkade and Cole (1966) which showed no traces of histidine, the preparations used in the present case contain small amounts of histidine. As expected (Stellwagen and Cole, 1968), the unchromatographed F1 histones (acetone powders) contained considerably more histidine than any of the purified fractions obtained by chromatography.

While the vast majority of published studies on lysine-rich histones are carried out with preparations containing traces of histidine residues, it is known (Stellwagen and Cole, 1968) that the histidine indicates contamination. This contamination probably occurs because the nuclei were not purified before TCA extraction.

The RNA composition of the acetone powders was determined to be 1.5 percent by weight, while the RNA compositions of the purified fractions were below the limit of detectability of the orcinol reaction.

TABLE II: Amino acid composition analysis of rabbit thymus Fl histone
acetone powder and chromatographically purified fractions.

| Amino Acid | Acetone Powder | Mole Percent[a] | | | |
		L1[b]	L2	13	14
Lyeins	22.0	27.4	28.8	29.7	31.3
Histidine	2.2	0.2	0.0	0.2	0.0
Arginine	3.3	1.6	1.5	1.8	1.0
Aspartic Acid	4.7	2.4	2.2	2.6	2.5
Threonine	4.4	4.2	4.5	3.8	4.1
Serine	6.1	7.4	6.9	6.4	7.5
Glutamic Acid	7.5	4.7	3.9	4.2	4.6
Proline	7.9	10.1	9.5	9.6	9.2
Glycine	6.6	7.0	6.9	6.9	7.9
Alanine	18.2	23.3	24.8	24.7	23.1
Half-cystine	1.7	0.0	0.0	0.0	0.0
Valine	5.2	5.3	5.0	4.0	3.6
Methionine	0.0	0.0	0.0	0.0	0.0
Isoleucine	2.1	1.1	1.0	0.8	0.8
Leucine	5.5	4.3	4.3	4.0	3.5
Tyrosine	1.1	0.5	0.5	0.5	0.3
Phenylalanine	1.3	0.5	0.5	0.7	0.5
Total Basic Amino Acids	27.5	29.2	30.3	31.7	32.3
Ratio of Lysine to Arginine	6.7	17.1	19.2	16.5	31.3

[a] Values are reported as moles of amino acids per 100 moles of all amino
acids recovered after 22-h hydrolysis at $110°$ in 6 N HCl.

[b] Nomenclature of Busin and Cole (1968).

TABLE III: Amino acid composition analysis of calf thymus F1 histone acetone powder and chromatographically purified fractions.

Amino Acid	Mole Percent[a]				
	Acetone Powder	L1[b]	L2	L3a	L3b
Lysine	20.7	28.8	28.5	28.9	
Histidine	0.9	0.1	0.1	0.4	0.4
Arginine	3.5	2.7	1.0	1.5	1.5
Aspartic Acid	6.0	1.7	2.6	2.3	2.7
Threonine	5.1	5.3	5.1	5.5	5.1
Serine	6.2	6.0	8.1	7.9	7.8
Glutamic Acid	8.0	3.6	5.0	4.7	5.6
Proline	8.7	7.6	7.5	8.8	7.4
Glycine	7.1	6.6	7.8	7.6	8.2
Alanine	17.4	26.2	20.5	23.8	23.3
Half-cystine	1.2	0.0	0.0	0.0	0.0
Valine	4.8	5.4	4.8	4.2	4.0
Methionine	0.0	0.0	0.0	0.0	0.0
Isoleucine	1.9	0.8	0.9	0.7	0.5
Leucine	5.7	4.3	3.5	3.8	3.8
Tyrosine	1.1	0.5	0.5	0.4	0.5
Phenylalanine	1.6	0.6	0.5	0.5	0.5
Total Basic Amino Acids	25.1	31.6	31.6	30.4	30.8
Ratio of Lysine to Arginine	5.9	10.7	30.5	19.0	19.3

[a] Values are reported as moles of amino acids per 100 moles of all amino acids recovered after 22-h hydrolysis at 110° in 6 N HCl.

[b] Nomenclature of Bustin and Cole (1968).

Experiments with Amberlite Fractions of F1 Histones.

Circular dichroism and DNAase hydrolysis of complexes between DNA
and F1 histone sub-fractions. Complexes of DNA and F1 histone sub-fractions
obtained by Amberlite cation exchange chromatography were prepared by
direct addition. The various fractions of calf and rabbit thymus F1 his-
tones differed in their respective efficiencies in protecting their
homologous DNAs from DNAase I hydrolysis. The sub-fractions also differed
in their ability to cause conformational changes in their homologous DNAs
as detected by CD. Table IV summarizes these results for complexes of
calf thymus and rabbit thymus histone fractions with DNA isolated from
the same tissue. The fractions from rabbit thymus were more effective
inhibitors of DNAase hydrolysis than were the sub-fractions from calf
thymus. The order of effectiveness for inhibition of DNAase hydrolysis was
CTL1 ≲ CTL3a ≂ CTL2 > CTL3b, and RTL3 > RTL1 > RTL2 > RTL4.
On the other hand, rabbit thymus histone fractions were less effective
than calf thymus fractions in causing CD spectral changes. The order of
effectiveness in inducing CD spectral changes was CTL3a > CTL1 > CTL2 >
CTL3b, and RTL1 > RTL4 > RTL3 > RTL2. There appeared to be no
obvious relationships between amino acid composition of the various histone
fractions and the order of effectiveness in inducing CD changes or in
protecting against DNAase hydrolysis.

Species specificity in DNA-F1 histone fraction complex formation.
Complexes of the fractionated histones with a variety of DNAs were pre-
pared by direct addition, and by urea-salt gradient dialysis. CD and
ultraviolet absorption spectra were obtained, and these complexes were
also assayed for resistance to DNAase hydrolysis. Complexes of histone
fractions and heterologous DNAs showed the same degree of resistance to

TABLE IV: Effect of Histone Sub-fractions on the Circular Dichroism and DNAase Sensitivity of Homologous DNAs.

Sub-fraction	Histone:DNA Ratio (w/w)[a] Required For 50% Change in $[\varepsilon_L - \varepsilon_R]_{275\ nm}$	Histone:DNA Ratio (w/w)[a] Required For 50% Inhibition of DNAase Hydrolysis[b]
Rabbit thymus L1	0.75	0.3
L2	1.35	0.5
L3	1.2	0.1
L4	0.85	0.7
Calf thymus L1	0.15	0.6
L2	0.2	1.0
L3a	0.1	1.0
L3b	0.3	2.9

[a]All complexes prepared by direct addition.

[b]Hyperchromic effect assays.

DNAase as did complexes of the same histone fractions with their homologous
DNAs (Figure 13). No evidence for species specificity was obtained, even
though from the data presented in Table IV, we may conclude that various
histone sub-fractions differ in their respective abilities to protect
DNA from DNAase I hydrolysis. Furthermore, from Figure 13 we conclude
that the amount of histone required to protect a given amount of DNA
from hydrolysis by DNAase I is affected neither by the base composition
nor the biological source of the DNA. The results in Figure 13 are
for complexes prepared by direct addition; similar results were obtained
with complexes prepared by dialysis.

Complexes of calf thymus, rabbit thymus, and salmon sperm DNA, and
poly d(AT), with calf thymus histone fraction L3a and rabbit thymus his-
tone fraction L3, were prepared by urea-salt graident dialysis. Figure 14
depicts the CD spectra of these complexes at a histone:DNA ratio of 0.5
(w/w); the CD spectra of the uncomplexed DNAs are also given. The most
unusual feature of these spectra is that rabbit thymus DNA remains largely
in the "B" conformation on complexing with fractions of rabbit and calf
histone. Notwithstanding, both of these histone fractions were capable of
inducing large changes in the CD spectra of other DNA preparations. All
of the DNA preparations contained less than 0.3% (w/w) protein. Differences
in the sedimentation patterns for the calf and rabbit thymus DNAs indicate
the calf DNA was more heterogenous (with respect to molecular weight) than
the rabbit thymus DNA (Figure 15).

The experiments reported in this section indicate that there is no
specificity directed toward the DNA in the binding of the individual
purified F1 fractions to DNA. It appeared interesting, therefore, to
combine the individual fractions in the same relative proportions
that they were found on chromatography of the acetone powders on

Amberlite IRC-50, and test the mixtures for species specificity. The results of assays of complexes of rabbit and calf DNAs with the mixtures of rabbit thymus and calf thymus histone fractions were the same, however, as the results obtained with the individual fractions. Fifty percent inhibition of hydrolysis of both calf and rabbit DNA was found at histone:DNA ratios of 0.9 (w/w) and 0.75 (w/w) for mixed histone fractions from calf thymus and rabbit thymus, respectively.

Concluding Remarks

We have shown that preparations of F1 histones (acetone powders) from a given species are more effective in protecting DNA of that species from DNAase I hydrolysis, than a similar preparation of histones from a foreign species. Furthermore, F1 histones (acetone powders) isolated from a given species protect DNA from that species from hydrolysis by DNAase I more effectively than foreign natural DNAs or a synthetic polydeoxyribonucleotide. Analogous results have been obtained using RNA polymerase assays. While each chromatographic fractioán of F1 histones differs from others in the extent to which it protects DNA against DNAase and RNA polymerase, it is indifferent to the nature of the DNA and thus does not exhibit any species specificity toward the nucleic acid. DNAase assays on complexes formed between DNA and a combination of all F1 histone sub-fractions also failed to show species specificity. We therefore conclude that some component present in the F1 acetone powders, and absent from the Amberlite fractions, accounts for the species-specific recognition of DNA, either acting alone or in concert with the histone.

From the amino acid analyses of the acetone powders and purified fractions we have concluded that the acetone powders contain certain protein components which are absent from the purified fractions. Furthermore, RNA determinations indicate the presence of significant amounts (1.5% w/w) of this nucleic acid in the acetone powders while the RNA composition of the purified fractions was found to be below the limit of detectability of the determination procedure used (less than 0.1% w/w). We have tested the possibility that the RNA component of the acetone powders imparts species-specificity on the histone component of the acetone powders by treating the acetone powders with ribonuclease. Ribonuclease treated acetone powders and untreated powders were then used in DNAase assays on DNA of the same species and foreign species. No differences were found in the inhibitory effectiveness of ribonuclease treated and control acetone powders. These results, however, must be viewed with caution, since the RNA component of the acetone powders could exist in a complex with protein that renders this nucleic acid resistant to hydrolysis by ribonuclease. This is analogous to the fact that F1 histones in complex with DNA render DNA resistant to DNAase hydrolysis.

At this time, we may conclude that purified F1 histones do not act as species-specific inhibitors of DNAase hydrolysis. F1 histones in the presence of, or complexed with, either RNA, non-F1 histone proteins or non-histone proteins, or some other unknown molecules, or with a combination of these molecular species, may act as species-specific inhibitors of the hydrolysis of DNA by DNAase. If we may extend our results with DNAase assays to RNA polymerase assays, we hypothesize that purified F1 histones do not act as species-specific regulators of

gene activity unless perhaps in the presence of, or complexed with, some non-F1 histone component of the acetone powders.

References

Allfrey, V. G., Littau, V. C., and Mirsky, A. E. (1963), Proc. Nat Acad. Sci. U. S. 49, 414.

Barr, G. C., and Butler, J.A.V. (1963), Nature (London) 199, 1170.

Bekhor, I., Kung, G. M., and Bonner, J. (1969), J. Mol. Biol. 39, 351.

Benjamin, W., Lenander, O., Gellhorn, A., and DeBellis, R. (1966), Proc. Nat. Acad. Sci. U. S. 55, 858.

Bonner, J., Dahmus, M. E., Fambrough, D., Huang, R.-C., Marushige, K., and Tuan, D.Y.H. (1968), Science 159, 47.

Bonner, J., Huang, R.-C., and Gilden, R. V. (1963), Proc. Nat. Acad. Sci. U. S. 50, 893.

Bonner, J., and Widholm, J. (1967), Proc. Nat. Acad. Sci. U. S. 57, 1379.

Burton, K. (1956), Biochem. J. 62, 315.

Bustin, M. (1969), Ph.D. thesis, Univ. of Calif. Berkeley.

Bustin, M., and Cole, R. D. (1968), J. Biol. Chem. 243, 4500.

Clark, R. J., and Felsenfeld, G. (1971), Nature New Biol. (London) 229, 101.

Crampton, C. F., Stein, W. H., and Moore, S. (1957), J. Biol. Chem. 225, 363.

DeLange, R. J., Fambrough, D. M., Smith, E. L., and Bonner, J. (1969), J. Biol. Chem. 244, 5669.

DeNooij, E. H., and Westenbrink, H.G.K. (1962), Biochim. Biophys. Acta, 62, 608.

Dorman, B., and Hearst, J. (1971), manuscript in preparation.

Dorman, B., and Maestre, M. F. (1971), unpublished results.

Fambrough, D. M., and Bonner, J. (1966), Biochemistry 5, 2563.

Fasman, G. D., Schaffhausen, B., Goldsmith, L., and Adler, A. (1970), Biochemistry 9, 2814.

Flamm, W. G., Birnstiel, M. L., and Walker, P.M.B. (1969), in Subcellular
     Components, Birnie, G. D., and Fox, S. M., Ed., New York, Plenum
     Press.

Glaser, M., and Singer, S. J. (1971), Biochemistry 10, 1780.

Gottesfeld, J. M., Adams, N. H., El-Badry, A. M., Moses, V., and
     Calvin, M. (1971), Biochim. Biophys. Acta 228, 365.

Gottesfeld, J. M., and Schwab, K. A. (1971), unpublished results.

Hatcher, D. W., and Goldstein, . (1969), Anal. Biochem. 31, 42.

Heyden, H. W., and Zachau, H. G. (1971), Biochim. Biophys. Acta 232, 651.

Huang, R.-C. (1967), Fed. Proc. 26, 1933.

Huang, R.-C., and Bonner, J. (1962), Proc. Nat. Acad. Sci. U. S. 48, 1216.

Huang, R.-C., and Bonner, J. (1965), Proc. Nat. Acad. Sci. U. S. 54, 960.

Kinard, F. E. (1957), Rev. Sci. Instr. 28, 293.

Kinkade, J. M., and Cole, R. D. (1966), J. Biol. Chem. 241, 5790.

Kunitz, M. (1950), J. Gen. Physiol. 33, 349.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951),
     J. Biol. Chem. 193, 265.

Marmur, J. (1961), J. Mol. Biol. 3, 208.

Marushige, K., and Bonner, J. (1966), J. Mol. Biol. 15, 160.

Olins, D. E. (1969), J. Mol. Biol. 43, 439.

Olins, D. E., and Olins, A. L. (1971), J. Mol. Biol. 57, 437.

Oth, A., Fredericq, E., and Hacha, R. (1958), Biochim. Biophys. Acta
     29, 287.

Panyim, S., Bilek, D., and Chalkley, R. (1971), J. Biol. Chem. 246, 4206.

Privat de Garilhe, M. (1967), Enzymes in Nucleic Acid Research, San
     Francisco, Holden-Day.

Rall, S. C. (1970), Ph.D. thesis, Univ. of Calif. Berkeley.

Shih, T. Y., and Bonner, J. (1969), Biochim. Biophys. Acta 182, 30.

Shih, T. Y., and Bonner, J. (1970), J. Mol. Biol. 50, 333.

Simpson, R. T., and Sober, H. A. (1970), Biochemistry 9, 3103.

Skalka, A., Fowler, A. V., and Hurwitz, J. (1966), J. Biol. Chem. 241, 588.

Spackman, D. H., Stein, W. H., and Moore, S. (1958), Anal. Chem. 30, 1190.

Stellwagen, R. H., and Cole, R. D. (1968), J. Biol. Chem. 243, 4456.

Tunis-Schneider, M.J.B., and Maestre, M. F. (1970), J. Mol. Biol.
  52, 521.

Yang, J. T., and Samejima, T. (1969), Prog. Nucleic Acid Res. Mol.
  Biol. 9, 223.

Zubay, G., and Doty, P. (1959), J. Mol. Biol. 1, 1.

Figure Captions.

Figure 1: DNAase assays on calf thymus DNA-calf thymus F1 histone
(acetone powder) complexes formed by the direct addition method.
Assays were carried out by the hyperchromic effect assay procedure
(closed circles), and by the TCA-soluble material assay procedure
(open circles). For details, see Materials and Methods section.

Figure 2: Inhibition of DNAase I (closed circles) and RNA polymerase
(open circles) by rabbit thymus F1 histones (acetone powder). Complexes
of histones and DNA were formed by the direct addition method. (a) Upper
graph: complexes of F1 histones and poly d(AT). (b) Lower graph:
complexes of F1 histones and rabbit thymus DNA.

Figure 3: Isolation of calf thymus DNA-calf thymus F1 histone (acetone
powder) complex by gel filtration chromatography on Bio-Gel P-30.
DNA (1.5 mg) and histones (3.75 mg of acetone powder) were mixed in
0.23 M NaCl in a final volume of 3.0 ml. This mixture was applied to
the column after a 5 min period of incubation at room temperature;
chromatography was carried out as described in Materials and Methods.
Protein concentrations (open circles) were determined by the method of
Lowry et al. (1951) on 0.25 ml of each fraction; the absorbance of each
fraction at 260 nm (closed circles) was taken as an indication of the
presence of DNA.

Figure 4: Circular dichroism spectra of calf thymus DNA-calf thymus F1
histone (acetone powder) complexes. (a) Upper grjph: complexes formed
by direct addition method. The molar ratios of histone amino acids:
DNA nucleotides were (a) 0, (b) 0.2, (c) 0.3, (d) 0.4, and (e) 0.75.
Abcissa on right refers to spectra (d) and (e) which have been reduced

Figure Captions (2)

in size by a factor of 5. (b) Lower graph: complexes with excess
histone formed in 0.23 M NaCl, and isolated by means of Bio-Gel
P-30 chromatography. Solid line, uncomplexed DNA chromatographed on Bio-
Gel P-30. Dashed line, DNA-histone complex with molar ratio of histone
amino acids:DNA nucleotides = 4.0.

Figure 5: Ultraviolet absorption spectra of (a) calf thymus DNA and
(b) calf thymus DNA-calf thymus F1 histone (Amberlite chromatogram.
peak 1) complex prepared by the urea-salt gradient dialysis procedure
(histone to DNA ratio of 0.2 [w/w]). All spectra were taken in 0.14 M
NaCl, 0.01 M Tris-HCl, pH 8, at a DNA concentration of approximately
35 µg/ml. Spectra were taken with a Cary 14 (dashed line), and with a
Cary 14R equipped with a Cary 1462 scattered transmission attachment
and fluorscat cell (solid line).

Figure 6: Inhibition of DNAase I hydrolysis of calf thymus DNA (-□-),
rabbit thymus DNA (-○-), salmon sperm DNA (-△-), and poly d(AT) (-■-)
by (a) calf thymus F1 histones (acetone powder), and (b) rabbit thymus
F1 histones (acetone powder). Assays were performed by means of the
hyperchromic effect procedure. Inhibition or activation was determined
by comparing the change in absorbance 2 min after addition of the enzyme
with a control sample of DNA in the absence of histone. Complexes were
prepared by direct addition.

Figure Captions (3)

Figure 7: Inhibition of DNAase I hydrolysis of (a) calf thymus DNA and (b) rabbit thymus DNA by calf thymus F1 histones (acetone powder) (-□-) and rabbit thymus F1 histones (acetone powder) (-○-). Hyperchromic effect assays were performed on complexes prepared by the direct addition method.

Figure 8: Effect of calf thymus F1 histones (acetone powder) on DNAase I hydrolysis of salmon sperm DNA. Complexes were prepared by the direct addition method, and assayed by the hyperchromic effect procedure.

Figure 9: (a) Inhibition by rabbit (-○-) and calf (-□-) thymus F1 histones (acetone powder) of transcription of rabbit thymus DNA. (b) Rabbit thymus F1 histones (acetone powder) as an inhibitor of transcription of rabbit thymus DNA (-○-), calf thymus DNA (-□-), and poly d (AT) (-△-). RNA polymerase assays on complexes formed by the direct addition method were performed as described in Materials and Methods.

Figure 10: Circular dichroism spectra of rat liver DNA-calf thymus F1 histone (acetone powder) complexes prepared by the direct addition method. The molar ratios of histone amino acids:DNA nucleotides were (a) 0, (b) 1.0, (c) 1.5, (d) 1.75, (e) 2.0, (f) 2.1, (g) 2.2, and (h) 3.0. Spectra are not corrected for histone contribution. Abcissa insert on right refers to spectrum (h), which has been reduced in size by a factor of 4.

Figure 11: Circular dichroism spectra of salmon sperm DNA-calf thymus F1 histone (acetone powder) complexes prepared by the direct addition method. The molar ratios of histone amino acids:DNA nucleotides are given on the spectra. The data are not corrected for histone contribution.

Figure Captions (4)

Figure 12: Circular dichroism spectra of T2 phage DNA-calf thymus F1 histone (acetone powder) complex (broken line) at a histone:DNA ratio of 3.0 (moles of histone amino acids:moles of DNA nucleotides), and of uncomplexed T2 phage DNA (solid line). DNA-histone complex was prepared by the direct addition method.

Figure 13: Effect of rabbit thymus histone fraction L1 on the hydrolysis of rabbit (-○-) and calf thymus (-□-) DNAs, and poly d (AT) (-△-), by DNAase I. Hyperchromic effect assays were performed on complexes prepared by the direct addition method.

Figure 14: Circular dichroism spectra of complexes of calf thymus histone fraction L3a (----) and rabbit thymus histone fraction L3 (-·-·-) with (a) calf thymus DNA, (b) rabbit thymus DNA, (c) salmon sperm DNA, and (d) poly d(AT). The histone:DNA ratio was 0.5 (w/w) for all complexes. Spectra of uncomplexed DNA are also shown. All complexes were prepared by urea-salt gradient dialysis. Insert abcissa in sections (a) and (d) refer to spectra of the complexes.

Figure 15: Sucrose gradient sedimentation patterns for poly d(AT), calf thymus DNA, and rabbit thymus DNA. The $S_{w,20}$ value for poly d(AT) is 8.0. Sedimentations carried out as described in Materials and Methods.
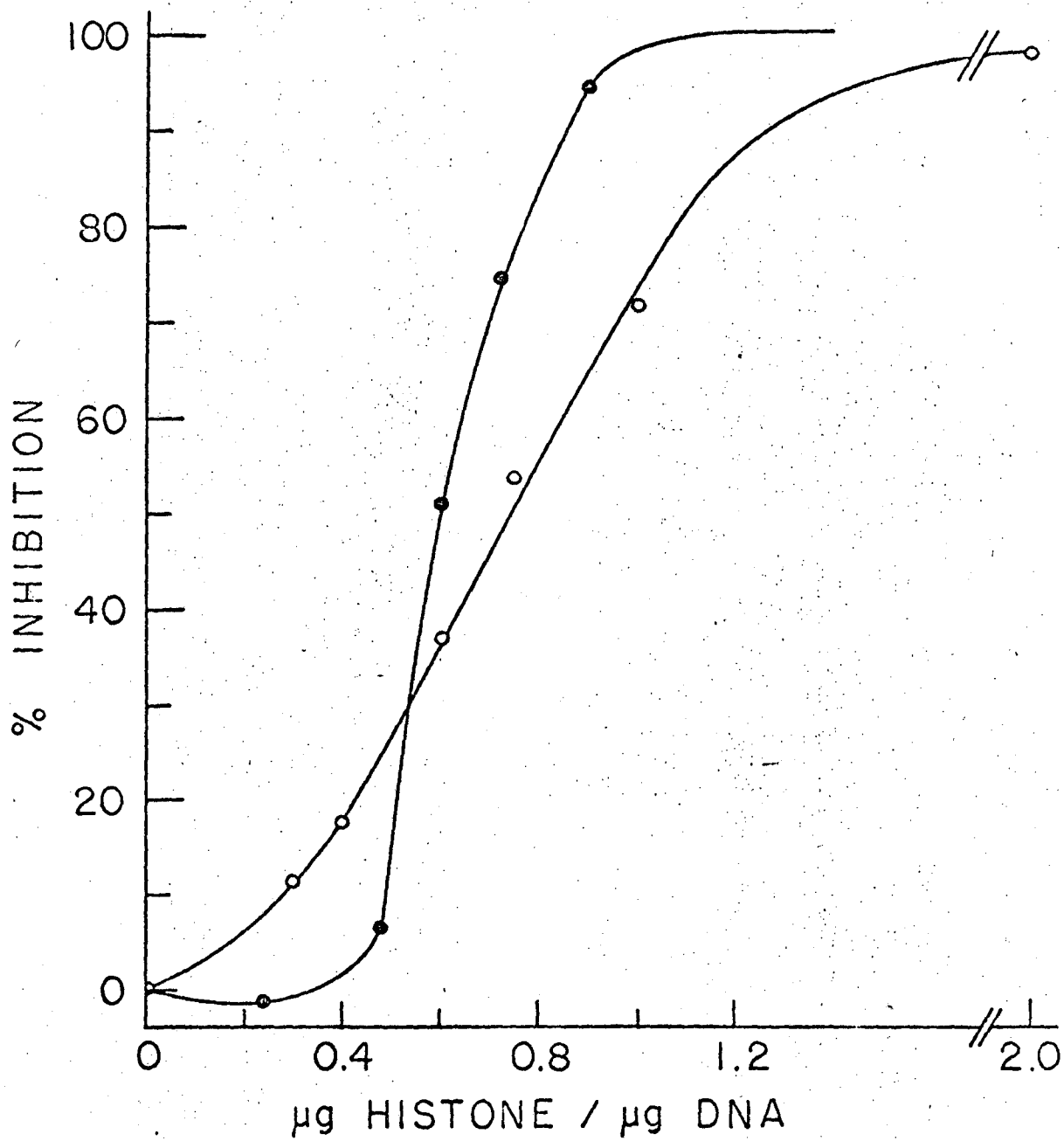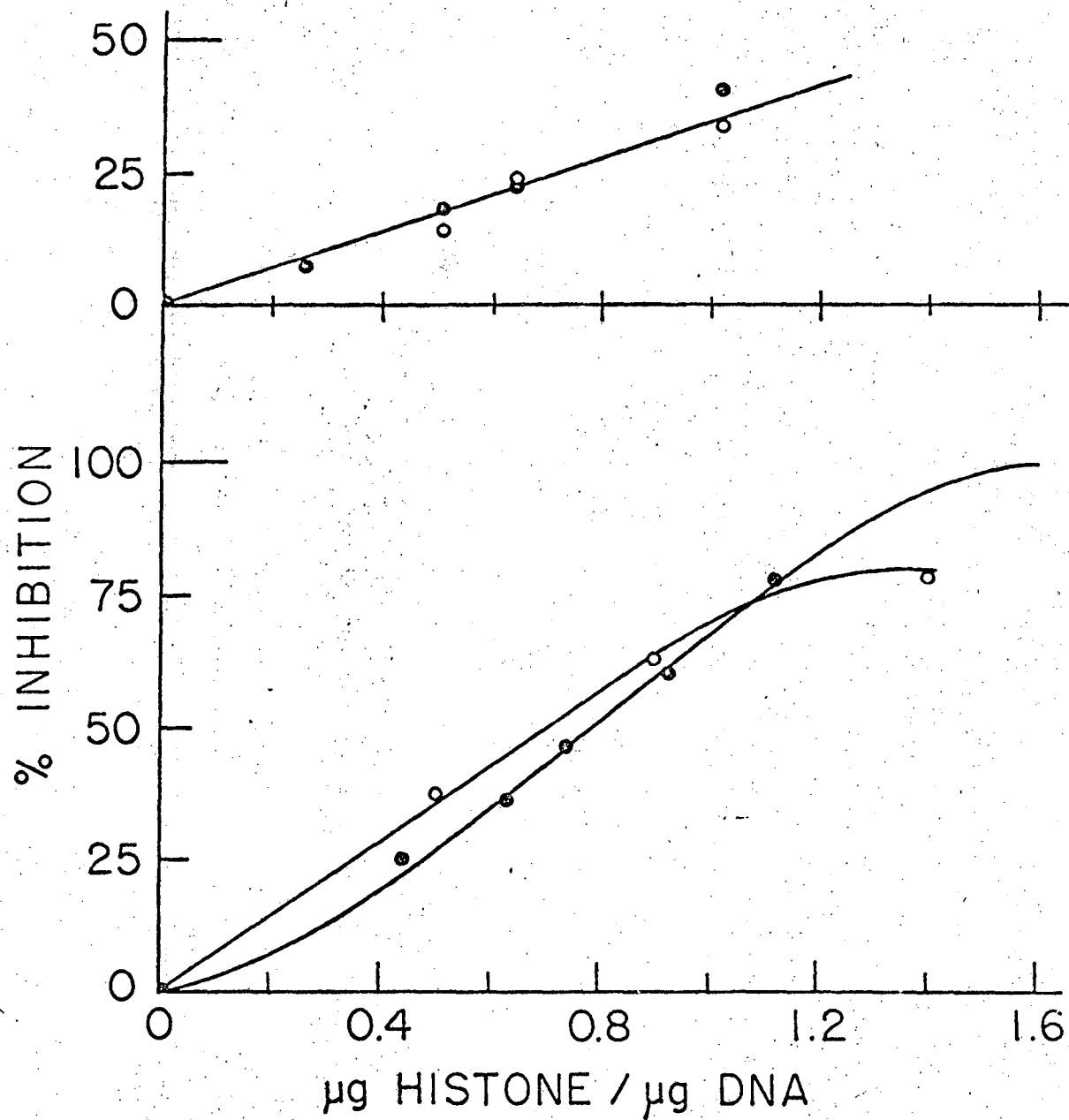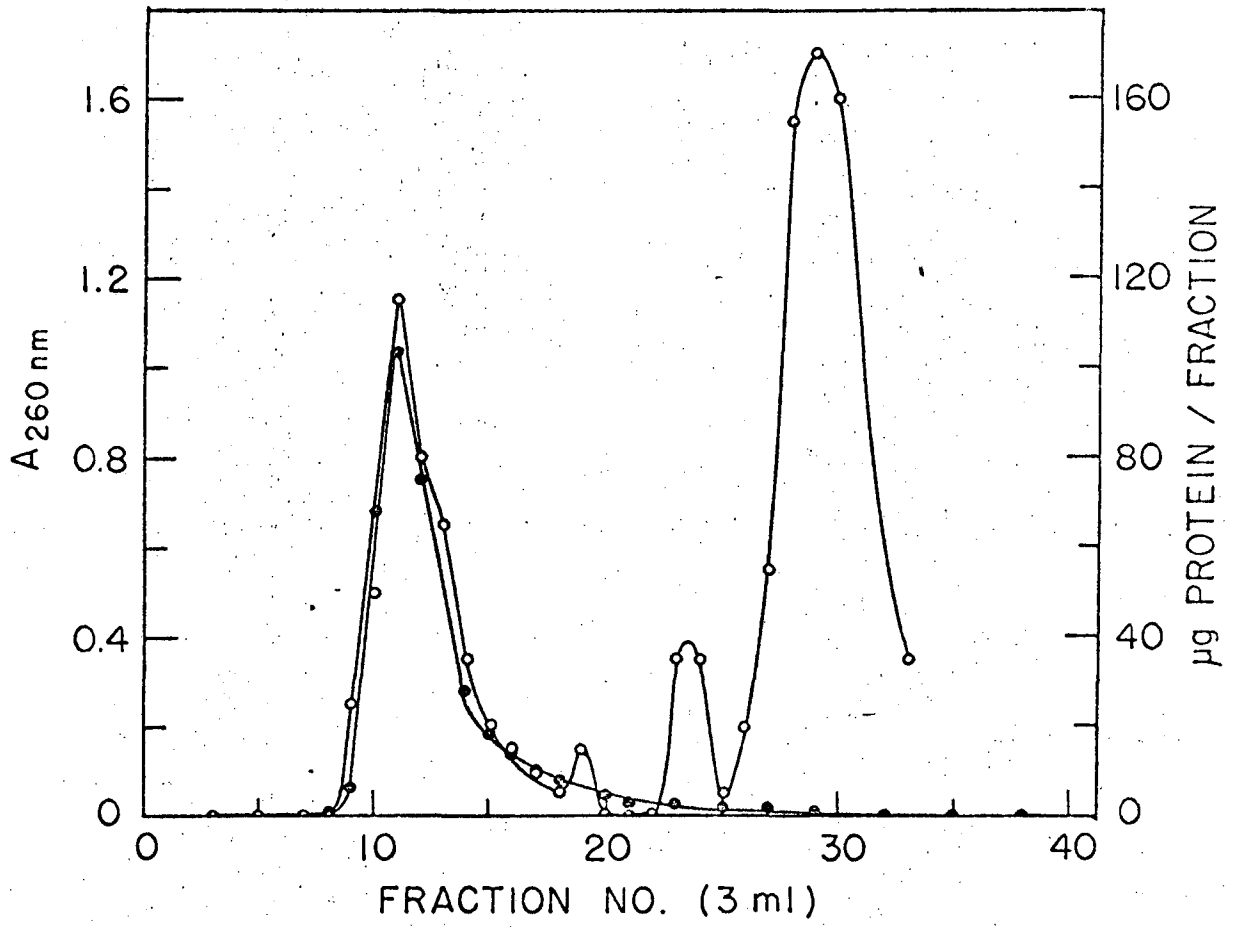
XBL 717-5275

Fig. 1

XBL717-5276

Fig. 2

XBL 717-5274

Fig. 3

CT DNA−CTL^V COMPLEXES

XBL716-5246

Fig. 4

-39-
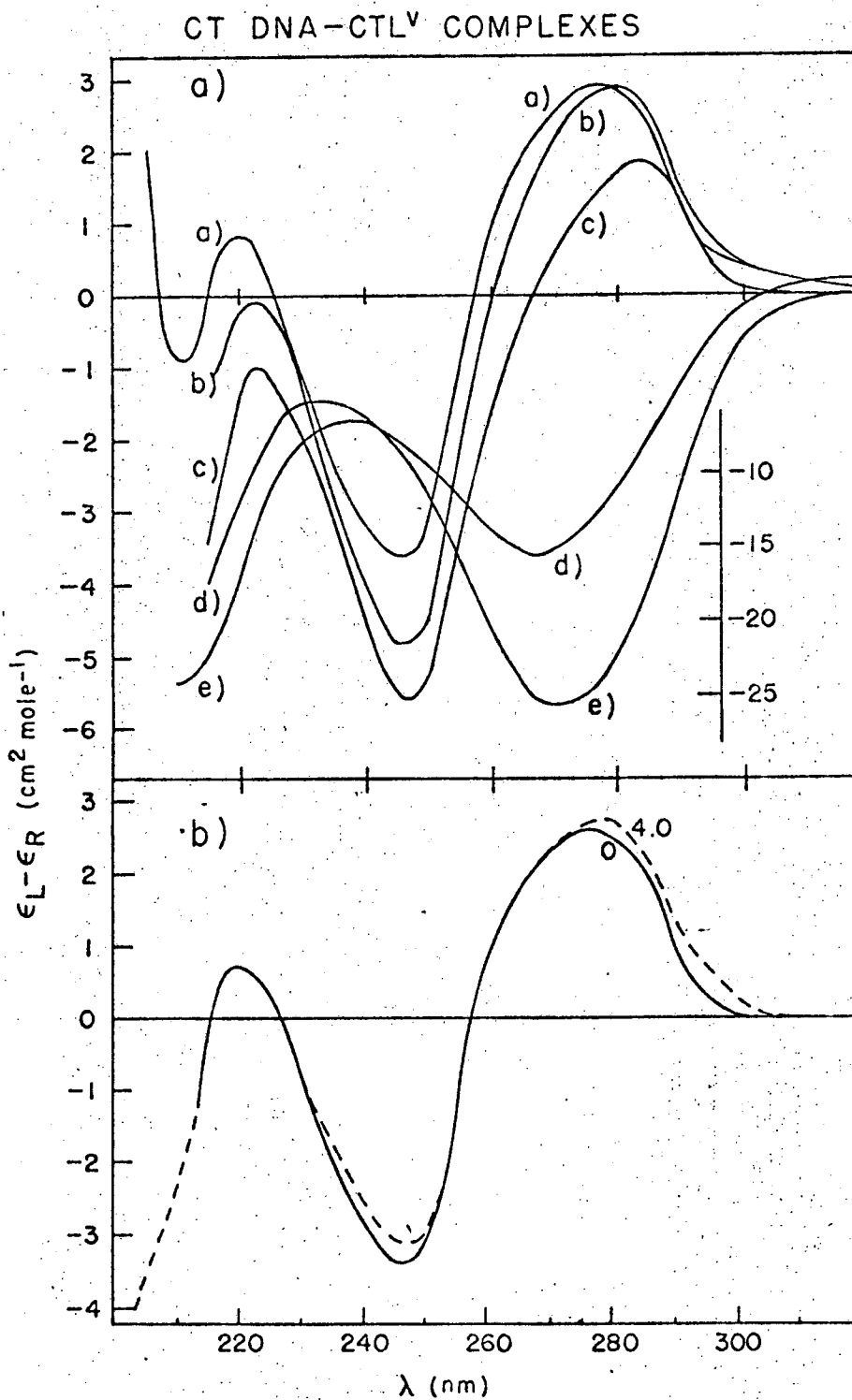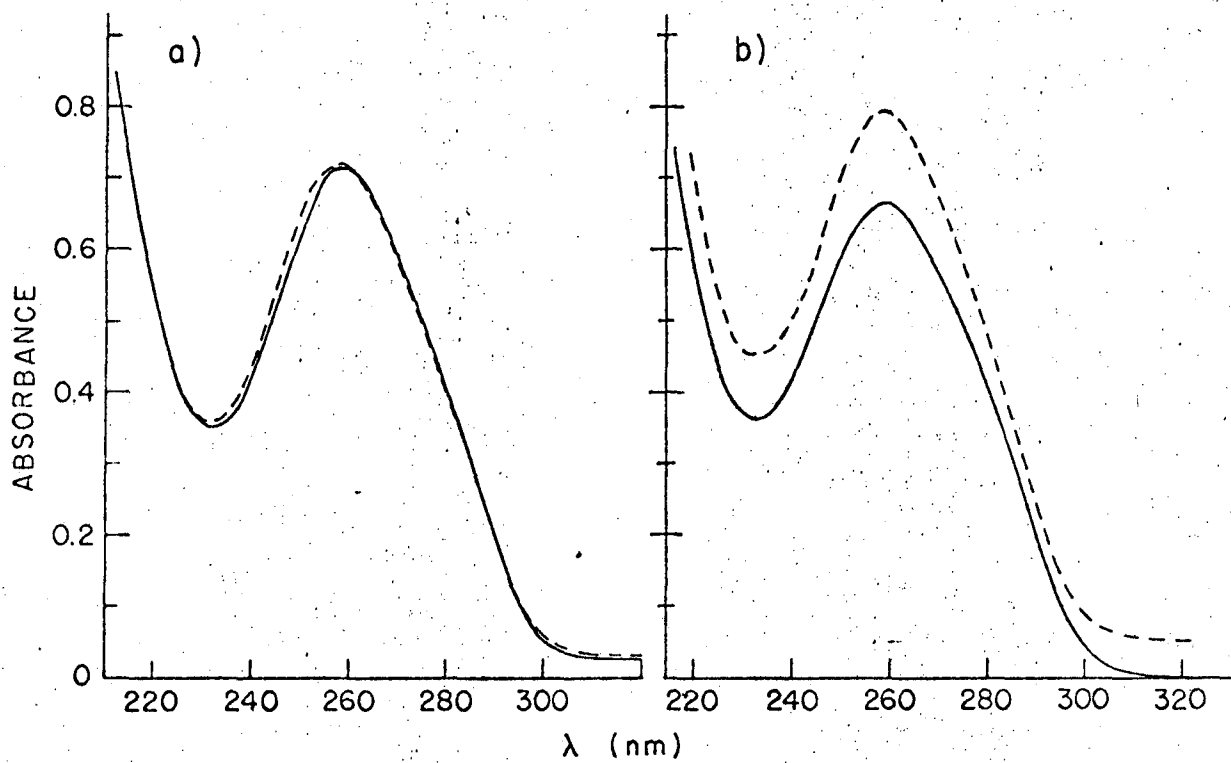
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Fig. 5
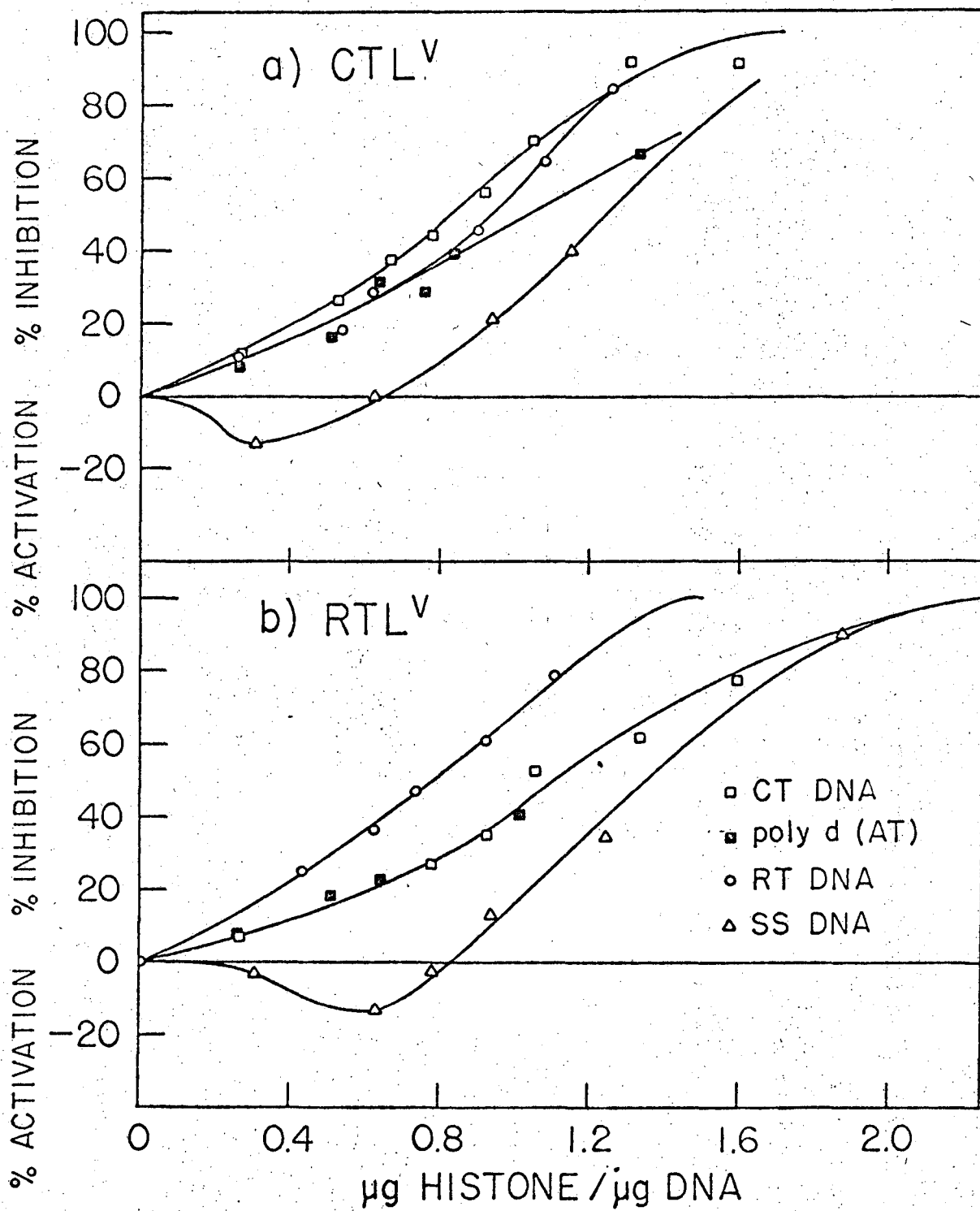
Fig. 6
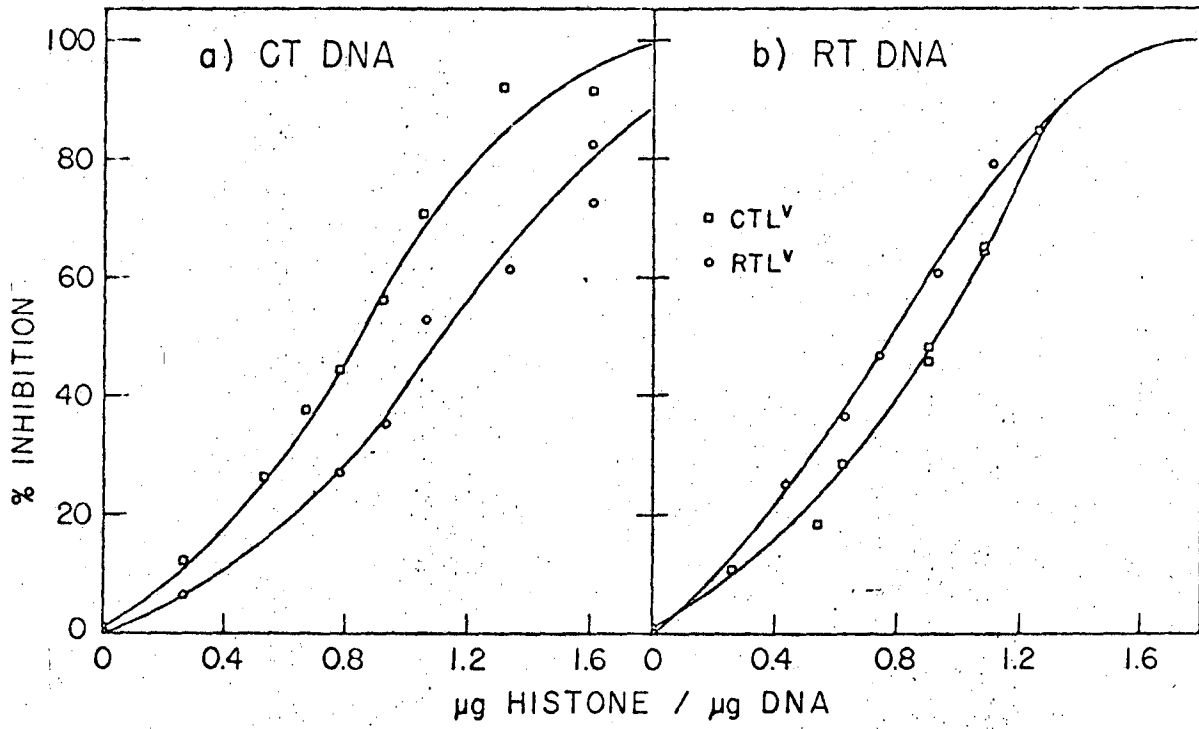
XBL717-5263

Fig. 7
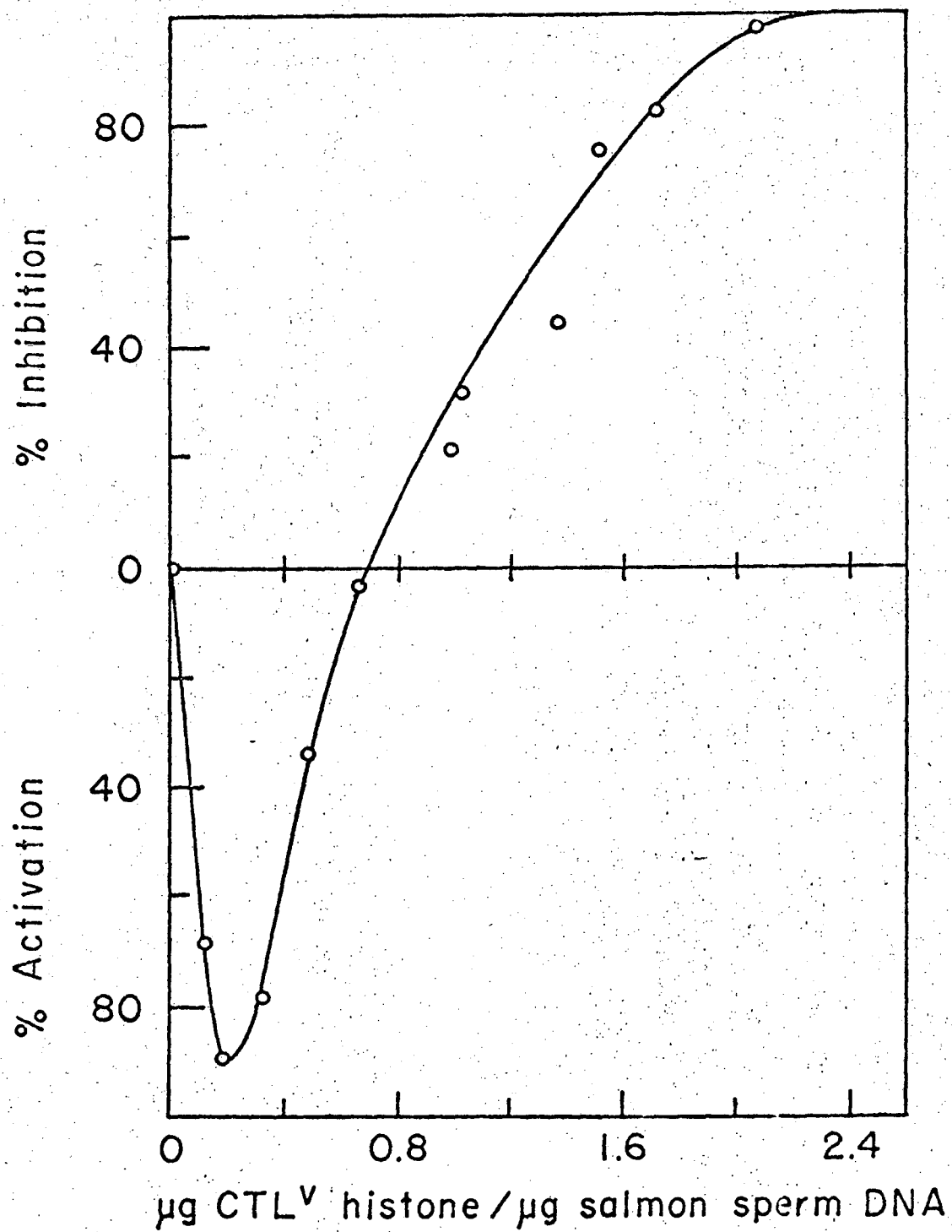
XBL717-5264

XBL716-5234

Fig. 8
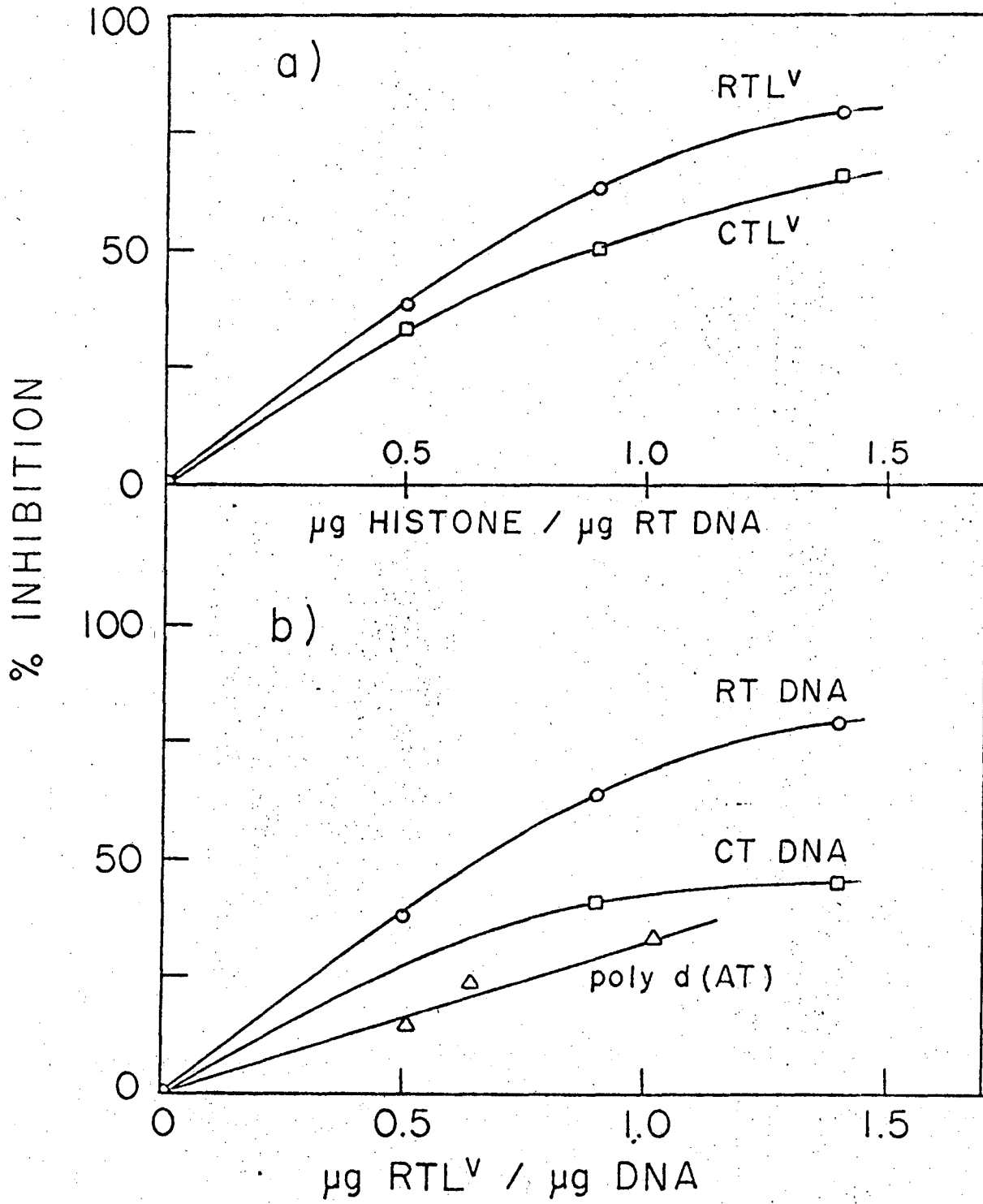
Fig. 9

XBL 717-5267
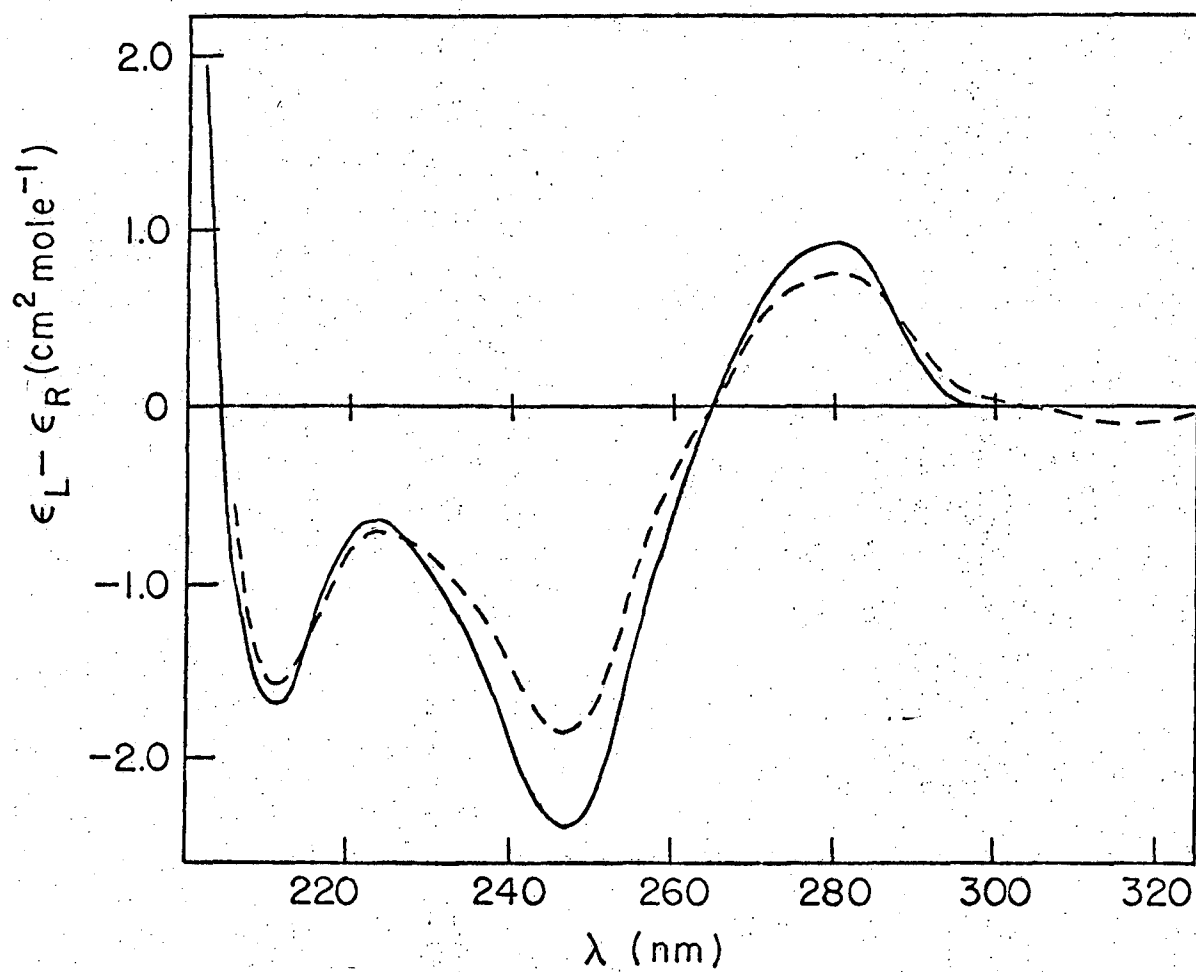
RAT LIVER DNA-CTL^V COMPLEXES

XBL 717-5265

Fig. 10

SALMON SPERM DNA−CTL^v COMPLEXES
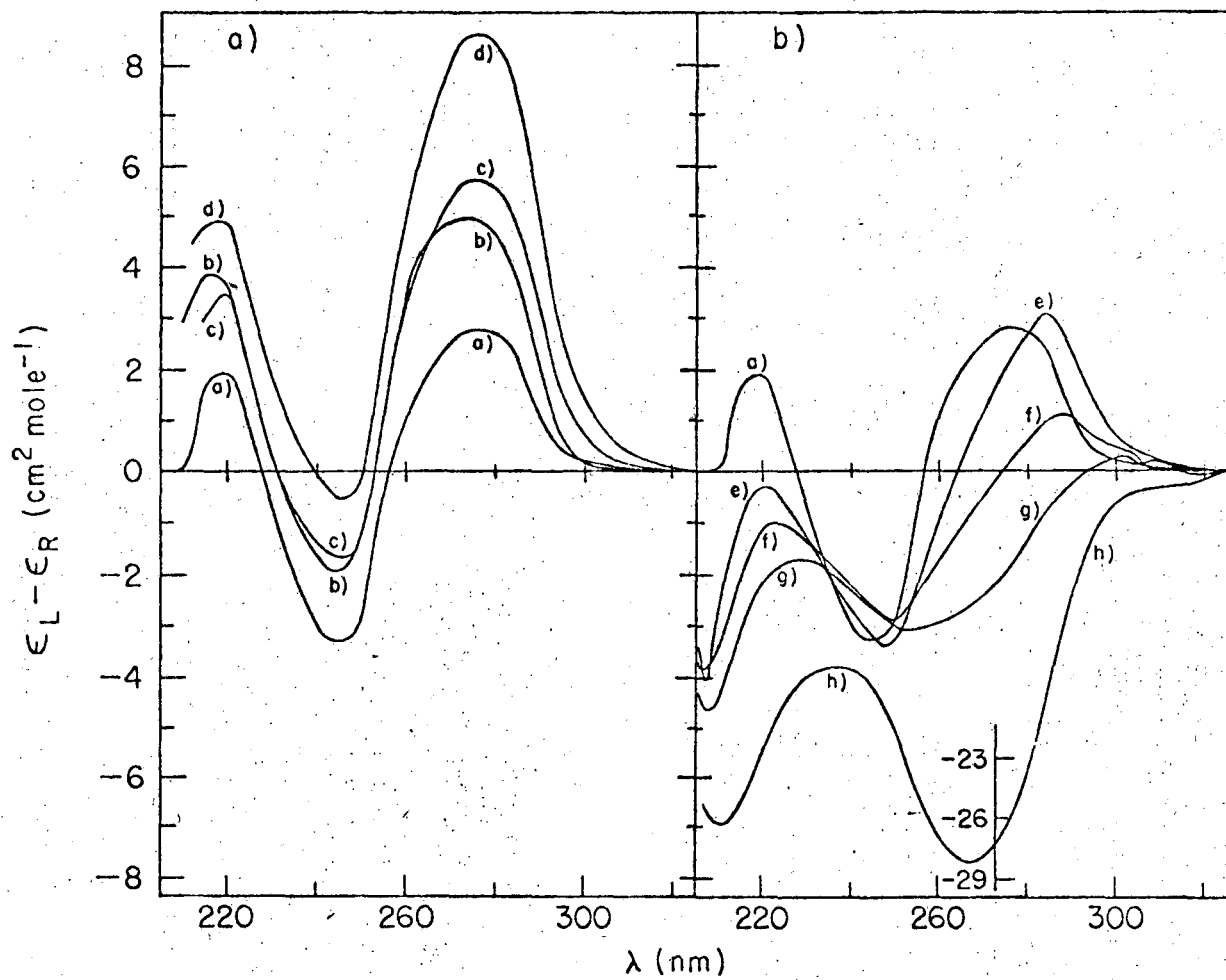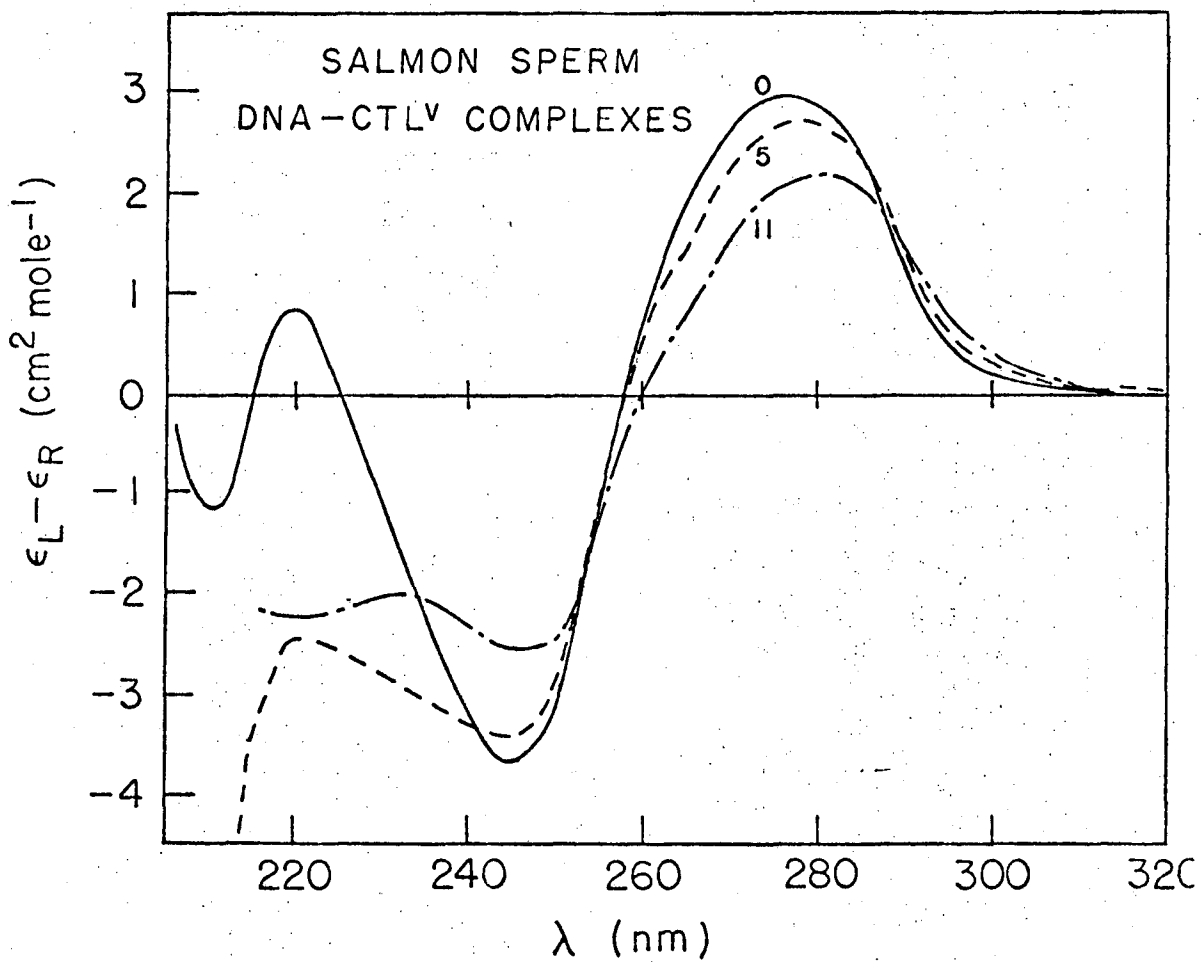
XBL717-5266

Fig. 11

XBL716-5233

Fig. 12

Fig. 13

XBL717-5262

Fig. 14

XBL717-5277

FRACTION NO. (0.2 ml)

XBL717-5280

Fig. 15