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CIRCULAR DICHROISM OF RAT UTERINE AND LIVER CHROMATIN FOLLOWING GENE ACTIVATION

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Summary

From circular dichroism studies, no detectable conformational change appeared in ovariectomized rat uterine chromatin up to 4 hours after 17β-Estradiol administration or in partial hepatectomied rat liver chromatin up to 22 hours after hepatectomy.

The DNA of chromatin is found complexed with histone proteins, non-histone acidic proteins, and small amounts of RNA. Furthermore, the template ability of DNA in chromatin is repressed with regard to purified DNA specifically by its interaction with histones (1). Circular dichroism studies of the conformation of chromatin have shown that the association of DNA with chromosomal protein causes conformational changes in the DNA associated with tilting of the bases relative to the helical axis (2).

When 17β-Estradiol is administered to the ovariectomized rat, increases in the genetic material available for transcription in the uterus are found concomitantly with a decrease in the relativistic amount of histone to DNA in uterine chromatin (3). Increases in template activity of liver chromatin after partial hepatectomy also occur (4). Specific changes in protein composition of chromatin accompany these changes in template ability as well as chemical modifications of already existing chromosomal proteins (5). We therefore thought it would be valuable to examine the conformation of chromatin

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in these two instances using circular dichroism.

MATERIALS AND METHODS

Animals: Long-Evans female rats (220 g) were ovariectomized through the dorso-lateral approach at least three weeks prior to use. 17β-Estradiol (10 μg in 0.2 cc of 1% ethanol-0.9% saline) was administered interperitoneally. Rats were killed by cervical dislocation at varying times ater injection, and the uteri were dissected and frozen in liquid N_2. Control (zero time) animals were given a sham injection immediately prior to sacrifice.

Sprague-Dawley male rats (250 g) were used for partial hepatectomy. Approximately 40% of the liver was removed by resection of the left lateral lobe. Metofane anesthesia was used during the operation as well as at the time of sacrifice (i.e., 3 hours, 9 hours, and 22 hours after partial hepatectomy).

Nuclei: Nuclei were isolated by a modification of the methods of Blobell and Potter (6). All steps were carried out at 4°C. Uteri from four animals were pooled, weighed, and homogenized in a Polytron homogenizer run at 60 volts for 90 seconds in 3 ml of homogenizing medium (0.1 mM PIPES-KOH buffer, pH 6.0; 1.5 mM $MgCl_2 \cdot 6H_2O$; 50 mM Na bisulfite; 3 mM KCl) containing 0.35 M sucrose. This was subjected to further homogenization in a Potter-Elvenhjem homogenizer run at 500 RPM with 4 slow up and down strokes. The homogenate was filtered through 2 layers of miracloth and the final volume was adjusted to 8 ml with homogenizing medium containing 2.4 M sucrose and 0.8% Triton X-100. After 15 minutes, the homogenate was overlaid on 4 ml of homogenizing medium containing 2.2 M sucrose and 0.5% Triton X-100 in cellulose nitrate tubes, and centrifuged in the SW 41 rotor of the Beckman Model L2 ultracentrifuge for 30 minutes at 33,000 RPM, yielding a pellet of nuclei which was examined for purity using phase microscopy. Liver nuclei were isolated in the same manner with the omission of Polytron homogenization.

<u>Chromatin</u>: Again all steps were carried out at 4°C. Chromatin was isolated by the method of Bonner <u>et al.</u> (7), the nuclei being lysed in 5 ml of 0.01 M Tris-HCl buffer (pH 8.0), and overlaid on 25 ml of 1.7 M sucrose (0.01 M Tris-HCl buffer, pH 8.0) in cellulose nitrate tubes. The upper 2/3 of the contents of each tube were stirred to form a rough two-step gradient; the tubes were then centrifuged in the SW 25.1 rotor at 20,000 RPM for 3 hours. The gelatinous pellet (purified chromatin) was resuspended in 0.01 M Tris-HCl buffer (pH 8.0) with a Potter-Elvenjhem homogenizer.

<u>DNA Concentrations</u> were estimated by absorbance at 260 nm corrected for scattering by the method of Leach and Scheraga (8). One O.D. unit/cm was taken as 45 μg of DNA/ml. This method yields DNA concentrations very similar to those obtained using the diphenylamine assay as described by Burton (9).

<u>Circular Dichroism Spectra</u> were recorded with a Cary Model 60 Spectropolarimeter equipped with a Cary Model 6001 CD attachment. The pathlength was 1 cm, with the cell located flush against the phototube lens. Spectra are reported in terms of mean residue ellipticity with the dimensions of deg cm^2/dmole; $[\theta] = \frac{\psi \cdot M}{100\ l\ c}$, where ψ is measured in degrees; M is the average gram molecular weight of a nucleotide (328 g); l is the pathlength in decimeters; and c is the concentration of nucleic acid in g/cm^3.

<u>Template Activity</u> was assayed by the method of Marushige and Bonner (10), using <u>E. coli</u> B RNA polymerase purchased from Miles Laboratories (Elkhart, Indiana). Each reaction mixture contained 0.025 μc of ^{14}C-UTP (Schwarz Bioresearch, Orangeburg, N. Y.). Radioactivity was counted in a Packard Tri-Carb Liquid Scintillation counter.

RESULTS AND DISCUSSION

Figure 1 shows the increases in uterine weight, and the template activity of uterine chromatin, after 17β-Estradiol stimulation; these responses are in

agreement with those reported in the literature as primary effects following hormone administration (3). However, as shown in Figure 2, no consistent change in the circular dichroism spectra of the purified uterine chromatin was apparent. Figure 2 shows the average spectrum for the control, 30-minute, 2-hour, and 4-hour chromatin samples, with standard deviations recorded every 10 nm. From our studies we conclude that the primary changes in template activity associated with hormone stimulation do not result in any detectable conformational change of the DNA in uterine chromatin.

Preliminary results with control, 3-hour, 9-hour, and 22-hour regenerating liver chromatin also show no apparent change in the circular dichroism spectra. It is of interest that the recent work of Matsuyama *et al.* (11) reports no conformational difference between normal rat liver chromatin and chromatin from hepatoma cells.

Our results suggest that the conformational alteration which results from the complexing of DNA with protein represents a more permanent repression of template activity than occurs during *in vivo* fluctuations of genetic activity. Whether this conformational change serves a merely structural role, or is indeed important during differentiation and selective gene activation, remains to be seen.

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FIGURE CAPTIONS

Fig. 1. (a) Uterine wet weight after 17β-Estradiol administration (in milligrams). (b) Template ability using E. coli B RNA polymerase of purified uterine chromatin after 17β-Estradiol administration as measured by acid-precipitable ^{14}C-UTP. Results are adjusted to constant DNA concentrations.

Fig. 2. Average circular dichroism spectrum of purified uterine chromatin zero, 0.5, 2, and 4 hours after 17β-Estradiol administration. Results are in terms of mean residue ellipticity (deg cm^2/dmole) with standard deviations recorded every 10 nm.

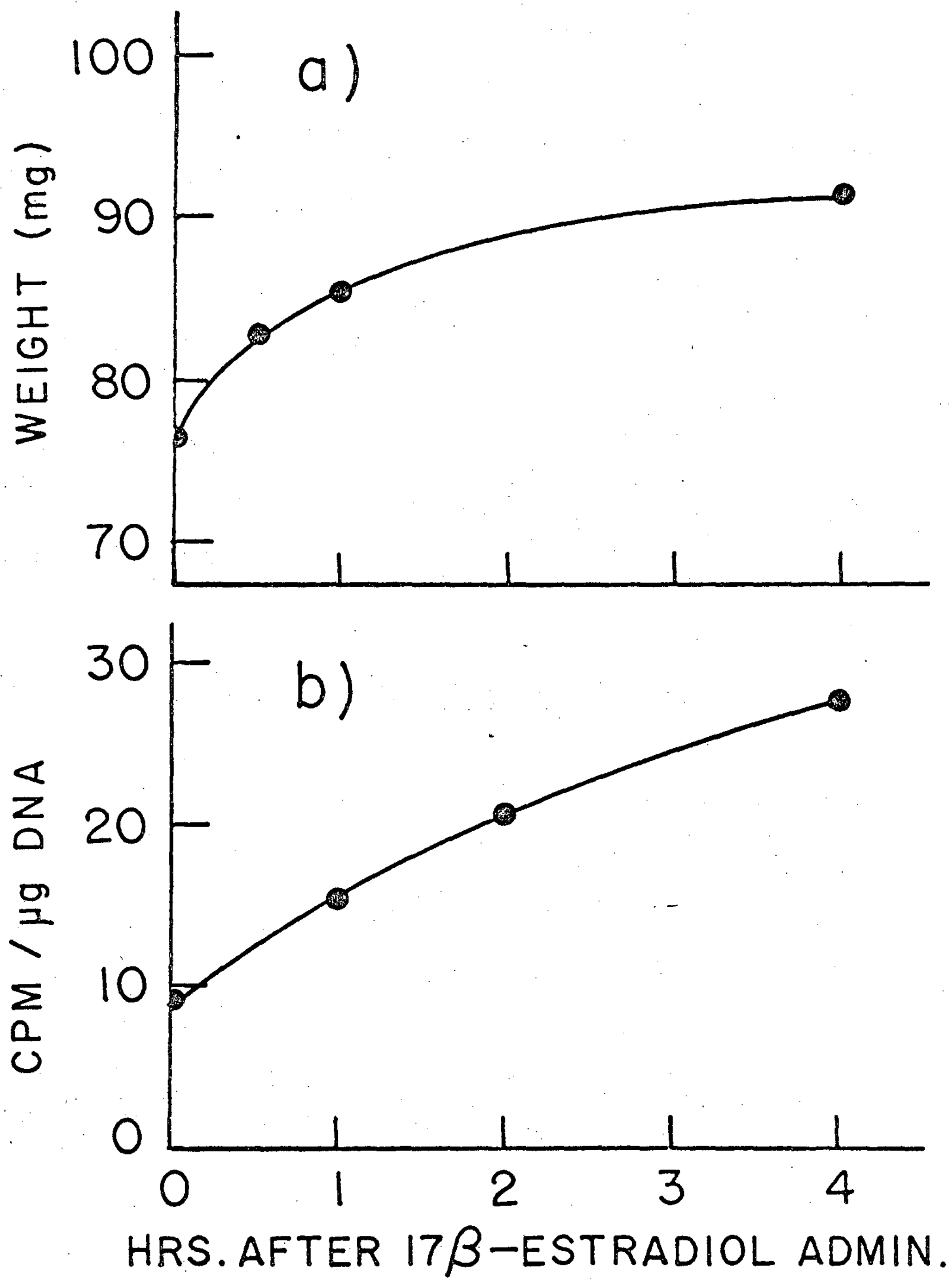

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Fig. 1

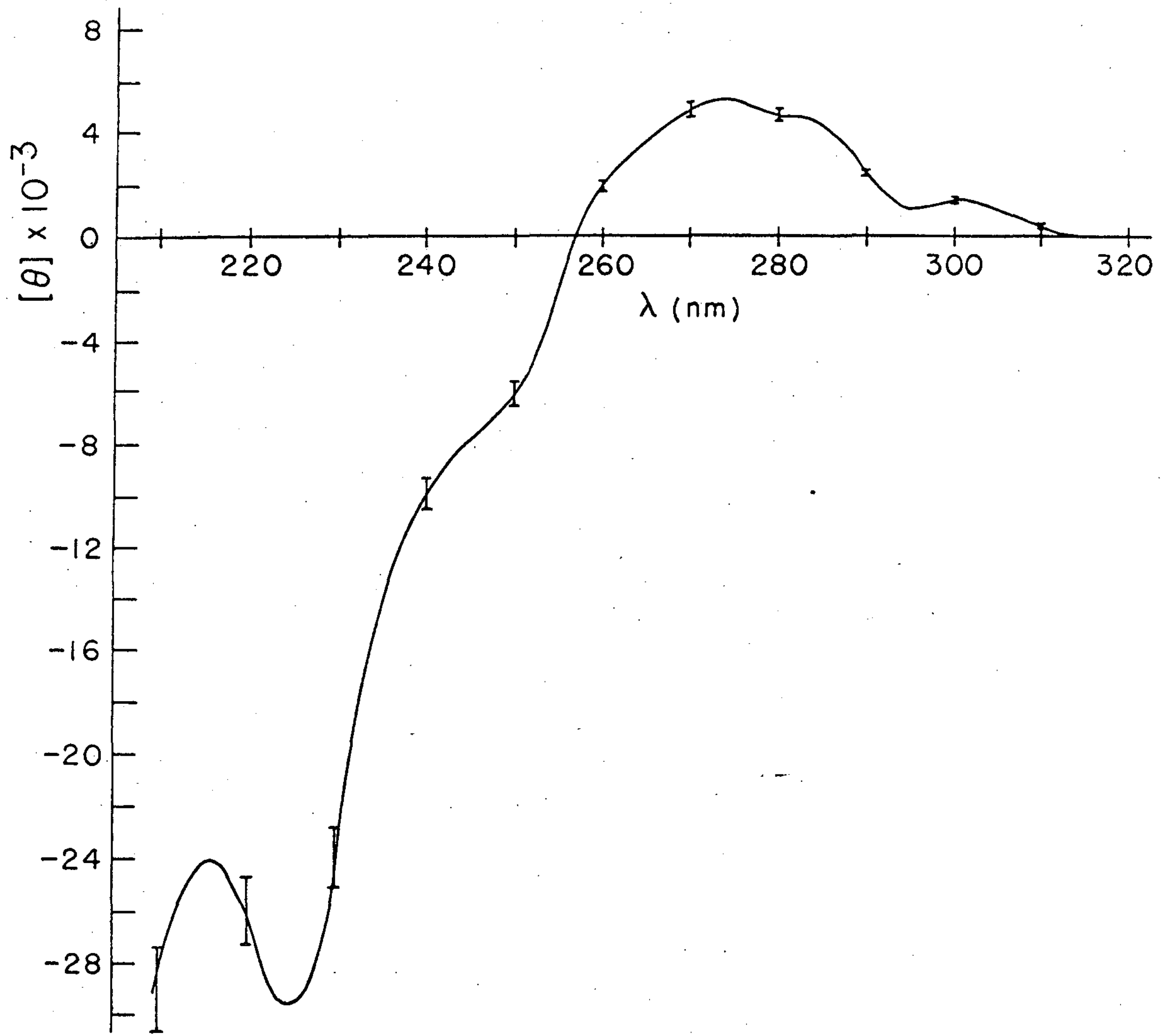

Fig. 2

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