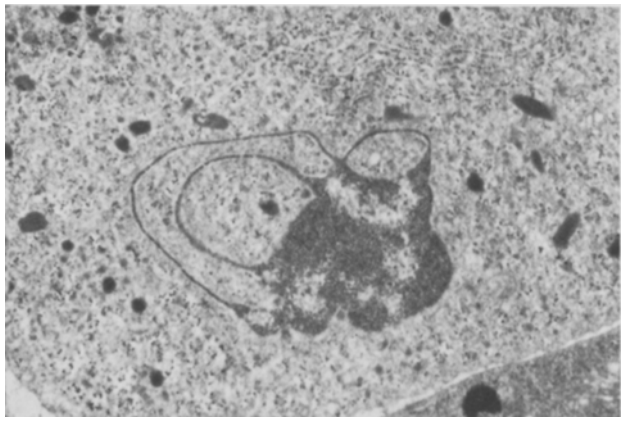

Fig. 1. Electron micrographs of a leukocyte from liver fixed first in glutaraldehyde and then with osmium tetroxide. Double stained with uranyl acetate and lead citrate. × 11,000.

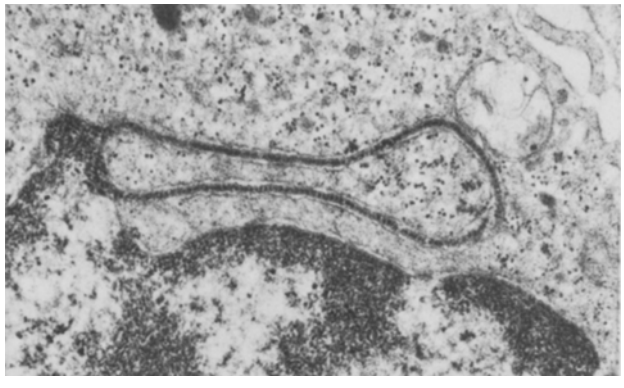

Fig. 2. Electronmicrograph of part of leukocyte from liver fixed first in glutaraldehyde and then with osmium tetroxide. Double stained with uranyl acetate and lead citrate. × 21,950.

each side by the nuclear envelope. In some sections blebs are found to be hollow spheres or tunnels of nuclear material, filled with cytoplasm.

We would like to draw attention to the chromatin organization within the extruded sheet of nucleus in the blebs which we have found. The chromatin is visualized in the sections as a row of discrete granules, each somewhat brick-shaped, and of dimensions of 20–40 nm across. Such a size suggests that they may represent a group of nucleosomes cut in sections. Such a stacking arrangement of chromatin granules has been previously visualized in the EM in the nuclear material immediately underlying the nuclear envelope in chicken erythrocytes[13] and in fish cells[3].

It has been proposed[14] that blebs of nuclear membrane may arise in nuclei which, for reasons unknown, produce an excess of nuclear envelope over and above what is needed to neatly enclose the chromatin. We are attracted by this view and cosider that this may occur in a low percentage of otherwise normal individual animals. The fact that in such nuclei chromatin is often extended between the sheets to reveal its beaded organization seems to offer a useful situation in which to study the organization of chromatin within the nucleus.

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Estradiol treatment reduces a cytosol androgen binding protein in male rat liver[1]

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Summary. The in vivo treatment of male rats with estradiol-17β resulted in a significant decrease in liver cytosol of the protein which binds specifically androstenedione and testosterone.

There is a sex difference in androgen metabolism in the livers of rats. We have observed that an injection of estradiol in male rats induces feminization of the hepatic metabolism of testosterone[2] and that the treatment of female rats with testosterone results in the induction of enzymes which are involved in the androgen metabolism[3,4]. We have recently demonstrated the presence of a protein which binds androstenedione and testosterone with moderate affinity in the cytosol of male rat liver[5]. These findings led to an attempt to clarify the effect of estrogen on the androgen binding protein in male rat liver. We report here that administration of estradiol reduces the androgen-binding protein in the liver cytosol of male rats.

Materials and methods. Estradiol-17β benzoate, dissolved in a small volume of ethanol and diluted with corn oil, was given s.c. to male adult Wistar rats weighing 200–250 g for 2, 4 or 7 days. The control animals received the vehicle only. The animals were castrated 15 h before sacrifice and were killed 24 h after the final injection. The liver was perfused with saline solution and quickly removed. The liver tissues were homogenized in 1 vol. of 0.01 M Tris-HCl buffer, pH 7.4, with 0.01 M KCl, 0.001 M EDTA and 0.001 M dithiothreitol (TKED buffer) in a glass-teflon homogenizer and then centrifuged for 10 min at 18,000 × g. Floating lipid was discarded and the supernatant was recentrifuged for 1 h at 105,000 × g. A clear supernatant (cytosol) was incubated with [1,2-^{3}H]androstenedione (sp. act. 60 Ci/mmole, New England Nuclear) or [1,2-^{3}H]testosterone (sp. act. 40 Ci/mmole, New England Nuclear). Separation of macromolecule-bound [^{3}H]steroids from free ones in the incubates was accomplished by a Sephadex G-100 column using 0.01 M TKED buffer and fractions of 5 ml were

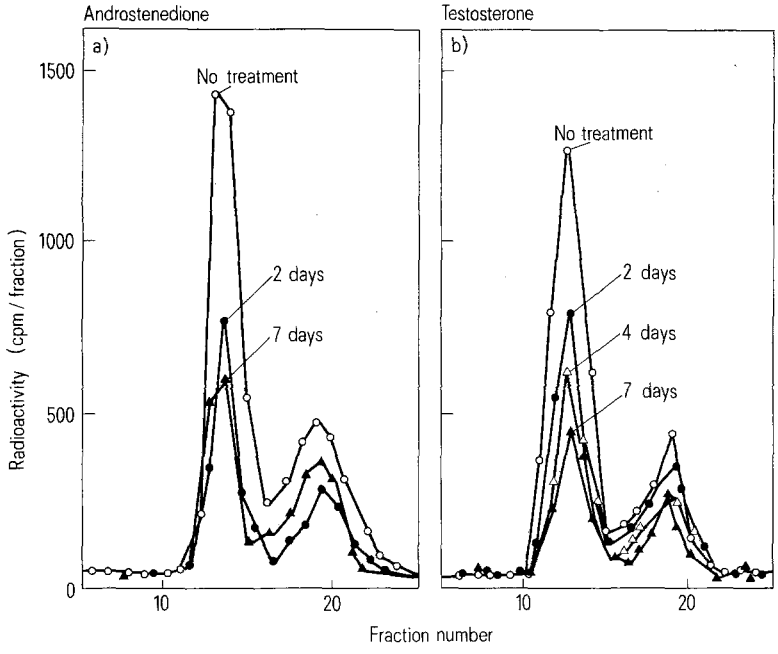

Fig. 1. Effect of estradiol on [^{3}H]androstenedione- or [^{3}H]testosterone binding protein. Rats were injected with estradiol-17β benzoate (100 µg as estradiol-17β/100 g b. wt/day) for 2, 4 or 7 days (3 animals each). The hepatic cytosol fraction (60 mg protein in 2 ml) was incubated with 16.7 pmoles [^{3}H]androstenedione or 25 pmoles [^{3}H]testosterone for 3 h at 0 °C and then the incubates were fractionated on a column of Sephadex G-100 (2.5×35 cm) at 4 °C with 0.01 M TKED buffer. Fractions of 5 ml were collected.

collected. The radioactivity in each fraction was counted by a Beckman liquid scintillation counter (LS-3155 T type) after the addition of dioxane scintillator. The counting efficiency was 35-40%. In the following experiment, the cytosol obtained from the liver homogenates of the control rats or estradiol-treated ones was chromatographed by a Sephadex G-100 column at 4 °C using 0.01 M TKED buffer. The fractions which appeared in and immediately after the void volume of the column (monitored by UV absorption) were pooled and concentrated by Centriflo CF-25 (Amicon Corp.). This concentrated fraction which contained the androgen-binding component was incubated with [^{3}H]androstenedione or [^{3}H]testosterone. The mixture was treated by the dextran-coated charcoal method[6] and the resultant fraction was concentrated by Minicon A25 (Amicon Corp.). Aliquots were layered on sucrose gradients and the gradients were centrifuged at 4 °C in a SW_{50} rotor of a Hitachi 65P ultracentrifuge.

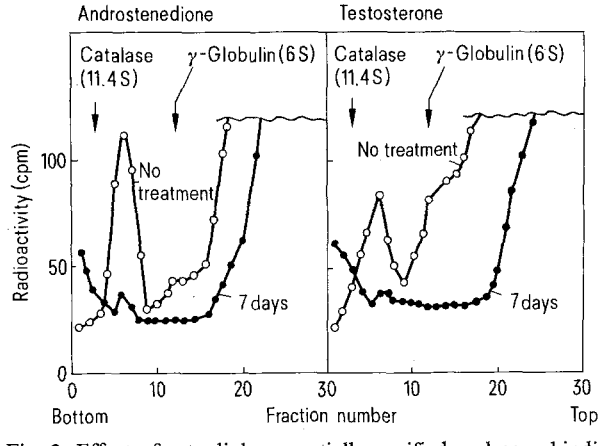

Fig. 2. Effect of estradiol on partially purified androgen-binding protein in male rat liver. Cytosol fractions which were obtained from the liver homogenates of control rats or estradiol-treated ones (details are shown in legends of figure 1) were chromatographed on a Sephadex G-100 column. The fractions, which were eluted in and immediately after the void volume of the column, were concentrated to contain 20 mg protein in 1 ml and incubated with 8.3 pmoles [^{3}H]androstenedione or 12.5 pmoles [^{3}H]testosterone for 3 h at 0 °C. The bound steroid (250 µl) obtained by the dextran-coated charcoal method was applied on a 5-20% (w/v) sucrose gradient (4.5 ml) in 0.01 M TKED buffer centrifuged for 8 h at 50,000 rpm. Fractions (10 drops about 150 µl) were collected from the bottom directly into counting vials. In parallel gradients, catalase and γ-globulin were included as standards.

Results and discussion. As seen in figure 1,a, the radioactivity of the [^{3}H]androstenedione-bound peak, which emerged earlier from a Sephadex G-100 column, was reduced in the rats treated with estradiol for 2 days, compared with that of the control, and it was more markedly reduced in the rats treated for 7 days. Similarly, the radioactive peak bound with [^{3}H]testosterone decreased remarkably with increasing duration of the estradiol treatment (figure 1,b). In both cases, no reduction of radioactivity was observed in the 2nd peak which emerged later from the column. As shown in figure 2, the sucrose density gradient profiles indicate that the [^{3}H]steroid-bound protein was markedly reduced by the injection of estradiol.

We have recently found that the cytosol androgen-binding protein is translocated into the nucleus in rat liver[7]. Thus, it is suggested that androgen acts on the gene via the receptor which is translocated into the nucleus in the liver; this is similar to the action of other steroid hormones on the target organ.

This study has provided evidence indicating that the decrease of androgen binding protein in male rat liver cytosol due to in vivo treatment with estradiol is an important feature distinguishing the female type from the male type in androgen metabolism of the liver.

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