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The Effects of Estrogen and Antiestrogen on Chromatin in Human Breast Cancer Cell Line, MCF-7. II.

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ABSTRACT. The effects of estrogen and antiestrogen on chromatin in a human breast cancer cell line, MCF-7, were studied in cells grown in media containing 10^{-8} M estradiol or 10^{-6} M tamoxifen for 1, 3 and 8 days. The effect on incorporation of thymidine into DNA was determined. Maximal incorporation occurred on day 1 after administration of estradiol, but was depressed to a low level within 3-8 days. A small increase in incorporation was observed on 1-3 days after administration of tamoxifen, but this was also depressed to a low level within 8 days. The uptake of [^{32}P]orthophosphate into an individual protein band was observed using sodium dodecyl sulfate-polyacrylamide gels in chromatin from the cells on day 1 after administration of estradiol and tamoxifen. The difference in the action mechanisms of estradiol and tamoxifen may be explained in part by the uptake of phosphate into individual protein. The results of the present experiments may be due to influence of either estradiol and tamoxifen on the cell cycle as well.

Key words : Estrogen — Antiestrogen — Chromatin — MCF-7 cell

Although the estrogen- and antiestrogen-receptor complexes interact with chromatin in target cells, the sites of interaction are unknown. In my previous paper,[1] the effects of estrogen and antiestrogen on chromatin in a human breast cancer cell line, MCF-7, were studied in order to clarify the action mechanisms of estrogen and antiestrogen. The receptor binding content in intact cell nuclei and the receptor binding capacity to chromatin in a cell-free system were determined using MCF-7 cells grown in the medium containing estradiol [1,3,5 (10)-estratriene-3,17β-diol] or tamoxifen [*trans*-(*p*-dimethylaminoethoxyphenyl)-1,2-diphenyl-but-1-ene citrate ; ICI 46474]. In addition, sodium dodecyl sulfate(SDS)-gel electrophoretic patterns of chromosomal proteins were compared.

In this paper, thymidine incorporation into DNA was compared in MCF-7 cells grown as mentioned above. Phosphorylation of chromosomal proteins in the cells was determined by SDS-gel electrophoresis.

MATERIALS AND METHODS

Cells and tissue culture

Details regarding cells and tissue culturing have been reported previously.[1]

Incorporation of thymidine into DNA

The cells were grown in 5% CO_2 in air at 37°C. At different times after

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administration of estradiol (Sigma Chemical Co.) (10^{-8}M), tamoxifen (obtained through Dr. Y. Omukai from ICI Ltd, Pharmaceutical Division) (10^{-6}M) or a vehicle alone, 0.5 μCi of [methyl-^{3}H]thymidine (20.0 Ci/mmol ; New England Nuclear) was added to each dish 2 h before the cells were harvested. The radioactivity of the acid-precipitable fraction in these cells was counted as previously described.[3]

Phosphorylation of chromosomal proteins

Phosphorylation of chromosomal proteins was determined by the method of Karn *et al.*[2] with modifications. At different times after administration of estradiol (10^{-8} M), tamoxifen (10^{-6} M) or a vehicle alone, MCF-7 cells were harvested by centrifugation at $400 \times g$ for 5 min and gently resuspended in 2 ml of 0.4 mCi of carrier-free [^{32}P]orthophosphate (Japan Atomic Energy Research Institute). After 20 min-incubation at 37°C, the cells were centrifuged as above. The chromatins were prepared as described previously.[1]

SDS-polyacrylamide disc gel electrophoresis

Electrophoresis was carried out as described previously.[1] Glass tubes were 15 cm long and 0.55 cm in diameter. The sample was mixed with sucrose to give a final concentration of 20% and with a [^{14}C]methylated protein mixture consisting of myosin (mol wt 200,000), phosphorylase-b (mol wt 92,500), bovine serum albumin (mol wt 69,000), ovalbumin (mol wt 46,000), carbonic anhydrase (mol wt 30,000) and lysozyme (mol wt 14,300) (Amersham). The gels were sliced into discs of 1.2 mm thickness. Every slice was collected in a scintillation vial. The gel was dissolved with the aid of 0.5 ml of 30% H_2O_2. The radioactivity in the gel was measured in a liquid scintillation spectrometer.

Measurement of radioactivity

Measurement of samples was done with a Searl Mark III liquid scintillation spectrometer (model 6880) using a toluene-based scintillation mixture containing 0.4% of 1,5-diphenyloxazole and 33% of Triton X-100 with a counting efficiency of 70-80% for ^{14}C and 30% for ^{3}H.

RESULTS AND DISCUSSION

The effect of estradiol and tamoxifen on incorporation of [^{3}H]thymidine into DNA was determined in MCF-7 cells in the medium containing 10^{-8} M estradiol or 10^{-6} M tamoxifen for 1, 3 and 8 days (Fig. 1). The incorporations in cells grown in the medium containing 10^{-8} M estradiol for 1, 3 and 8 days were 36,800, 15,500 and 6,000 dpm/mg DNA, respectively. Those in cells grown in the medium containing 10^{-6} M tamoxifen for 1, 3 and 8 days were 12,200, 12,900 and 3,900 dpm/mg DNA, respectively. Incorporation in cells grown in the medium to which the vehicle alone had been added was 8,300 dpm/mg DNA (control value). Maximal incorporation occurred on day 1 after administration of estradiol, but was depressed to a low level within 3-8 days. A small increase in incorporation was observed in cells to which tamoxifen had been administrated on day 1-3, but this was also depressed to a low level within 8 days. Tamoxifen is possibly a weak estrogen as previously mentioned.[4] It has been reported that estrogen initially stimulates uterine DNA synthesis and subsequently inhibits the responsiveness of the uterus to further estrogen treatment.[5] The results in the present experiment seem to agree with those

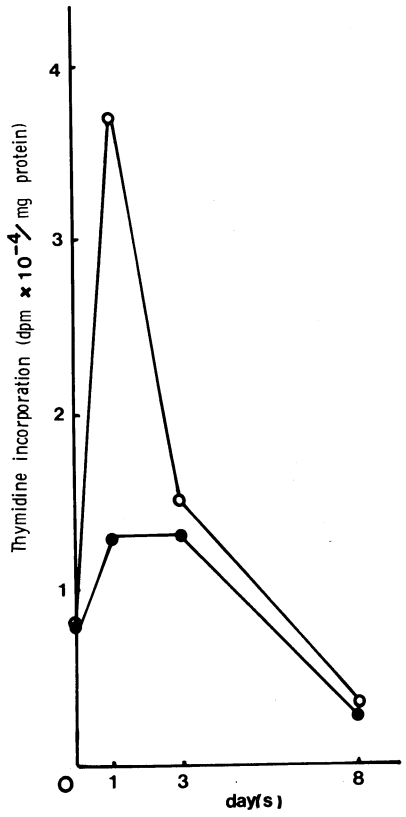
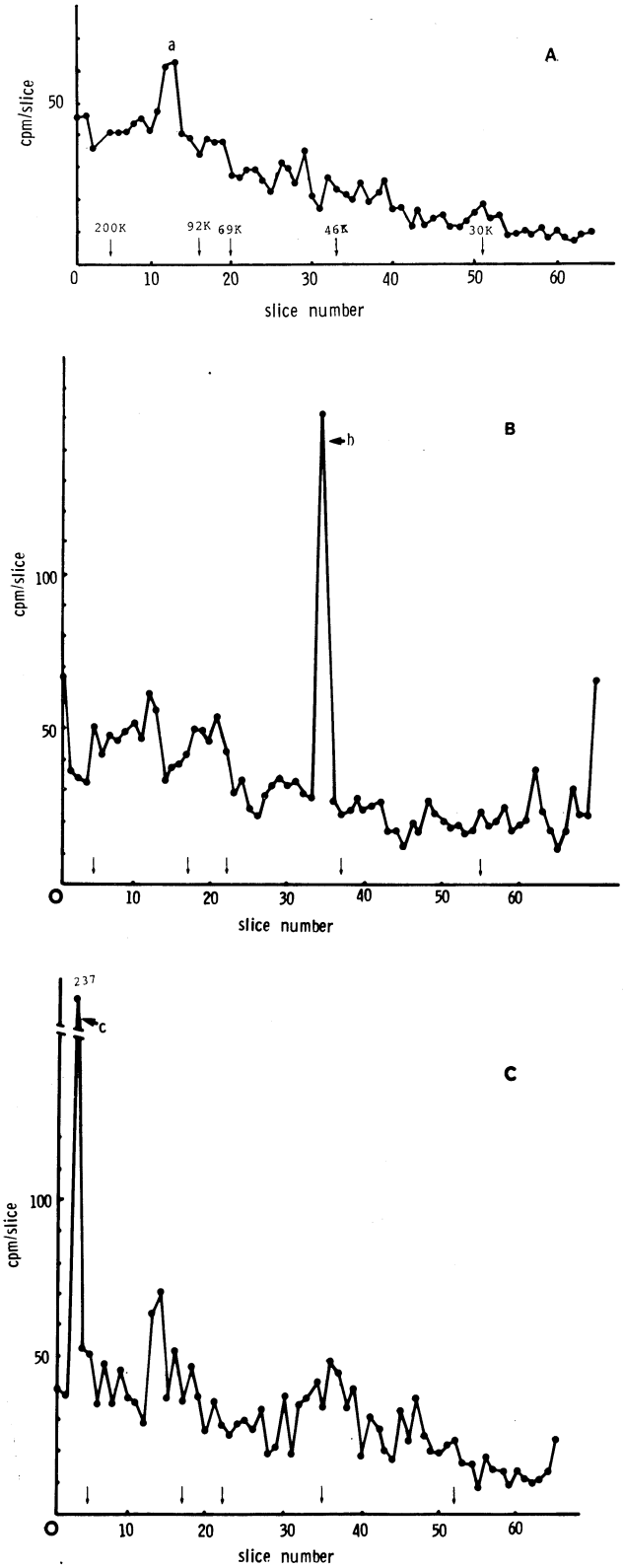


Fig. 1. Effect of estradiol and tamoxifen on thymidine incorporation into DNA. Cells were incubated for the indicated times in medium containing 10^{-8} M estradiol or 10^{-6} M tamoxifen, and each dish was pulsed with 0.5 μCi of [^{3}H]thymidine for 2 h prior to harvesting of the cells. All values are means of duplicate determinations.

observations.

Phosphorylation of chromosomal proteins was determined using cells grown as mentioned above (Fig. 2). A small peak a was always observed in chromatin from cells grown in the absence (control) or presence of either estradiol and tamoxifen, as may be seen in Fig. 2, A–G. Peaks b and c appeared as prominent peaks in chromatin from the cells on day 1 after administration of 10^{-8} M estradiol and 10^{-6} M tamoxifen, respectively. These peaks practically disappeared within 3–8 days. Some small peaks were observed in cells grown for 3–8 days in the presence of estradiol or tamoxifen. Some peaks were a little large in cells grown for 8 days in the presence of tamoxifen.

It was found that the independent chromosomal protein was phosphorylated and that the effect on thymidine incorporation into DNA was also different on day 1 after administration of estradiol or tamoxifen. It has been reported that phosphorylation of chromosomal protein enhances steroid hormone-receptor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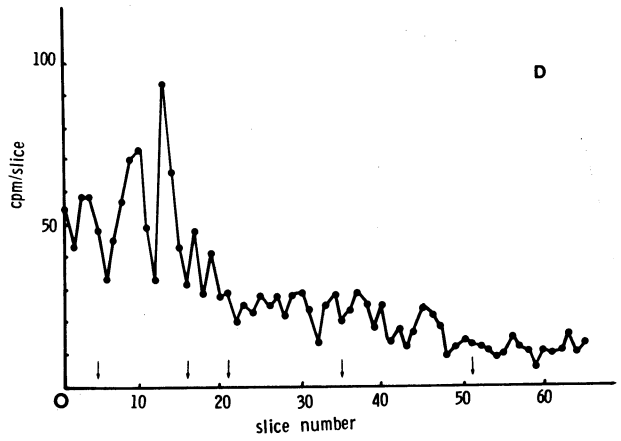
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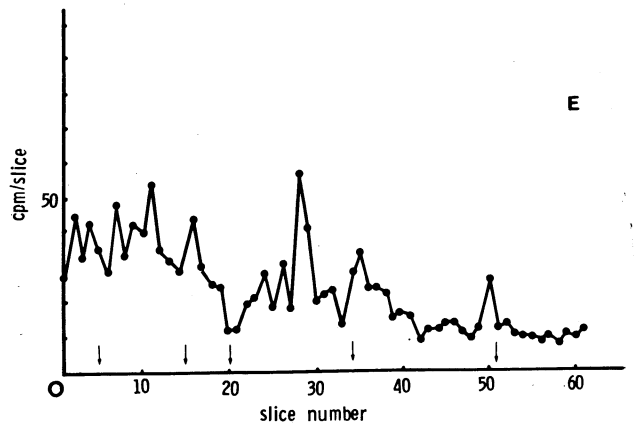
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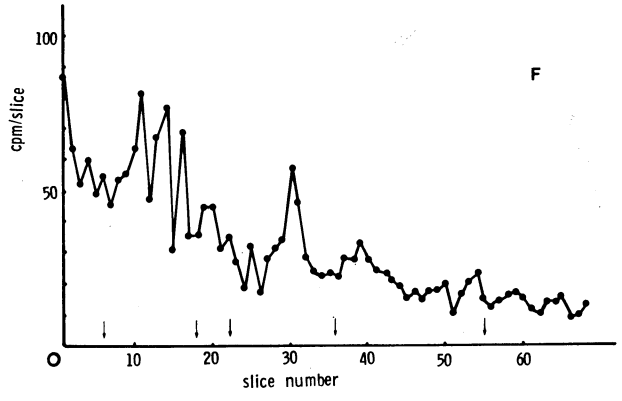
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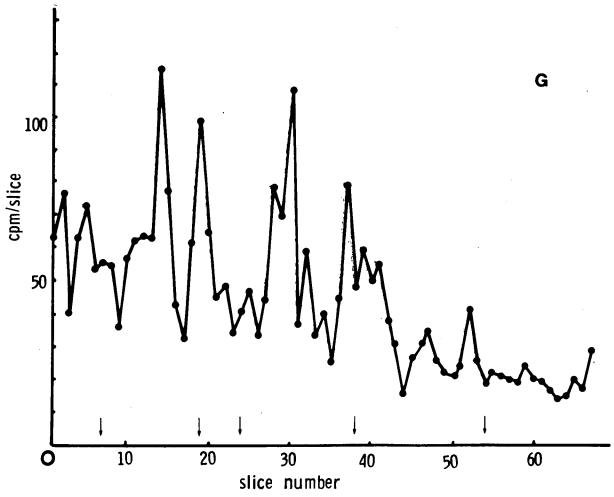

Fig. 2. Uptake of [^{32}P]phosphate in the chromosomal proteins of MCF-7 cells. Cells were incubated for 1 day (B,C), 3 days (D,E), or 8 days (F,G) in media containing 10^{-8} M estradiol (B,D,F) or 10^{-6} M tamoxifen (C,E,G) or a vehicle alone (A). The cells were incubated in 2 ml of 0.4 mCi of carrier-free [^{32}P]orthophosphate for 20 min at 37°C. The chromatins were prepared and the electrophoresis was carried out as described in "Materials and methods". The small arrows signify the mobilities of the internal standard proteins.

complex binding capacity.[6] Although the receptor binding capacity of chromatin was approximately the same,[1] thymidine incorporation into DNA was increased in the cells on day 1 after administration of estradiol in comparison with tamoxifen (Fig. 1). The difference in the action mechanisms of estradiol and tamoxifen may possibly be explained in part by the appearance of peaks b and c, respectively. It has been noted that tamoxifen reduces the S and G_2+M phases and increase the G_1 phase of the cell cycle in MCF-7 cells grown in medium with charcoal-stripped bovine serum.[7] Stimulation of the progression of the cells in the G_1 phase to enter the S phase has also been observed at 12–18 h after the addition of estradiol to cells maintained for 4 days in medium with stripped serum.[7] It has also been reported that the rates of phosphate uptake into most major phosphoprotein species increases during the early G_1 and early S phases and are minimal during the late G_2 to M periods.[2] The results of the present and the previous[1] experiments, it is believed, reflect a phase of the cell cycle as well. It is possible that the S and the G_2+M phases increased on days 1 and 3–8, respectively, after administration of estradiol. The G_1 phase is thought to have increased on day 1–8 after administration of tamoxifen and a portion of the cells may have been synchronized in the S phase on day 1.

Acknowledgment

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