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PHYSICOCHEMICAL AND DNA-BINDING PROPERTIES OF THE ESTRADIOL AND HYDROXYTAMOXIFEN BOUND ESTROGEN RECEPTOR FROM MCF-7 CELLS SOLUBILIZED BY MICROCOCCAL NUCLEASE.

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In order to get insight into the molecular mechanism of estrogen and antiestrogen action at the chromatin level, we compared the physicochemical properties of the estrogen receptor-chromatin fragments released by micrococcal nuclease digestion of nuclei isolated from MCF-7 cell line previously exposed to (^{3}H)estradiol or (^{3}H)hydroxytamoxifen. The following parameters were determined: sedimentation constants (S) on a sucrose gradient, stokes radii (Rs) by gel filtration on a Sephadex G-200 column and the binding ability to a DNA--cellulose column. The molecular weights (Mr) and frictional ratios (f/fo) were calculated from the S and Rs values.

	Buffer	S	Rs(nm)	Mr	f/fo
Estradiol	Low Salt	5.2±0.07	7.04±0.13	151,000	1.85
	High Salt	4.2±0.07	4.45±0.10	77,000	1.46
	High Salt and Urea	3.4±0.05	5.60±0.08	79,000	1.82
Hydroxy-	Low Salt	5.2±0.06	7.25±0.28	155,000	1.88
tamoxifen	High Salt	4.9±0.04	5.87±0.34	119,000	1.66
	High Salt and Urea	3.5±0.03	5.78±0.32	83,000	1.85

About 40% of either the estradiol or hydroxytamoxifen ligated receptor released by the nuclease bound to a DNA-cellulose column, could be eluted by high salt concentrated buffer.

Conclusion: 1. A similar receptor containing particle was released by micrococcal nuclease digestion from the chromatin of cells exposed to estrogen or antiestrogen. 2. High salt dissociation revealed that the antiestrogen bound receptor form was of higher molecular weight than the estrogen bound receptor form. 3. Further treatment with urea dissociated the antiestrogen bound receptor form into a receptor similar to the estrogen bound receptor.

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Determination of tamoxifen and three metabolites by HPLC in serum of post-menopausal breast cancer patients

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Tamoxifen (Tam) is currently the compound of choice for hormonal treatment of patients with breast cancer.In a collective of 50 postmenapausal breast cancer patients treated with 20 mg Tam per day were the serum levels of Tam, 4-hydroxy-tamoxifen (OHT), N-desmethyl-tamoxifen (NDT) and N-didesmethyl-tamoxifen (NDDT) quantified.

The serum samples were deproteinized with dioxane in the cold and after centrifugation were the supernatants dried under nitrogen.After dissolving the residues in solvent A (acetonitrile/0.3 mol/l H_3PO_4/10 mmol/l KH_2PO_4 190/50/280),chromatography was performed using a CN reversed phase column (25cm,10µ) with solvent A.Metabolites were detected by fluorescence after photocyclisation to their corresponding phenanthrene derivatives.

The aim of this investigation was to evaluate the serum profiles of Tam and its metabolites under dayly Tam treatment with respect to estrogen and progesterone receptor concentrations of the primary tumor and to clinical response.