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ESTROGEN REGULATED PROTEINS FROM THE HUMAN BREAST CANCER CELL LINE MCF-7.

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The human breast cancer cell line MCF-7 has been propagated in monolayer culture in presence as well in absence of serum. During growth in tissue culture the cells will synthesize and secrete a number of proteins to the surrounding medium. By polyacrylamide gelelectroforesis and autoradiography we have studied the synthesis of secreted proteins in cells grown with and without estradiol and find that the synthesis of at least six proteins is regulated by estradiol. Three proteins with approximate molecular weights 70K, 65K and 52K are stimulated by estradiol, whereas three proteins with molecular weights 46K, 43K and 39K are inhibited by estradiol.
An investigation of the function of these proteins are in progress.

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FLOW CYTOMETRIC EVALUATION OF RADIATION INDUCED DAMAGE IN MICE SPERMATOGONIAL STEM CELLS

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The ability of flow cytometry to detect and quantify damage on mice spermatogonial stem cells following radiation has been investigated. After pepsin treatment and staining with ethidium bromide for 24 hours the DNA distribution of the testis revealed four peaks representing: 1) haploid cells with condensed chromatine (30%), 2) haploid cells staining stoichiometrically (43%), 3) diploid cells (13%), and 4) tetraploid cells (11%). After irradiation there was a dose-dependent decrease in the number of cells in all peaks. The absolute number of cells in each peak per testis should therefore be calculated in order to express results as a fraction of untreated controls. Survival curves obtained 42, 56, and 70 days after irradiation using total number of haploid cells as the end point showed a survival response with 2 shoulders, the second shoulder occurring at around 8 Gy. Do values for doses between 8 and 14 Gy were 2.3-2.6 Gy.

Supported by the Danish Cancer Society.

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FLOW CYTOMETRIC METHODS FOR DNA ANEUPLOIDY AND CELL CYCLE ANALYSIS

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Flow cytometric single parameter DNA analysis is an important method for detection of tumor aneuploidy. The efficiency depends on the methods of sampling, staining and statistical deconvolution of the DNA histogram. Statistical estimation of the fraction of nuclei with G1, S and G2+M content and the DNA index is calculated using a maximum likelihood method. The distribution of the S-phase nuclei is fitted with a function allowing the estimation of synchronized and asynchronous populations. Distributions with overlapping nondiploid cell populations can be fitted under certain restrictions. In this case, the proportion of nuclei in each population is estimated. Further information on cell cycle kinetics requires dual parameter measurements. The fraction of mitotic cells is measured by dual parameter flow cytometry of nuclear suspensions fixed in formaldehyde (Larsen et al. 1986, Cytometry 7:54). For dual parameter analysis of nuclear DNA content and the cycling cell antigen Ki-67 we have established a method for cell lysis and staining based on sequential addition of detergent/propidium iodide, Ki-67 antibody, and FITC-conjugated antibody, without fixation or intermediate washings. With these methods it is possible to measure the mitotic index and the cycling/noncycling distribution in subpopulations in a heteroploid mixture.

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Circumvention of anthracycline and vinca alkaloid resistance in Ehrlich ascites tumour (EHR/DNR$^+$) in vitro by anthracycline analogues.

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Mutual cross resistance is a common phenomenon between anthracyclines and vinca alkaloids. Experimental resistance to these drugs are characterized by a decreased drug accumulation in the resistant cells.
25 different anthracycline analogues were tested for influence on [^{3}H]-DNR accumulation in resistant cells. The respective analogue was added to the suspension of cells at zero time. Cellular accumulation of [^{3}H]-DNR after incubation for 60 min.was determined by liquid scintillation compared to control. At equimolar concentrations 4 of the analogues enhanced [^{3}H]-DNR accumulation more than 200%. No specific structural feature separated the 4 compounds which influenced DNR accumulation from the 21 analogues which exerted no or minor effect. To further characterize the analogues, their lipid solubility was examined by measuring the partition coefficient in octanol/phosphate buffer (pH = 7,45). High lipid solubility of the anthracycline analogues resulted in enhanced [^{3}H]-DNR accumulation in resistant cells. A comparison with 3 low and 3 high lipid soluble analogues on vincristin (VCR) accumulation certified good correlations between effect on [^{3}H]-DNR accumulation in resistant cells and lipid solubility of the analogues. In conclusion, anthracycline analogues with high solubility are able to enhance [^{3}H]-DNR and [^{3}H]-VCR accumulation in resistant cells.
This indicates that different anthracyclines analogues may be able to circumvent resistance to anthracyclines and vinca alkaloids.