showed excellent overall correlation in both cell systems, was highly specific, and matched microscopic observation of the cells. This work was supported by NIH grants AI28281 and DA05161.

CB 83

Lead Effect on Viability Estimates in Dutch Belted Rabbit Sperm

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(Authors): National Institute for Occupational Safety and Health

The use of vital stains for assessing sperm viability is a subjective measure of the number of live vs dead cells. In some cases it may be difficult to determine the uptake of the dye by the cell and only a limited number of cells may be scored. In this study an objective method was assessed using a flow cytometer. A Coulter Epics Elite flow cytometer with an air cooled argon laser was used. When cells are mixed with the LIVE/DEAD FertiLight™ sperm viability kit reagents (Molecular Probes, Eugene, OR) and excited with visible-wavelength light the cells emit fluorescence. The two components of the kit SYBR 14 and propidium iodide(PI) bind to the DNA of the sperm. SYBR 14 is a membrane-permeant nucleic acid stain and PI is the conventional dead-cell stain. This method was then applied to four groups (15/group) of Dutch Belted rabbits to determine the effects of lead on sperm functions. The rabbits were dosed with lead acetate to maintain blood lead concentrations of 0, 20, 40, and 80 µg/dl for the spermatogenic cycle. The semen was collected weekly for 20 weeks with an artificial vagina, 10 µl of the first ejaculate was diluted with 500 µl of Ham's F-10 and used to measure viability. A mixed model, repeated measures analysis was used to assess the impact of blood lead levels on percent viable sperm for weeks 16-20. Increasing blood lead resulted in a significant (p=0.0003) linear decrease in rabbit sperm viability.

CB 84

A study of correlation between chromosome 8 aberrations and Ki-67 in human breast cancer.

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There are many prognostic factors in breast cancer. However, because of heterogeneity of cancer cells, it is understood that only axillary lymph nodes involvement is useful to prognosis. So we studied the simultaneous detection of Ki-67 antigen(MIB-1) and centromere specific DNA probe (D8Z2/biotin labeled, Oncor) for human chromosome 8 in 50 breast cancer to investigate the correlation proliferating cells and chromosome aberrations. Ther was a significant correlation between the nodal involvement and the rate of Ki-67 positive cells with chromosome 8 numerical aberrations($p<0.05$). However, the nodal involvement was not correlated with tumor size, estrogen and progesterone receptor status, DNA ploidy pattern, and the rate of Ki-67 negative cells with or without chromosome 8 numerical aberrations. This data shows that Ki-67 positive cells with numerical chromosome 8 aberration tends to involve lymph nodes.

CB 85

Flow Cytometry Contributions to Understanding Mechanisms of Drug Action

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Recent accomplishments in understanding mechanisms of growth regulation and the action of cytotoxic agents have allowed us to develop a conceptual framework at the cellular and molecular lev death, is considered to be the n death. We are able to demonstrat compartment of perturbed cells, r through the cell cycle but also l showing DNA fragmentation, or HL60 cells grown exponentially ir with equimolar concentrations of Taxol (0.45µM). At 3 hours, on (pulse chase) and the other was c 3, 6, 12, 24, and 48 hours, cells were washed in PBS and fixed in 70% ethanol. Controls with no d harvested and fixed. Detection o plished by a two-step TDT assa propidium iodide and RNase wer RESULTS—Camptothecin, a t 12%, 38%, 26% and 17% apopt 3, 6, 12, and 24 hours, respectivel uous exposure, the apoptotic frac at 24 hours, 89% of cells were a continuous incubation with CP active analog, SN-38 showed fi tothecin. In contrast, HL 60 cel delay in apoptosis for 12 hours. drug incubation showed a progr apoptotic cells in pulse chase set hours, respectively.

We have demonstrated differenc Camptothecin caused apoptosis was cell cycle dependent. 2) CPT- but SN-38, the active componen tothecin. 3) Taxol, a microtubule response", and the apoptosis seen tive of the duration of drug expos The findings of duration of dru affected can be an important con findings suggest that Topoisom proliferating cells, and daily to w more efficacious than monthly more prolonged intervals between utility of in-vitro studies of new a schedules. In addition, we have sh activity; however, its active form.

This work was supported by the Grant P30 CA 54174.

CB 86

Phenotypic heterogeneity of PH lymphocytes: APO-1/Fas antige expression identifies different fu

Carlo Rumi, Sergio Rutella, Mo Puggioni, Giuseppe Leone,

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(Author 5):Chair of Hematology, Catho.

We used an in vitro model of lym expression of APO-1/Fas (CD9 normal CD69+ lymphocytes. H subjects were activated by incubati to 25 µg/ml) for 4h at 37° C. Cell

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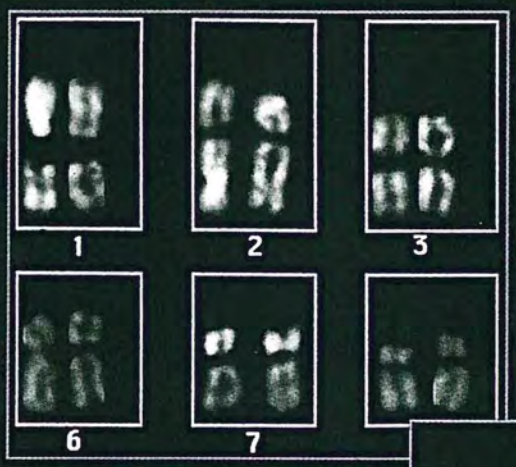
Cytometry



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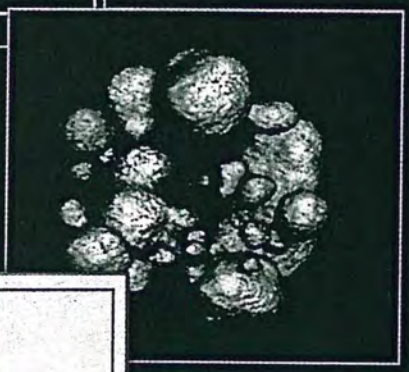

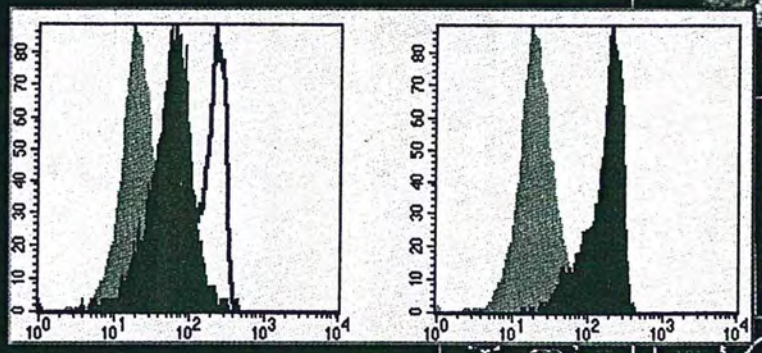

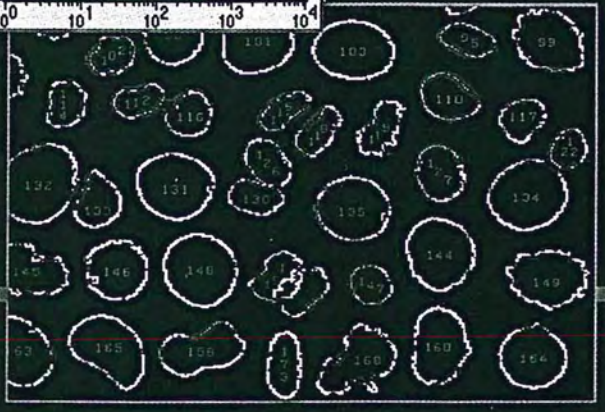

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