reveals growth regulation and cell density dependent reactions. The culture system described for growing L929 cells in feeder layers promotes colonies of homologous cells, cultured on the surface agar layer. A cell density of $0.5x10^6$ cells per ml reach confluency at 24 hr and these stationary cultures cause a complete inhibition of colony formation. This inhibition is extended not only to homologous cells but to prokaryotic cells as well. Streptococcus grows slowly and forms tiny colonies on stationary cultures, on the contrary a growing cell culture promotes development of greater diffused colonies. Such model systems of colony morphogenesis on stationary and growing feeder cells may prove more sensitive estimating regulatory actions of pharmacological and biological substances or cell to cell interactions.

OCHRATOXIN A IN HUMAN BLOOD IN RELATION TO BALKAN ENDEMIC NEPHROPATHY AND URINARY SYSTEM TUMOURS IN BULGARIA

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In an effort to provide further evidence for the hypothesis that a mycotoxin is involved in the aetiology of Balkan endemic nephropathy(BEN) and that the later is associated with the occurrence of urinary system tumours (UST), a survey was made for the occurrence of ochratoxin A(OA) in human blood samples collected from people living in an area with BEN and high incidences of UST compared with those from another non-endemic area in Bulgaria. In all, 312 people were analysed and OA was found in the serum of people from both endemic and non-endemic areas. But a much greater proportion of samples containing OA (26.3%) was found in the serum of patients with UST and/or BEN whereas the proportion of OA in the serum of people from the non-endemic area approximately to 7.7%. The highest concentration found was 35 ng ochratoxin A /g serum.

A TRANSFORMING GROWTH FACTOR PRODUCED BY SV40-3T3 CELLS

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A growth promoting activity was purified from serum-free medium conditioned by SV40-transformed 3T3 cells seeded at high density (CM). The purification steps consisted of gel permeation chromatography of the acid-soluble CM fraction followed by cation exchange and reverse-phase high pressure liquid chromatography. A partially purified preparation of growth factor was found capable of stimulating both thymidine incorporation as well as the proliferation rate of quiescent 3T3 cells. This fraction also induced anchorage-dependent non-transformed cells (NRK) to form colonies in soft agar. The presumptive transforming properties associated with the growth promoting activity as well as its possible relationship with known TGFs or PDGF-like factors are currently under investigation.

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IGF1 RECEPTORS (IGF1-R) IN 72 PRIMARY HUMAN BREAST CANCER. RELATION WITH ESTRADIOL AND PROGESTERONE RECEPTORS (ER, PgR)

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IGF1 (Insulin-Like Growth Factor (1) stimulates the proliferation of human breast cancer cells. We have characterized the R-IGF1 in four breast cancer cell lines in long-term tissue culture: we followed this work determining the R-IGF1 concentration in 76 primary breast cancers. The labeled IGF1, 200 uCi/ug (Pr Humbel-Zurich and Amersham-France) was incubated for 5 hr at 4°C with 400 ug of breast cancer membrane proteins, in the presence or absence of a partially purified IGF1 preparation. Only 6.6% of the tumours bound less than 1% of the total radioactivity (IGF1-R-); 18.4% bound 1 to 2% (IGFR+), 75% of the tumours bound more than 2% (IGF-R+). The range was 0 to 16.4%. There is a relation between IGF1-R+ and RPg+ (χ^2=8.6,p=0.003) and between IGF1-R+ and the menopause (χ^2=6.8, p=0.009). The concentration of IGF-R is correlated (Spearman test) to RE (p=0.0018) and to RPg (p=0.0011). There is a linear positive correlation between log IGF-R and log RE (n=59, p=0.025) and between IGF-R and log RPg (N=54, p=0.0025). These results suggest that (1) as breast cancers contain IGF-1-R they could be sensitive to this growth factor, and (2) an IGF-R lowering drug could be a beneficial treatment for these patients.

MODULATION OF THE HUMAN Ha-ras-1 ONCOGENE EXPRESSION BY DNA METHYLATION

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The promoter region of the Ha-ras oncogene contains CG clusters similar to those found in eukaryotic house-keeping genes, whose expression has been found to be modulated by DNA methylation. We have inserted fully methylated Ha-ras oncogene in NIH-3T3 cells by co-transfection with a plasmid containing a selectable marker. The NIH-3T3 transfected cells remained normal in morphology and were not tumourigenic in vivo. A cloned NIH-3T3 cell line, containing an integrated, methylated and silenced Ha-ras gene, was treated with the demethylating analog 5'-azacytidine. After the treatment: (1)the morphology of the cells turned from flat to reflactile spindle shape; (2)the cells acquired the property to growth in 0.3% agar and to form tumours when injected in nude mice; (3)the promoter region (0.8 SacI fragment) of the Ha-ras gene was specifically demethylated; (4)the cells produced Haras associated mRNA and p21. These data show that DNA methylation can modulate the expression of a genetically altered human ras oncogene.

MECHANISM OF ACTION OF IMMUNOTOXINS

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Immunotoxins, ITs, represent a new class of pharmacological agents designed to have cell specific activity. They are conjugates of antibodies against cell surface antigens with highly active toxins, such as diphtheria toxin and the plant toxins ricin and abrin (or their active subunits), which act by inhibiting cellular protein synthesis. The mechanism of action of ITs, and their potential use in cancer treatment have been evaluated.

The ITs are internalized by receptor mediated endocytosis. The subsequent penetration of the toxic moiety, the A-chain, through the vesicular membrane into the cytosol is somehow facilitated by the toxin B-chain and seems to involve several pathways. The toxicity and specificity of ITs depends, not only on the antigen, the antibody and the toxin used, but also upon inherent metabolic properties of the cells. Thus, different melanoma cell lines differed widely in sensitivity to abrin-IT and these differences were associated with concomitant differences in sensitivity to native abrin, probably reflecting different abilities of the cell lines to translocate the toxin from vesicles to the cytosol.

ORAL CONTRACEPTIVES AND HORMONE-RELATED CANCERS

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Our understanding of endometrial cancer, the 'simplest' hormone dependent cancer, has become ever more clear over the last 2 decades. We now have a very satisfactory semi-quantatitive explanation of the major risk factors, and in particular the markedly protective effect of combination-type oral contraceptive (COC) use, in terms of hormone levels and cell cycling times. Our understanding of breast cancer has remained, however, at a 'primitive' level. Breast cancer shares many features with endometrial cancer and the biology of the 2 diseases was until quite recently thought to be similar. Breast cancer fails, however, to show a very significant increase with oestrogen replacement therapy, or a decrease with COC use. The basis of these discrepancies has been the subject of the present investigation, and specifically, possible ways in which a breast cancer protective OC may be formulated, are under evaluation.

EFFECT OF IFN ON THE EXPRESSION OF N/C-myc AND CLASS I ANTIGENS

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The MHC class I antigens play an important role in the host defence mechanisms against tumour cells. In several laboratories it has been shown that expression of these antigens is down-modulated by some oncogenes (e.g. myc or E1A) and increased by interferon.

By immunoblotting experiments we found that after gamma-IFN treatment the class I antigens are greatly enhanced in all cell lines examined. Unlike MHC class I antigens, myc proteins appear to be slightly decreased (c-myc in melanoma and COLO 320 cells) or unaffected (N-myc in neuroblastoma and retinoblastoma Y 79 cells).