EARLY SEVERE SHOCK!

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The stathmokinetic method of measuring crypt cell production rate (CCPR) acurately assesses cell turnover in intestinal epithelium but ethical constraints limit its application in man. Organ culture might overcome this problem, although the exact cytokinetics of colorectal mucosa within this system have yet to be determined. We therefore studied 2 x 2 mm mucosal explants from the lower descending colon of male Sprague-Dawley rats (n=35), which were placed on still grids in culture dishes containing supportive medium and gently rocked in an atmosphere of 95% O_2, 5% CO_2. After 18 hr, vincristine (0.5 µg/ml) was added to the medium, and 6 sequential specimens were removed during the next 3 hr. A linear increase in metaphase arrests was observed with a CCPR of 4.78±0.41 cells/crypt/hr (means±S.E.M.). By contrast, in further experiments vincristine was added either ab initio or 3, 6 or 9 hr after the commencement of culture. During the first 5 hr of organ culture there was almost no increase in arrested metaphase figures per crypt (CCPR=0.03; $p<0.0001$). However, if 6 or 9 hr culture preceded addition of vincristine, CCPR was 4.01 and 4.06 respectively (p=n.s. vs 18 hr). Colorectal mucosa undergoes severe shock during the initial 5 hr of organ culture. A 6 to 9 hr period of culture yields satisfactory data on CCPR and could reflect original proliferative rates more closely than an 18 hr culture.

TOXICITY OF COMPOUNDS RELATED TO DENTAL MATERIALS IN CULTURED HUMAN BUCCAL CELLS

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Clinical reports have clearly associated different dental materials with pathological effects in buccal mucosa. Therefore in vitro models using cultured normal human buccal epithelial cells and fibroblasts have been developed. Adult human buccal mucosa, obtained from surgery, was either maintained in a serum-free growth medium to derive epithelial cells, or in a low-serum (0.6%) culture medium to derive fibroblasts.

The effects of several metal-ions corresponding to metals commonly used in dental materials were investigated. The doses required to decrease the colony forming efficiency (CFE) of fibroblasts to 50% after 1 hr exposure were: Hg(II), 1 µM; Ni)II), 1 µM; Cr(VI), 1µM; Cd(II), 3 µM; Cr(III), 100 µM; and Co(II), 300 µM. Formaldehyde, a reactive compound known to be released from denture base polymers, was also found to decrease CFE of fibroblasts; a 50% inhibition was found at 30 µM. Preliminary experiments indicate that individually these agents were equally toxic to buccal epithelial cells grown at clonal density. The results show that different cultured human buccal cell types can be grown and used for studying pathobiological effects of dental materials.

HUMORAL ENHANCEMENT OF METASTASIS : IgG BINDING BY TUMOUR-BEARER T LYMPHOCYTES AND CONCURRENT CHANGES IN HELPER/SUPPRESSOR RATIOS

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Using the RT7-4b hepatocarcinoma of the inbred BD-IV rat, we have previously shown that metastasis can be enhanced using the IgG2b fraction of tumour-bearer serum. Flow cytometric analyses of lymphocytes from tumour bearing, serum enhanced tumour-bearing, and naive rats revealed that IgG from tumour-bearer serum bound to a subset of T lymphocytes. PBLs from serum enhanced tumour-bearing rats bound predominantly the IgG2b isotype (74% T cells exhibiting this isotype specific fluorescence) and this binding was saturated in vivo. Tumour-bearer T splenocytes, sorted on their IgG binding parameters, enhanced metastasis (approximately 2-fold) in the lung colony assay. As well as the additional macrometastases, numerous micro-metastasis were seen in the enhanced rats. During the development of metastasis, helper:suppressor T cell ratios fell progressively, being most rapid for PBLs and serum enhanced animals. Suppressor cells appear to be involved in humoral enhancement of metastasis.

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THE EFFECT OF HYROXYUREA ON GENE AMPLIFICATION IN HUMAN NEUROBLASTOMA CHP-100 CELLS

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Evidence exists to suggest that

anti-cancer drugs which inhibit DNA synthesis can increase the frequency of gene amplification. The currently favoured model for the mechanism of gene amplification is that of saltatory replication whereby unscheduled DNA replication creates strands of DNA that are not attached to the chromosome; such re-replicated DNA may be observed cytologically as double minutes (DMs) or homogeneously staining regions (HSRs).

We have investigated whether the anti-cancer drug hydroxyurea can induce this mechanism of gene amplification in human neuroblastoma CHP-100 cells. DNA double labelling techniques have revealed no evidence of re-replication of DNA following hydroxyurea treatment. Therefore, it is unlikely that hydroxyurea can induce gene amplification by this mechanism.

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THIOL STATUS OF NORMAL HUMAN BRONCHIAL EPITHELIAL CELLS AND FIBROBLASTS

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The content of total sulphydryls (SH) and low molecular weight thiols (LMWT) including reduced glutathione (GSH), and cysteine, oxidized glutathione (GSSG), cystine and mixed protein disulphides as determined in human bronchial epithelial cells and fibroblasts. Epithelial cells had signficantly higher levels of total SH than fibroblasts, 75 as compared to 53 nmol of SH per 10^6 cells, respectively. In both types of cells, qualitative analysis indicated similar proportions among the various LMWT where GSH was found to be the major thiol. For both cell types, passage in culture caused an immediate decrease in total thiols and also changed ratios among different LMWT. Continued culture caused a marked peak in GSH synthesis which preceded cellular proliferation. Furthermore, the proportion of GSSG plus mixed disulphides was significantly higher before the cells entered the growth phase. During logarithmic growth, the amount of GSH was markedly decreased. Prolonged maintenance of fibroblasts at confluence, did not cause further change in SH. The results indicate variations in SH content between different human cell types and implicate the importance of LMWT in growth regulation.

DNA MEASUREMENTS FOR EFFECTIVE CHEMOTHERAPY OF HÜRTHLE CELL CARCINOMA

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Inoperable or disseminated Hürthle cell carcinoma is a therapeutic challenge as therapy with [131]I or radiation or chemotherapy is usually ineffective. In order to find an effective chemotherapy, the influence of vinblastine (VLB) (2 mg bolus or infusion over 6, 12, 24 hr) was studied in 5 patients (4 women, 1 man, aged 43 to 69 yrs). Four patients had distant metastases, one locoregional disease only. Thin-needle aspiration biopsies of tumours (1 primary, 4 metastases) were performed before and repeatedly after VLB applications. The smears were stained after Feulgen and were used for cytophotometric DNA measurements. VLB produced an increase of cells in S phase compartment. On the basis of changes produced in the DNA distribution pattern by the test dose of VLB, chemotherapy was planned: either a sequence of 3 VLB infusions with individual intervals or a combination of VLB, cisplatinum, methostrexate, bleomycin or adriamycin was used. All 5 patients responded - 1 CR, 4 PR. Chemotherapy was combined with surgery in 1 and radiation in 2 patients. Two out of 5 patients show no evidence of disease 3.1 years after therapy, 2 continue chemotherapy, 1 patient is dead of other causes.

DETECTION OF ANTIBODIES AGAINST AFLATOXIN-CONJUGATE IN SERA FROM AFRICAN AND DANISH POPULATIONS

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A sensitive ELISA assay has been developed to detect antibody activity against aflatoxin (AFB) in human sera. Antibodies to an epitope on AFB-BSA were detected in all sera collected in Kenya. The specific activity showed a trimodal distribution. The high activity group had a higher frequency of recent AFB exposure, as measured by urinary excretion of aflatoxin-guanine, than the low activity group. Little or no activity was detected in Danish sera. Animal experiments indicate that the specific activity depends on the metabolism of AFB to its ultimate carcinogenic form. The activity in rat sera was inhibited in a competitive assay by an aflatoxin-like antigenic material found in