for the determination of NOR and its hydrolysis products. The method was based on derivatization by heptafluorobutyric anhydride. The structures of the derivatives were established by GCMS. The rate of disappearance of NOR at 37 degrees and pH 7.4 was about 20 min, and a similar rate was noted irrespective of whether NOR or PAM was used as starting material. N-(2-chloroethyl)-N-(2-hydroxyethyl)amine (NOR-OH) appeared with a half-life of 19 min when NOR was used, but with a half-life of 23 min when PAM was used as starting material. The main difference in product yields was the relatively higher amounts of NOR-OH and N,N-bis(2-hydroxyethyl)amine (NOR-OH-OH) formed when PAM instead of NOR was used as starting material. This suggests the formation of NOR-OH and NOR-OH-OH from NOR as well as from the hydroxylated derivatives of PAM.

REDUCTION TO HOMOZYGOSITY OF GENES ON CHROMOSOME 11 IN HUMAN BREAST NEOPLASIA

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There is increasing evidence that recessive genetic lesions might be involved in the genesis of several paediatric and adult tumours. These recessive mutations are unmasked when tumour cells attain hemi- or homozygosity for particular genes. In primary breast tumours an allelic loss of c-Ha-ras-1 locus (chromosome 11p) was detected in 27% of patients heterozygous for this proto-oncogene. Restriction fragment length polymorphism analysis of tumour DNAs provided evidence for reduction to homozygosity of not only c-Ha-ras-1 gene but also of several markers on the short arm of chromosome 11. This loss of normal cellular sequences was specific for chromosome 11 and had a significant correlation with the most aggressive form of the disease. Our analysis also suggested that the deletion of the region between the B-globin and PTH loci might be important in this subset of tumours.

A systematic study of the possible alterations in other proto-oncogenes strongly suggests that human breast neoplasia, a highly complex and genetically heterogeneous disease, might involve abnormalities of several proto-oncogenes.

GROWTH FACTOR AND ONCOGENE EXPRESSION DURING MEGAKARYOBLASTIC DIFFERENTIATION OF K562 LEUKAEMIA CELLS

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Platelets contain PDGF and TGF-beta, but their site of synthesis has not been proven since megakaryocytes are difficult to obtain for studies of this nature. Our results of studies with the chronic myeloid leukaemia (CML) cell line K562 suggest that the genes encoding the two PDGF chains and TGF-beta (1) and the synthesis of the corresponding proteins are induced during the megakaryoblastic differentiation process. The expression of the bcr-c-abl oncogene mRNA remained unaltered during the differentiation of K562 cells, but the kinase activity of the corresponding fusion protein is almost completely shut off suggesting that an active c-abl oncogene is incompatible with K562 cell differentiation.

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MONOCLONAL ANTIBODIES AGAINST NIH 3T3 CELLS TRANSFORMED BY HUMAN THYROID CARCINOMA DNA

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First-cycle transfectants of NIH 3T3 transfected with human metastatic thyroid carcinoma DNA were used as an immunogen to obtain monoclonal antibodies against antigens induced by the transfected tumour DNA. The transfected cell line (M33) was shown to contain ALU sequences. Two monoclonal antibodies were selected on the basis of their differential reactivity toward NIH 3T3 or M33 cell lines. By biological and biochemical analysis, the first monoclonal antibody (MTr1) recognised an epitope on cytoskeletal filaments of proliferating murine fibroblasts. Similar filaments labelled by MTr1 were also found to accumulate into cytoplast-like structures produced by M33 cells.

Characterization by immunofluorescence of the second monoclonal antibody, MTr2 indicated that it recognizes a specific human antigen associated with normal thyroid tissues and differentiated thyroid tumours.

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FATTY ACID COMPOSITION OF PHOSPHOINOSITIDES IN RAT LIVER NODULES

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Rat liver nodules produced by treatment wth carcinogens exhibit elevated proliferation rate and differs from normal liver in several biochemical properties.

The composition of individual phospholipids in nodular tissue is changed with a two-fold increase in phosphatidylinositol (PI) compared to normal liver. Since PIs play a critical role in cell regulatory mechanisms, it is of great importance to understand the action of PIs in nodules. Earlier studies show a connection between cell proliferation, PI-metabolism and arachidonic acid release.

The fatty acid composition of the phosphoinositides in nodules was studied, with special interest focused on arachidonic acid (20:4) and its precursor linoleic acid (18:2). Preliminary results indicate no difference in fatty acid composition in phosphoinositides between nodular and normal liver.

INHIBITION OF COLONIC NEOPLASIA AND CRYPT CELL PRODUCTION RATES BY INTRALUMINAL CALCIUM

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Small bowel resection and intrarectal administration of sodium deoxycholate each stimulate cell proliferation and promote carcinogenesis in the large intestine; oral supplements of calcium reduce the mitogenic effect of bile acids on colorectal mucosa. Potential suppression of intestinal adaptation and carcinogenesis by intraluminal calcium was tested in 120 male Sprague-Dawley rats weighing 186±9 g. Rats were randomised to receive azoxymethane s/c 15 mg/kg/week for 6 weeks or vehicle, followed by 80% mid small bowel resection or transection with reanastomosis. Half the animals in each group received supplemental calcium in the drinking water (calcium lactate 24g/1). Crypt cell production rate (CCPR) in descending colon was determined 7 weeks postoperatively in vehicle-treated rats; in the remainder colonic tumour yield was assessed at 26 weeks. Among rats with transection calcium supplements reduced colonic CCPR by 26% from 4.49±0.33 to 3.32+0.40 cells/crypt/hr ($p<0.05$) and more than halved tumour yield from 4.3 to 1.8 tumours/rat ($p=0.0007$). Jejunoileal resection increased both CCPR (by 51 to 61%; $p<0.001$) and tumour yield (by 65 to 105%; $p<0.005$), but again calcium lowered CCPR by 31% (7.23+0.44 vs 4.98±0.70; $p<0.02$) and tumour yield by 46% (6.9 vs 3.7; $p=0.0006$). Increased dietary levels of calcium diminish both adaptive and neoplastic growth in the colon, and calcium also blunts the co-carcinogenic stimulus of massive enterectomy.

ASSESSMENT OF RAT COLONIC TUMOURS BY FLOW CYTOMETRY: METASTASES ARE COMMONLY DNA ANEUPLOID

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The study of azoxymethane-induced colonic carcinogenesis in rats helps in understanding human colorectal cancer. We have used flow cytometry to investigate the presence of DNA aneuploidy in rat intestinal tumours, and to evaluate the rat model as an experimental system. Fifty male Sprague-Dawley rats weighing 185.6+9.2 g were given azoxymethane 15 mg/kg/week s/c for 6 weeks and then underwent 80% small bowel resection (n=25) or jejunal transection (n=25). Half the animals in each group had calcium lactate 24g/1 added to the drinking water. Ten further non-operated rats (NOP) received azoxymethane 10 days later than the others. Forty-three rats survived 26 weeks and yielded 149 colonic and duodenal tumours of which 140 were measurable by flow cytometry. The incidence of DNA aneuploidy was 43% in NOP which was higher than in rats with resection (9%; $p<0.0005$) or transection (24%; $p<0.0005$). There was no significant difference in the prevalence of DNA aneuploidy between adenomas (32%) and carcinomas (17%) or between calcium treated (11%) and non-calcium groups (12%). However metastases were more commonly DNA aneuploid than the primary tumours (62% vs 20%; $p<0.005$). DNA aneuploidy is present in rat intestinal tumours and levels can vary widely with manipulation of the model. Metastases are associated with a high incidence of DNA aneuploidy.

ASSESSMENT OF COLONIC ADAPTATION BY CRYPT CELL PRODUCTION RATES IN ORGAN CULTURE: AN