A B S T R A C T S

XXIV

Annual Meeting

of the

Cell, Tissue and Organ Culture Study Group

(C.T.O.C.)

affiliated with the International Union Against Cancer

(U.I.C.C.)

Special Seminars on:

- Models for Invasion and Metastasis
- The Cell Biology of Trophoblast Invasion in vivo and in vitro

Heidelberg (FRG)

10 - 11 October 1986

Abbreviations and numbers indicate where the contribution is placed in the program:

IM : Seminar on "Models for Invasion and Metastasis" (Friday afternoon, October 10)

TI : Seminar on "The Cell Biology of Trophoblast Invasion in vivo and in vitro" (Saturday morning, October 11)

FC : Free Communications (Saturday afternoon, October 11)

TI 5 **Evidence for the Involvement of Specific Membrane Components in Embryo-Uterine Interactions During Implantation.**

Ted Anderson, Loren Hoffman*, Gary Olson*, James Simon, and Gary Hodgen. Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, Virginia 23507 USA, and *Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232 USA.

An increase in galactose-containing glycoconjugates of the uterine luminal surface (RCA-I lectin binding) has been demonstrated in mice and rabbits to be related temporally to implantation (Biol. Reprod. 32:1135 and 34:701). In rabbits, this has been correlated with expression or increased synthesis of membrane glycoproteins (from 42 kD to 245 kD), identified by RCA-I binding to Western blots of solubilized membrane components resolved by SDS-PAGE. Lectin binding to the uterine surface and to isolated glycoproteins is reduced at implantation sites, compared with nonimplantation areas, providing evidence for local modification of these membrane components by implanting embryos. More recently, RCA-I binding to the uterine luminal surface of cynomolgus monkeys was examined throughout the normal (28-day) menstrual cycle, and was noted during the peri-implantation period (6 days post-ovulation) but not during the proliferative (cycle days 4-6) or late secretory (cycle days 26-28) intervals. Specific effects of embryos on the uterine surface have not been examined in this model, but galactose-containing glycoproteins (from 30 kD to 155 kD) have been isolated using biotinylated lectins and avidin affinity chromatography. These results are consistent with the known involvement of galactose-containing glycoproteins in cellular interactions such as recognition and adhesion, and suggest that certain molecular aspects of uterine receptivity may be common among a variety of species. Regulation of expression of these membrane components by steroid hormones is being investigated in vivo and in vitro. Additionally, interactions between peri-implantation embryos and uterine epithelial cells or isolated membrane components are being examined toward the elucidation of a unified concept of embryo-uterine interactions.

IM 6 **Effects of TPA and misonidazole on growth, DNA synthesis and plasminogen activator production of human cancer nodules maintained organ culture.**

René Beaupain, Sylvie Chevillard, Henri Magdelenat and Jacques Coppey. Institut Curie, 26 rue d'Ulm, 75231 Paris cedex 05, France.

The organotypic culture method of human tumor cells has been found to be a useful tool for assessing the effects of cytostatic treatments on tumor growth and longterm survival. The extremely low traumatizing culture conditions offer to injured cells after the different cytostatic treatments, a maximal survival chance, close to the in vivo situation. Using this culture system, concentrations of misonidazile (MISO) corresponding to the serum levels (40-160 µg/ml) reached during the course of phase I clinical trials, showed a 1.6-fold growth stimulation of lung carcinoma (A549) nodules as compared with the untreated controls. The stimulation may be related to the transformed state of the tumor cells and the close cell to cell contacts in the nodules. DNA synthesis was increased starting 24 h after the treatment with MISO and the rate of sister chromatid exchange were also enhanced. These stimulatory effects could suggest a tumor-promoter-like property of MISO. For this reason, a comparison was made with the known tumor promoter 12-0-tetradecanyol phorbol-3-acetate (TPA) on A549 nodules. TPA actually stimulates growth of A549 nodules but here the rate of DNA synthesis is enhanced within 24 h. A large amount of urokinase-like plasminogen activator (PA) is secreted after TPA treatment while the rate of PA production is only slightly increased by MISO. So, in spite of similarities in some effects of MISO and TPA, the origin of the growth stimulation of human tumor nodules by MISO seems not to be due to a straight tumor promoter-like effect.

IM 13 **Separation of tumor cell subpopulations with high and low motility from a metastatic tumor line**

Rosemarie Benke, Georg Brunner, Lars J. Erkell and Volker Schirrmacher. Institut für Immunologie und Genetik, Deutsches Krebsforschungszentrum, D-6900 Heidelberg, Federal Republic of Germany.

Using a modified Boyden chamber assay we have separated high from low motile subpopulations from high metastatic EbF1 mouse hybridoma cells (L. Larizza, V. Schirrmacher, Cancer Met.Rev. 3, 193, 1984). For selection the cells had to migrate through a nucleopore filter to reach a second nitrocellulose filter. The high motile subpopulation was obtained by culturing the cells which had penetrated the nucleopore filter while the low motile subpopulation was obtained by culturing the cells remaining on the nucleopore filter.

The high motile fraction migrated twice as far as the low motile or the non-separated EbF1 cells. Furthermore, the high motile subpopulation produced twice to four times more plasminogen activator than the low motile or the non-separated EbF1 cells.

Finally, in an in vitro gel invasion assay (L.J.Erkell, V. Schirrmacher, in preparation) preliminary data indicated that the high motile subpopulation has increased invasiveness.

In vivo, the low motile fraction caused larger tumors (about 20mm) after subcutaneous injection than the EbF1 control cells (about 14 mm).

These data may indicate that EbF1 cells are heterogeneous and contain a subpopulation of motile and rapidly disseminating cells which have been partially depleted by the selection for low motile cells.

IM 7 The interaction of invasive cells with laminin can be altered by conversion products of (+)-catechin in liquid culture media

Marc Bracke[1], Vincent Castronovo[2], Rita Van Cauwenberge[1], Guido De Pestel[3], Peter Coopman[1], Jean-Michel Foidart[2] and Marc Mareel[1]. (1) Laboratory of Experimental Cancerology, Department of Radiotherapy and Nuclear Medicine, State University of Ghent, B-9000 Ghent, Belgium, (2) Department of Obstetrics and Gynecology, Laboratory of Dermatology, University of Liège, B-4020 Liège, Belgium and (3) Laboratory of Embryology, State University of Ghent, Belgium.

The flavonoid (+)-catechin is a tool to study the mechanisms of tumor invasion, since it inhibits the invasion of a number of cell types in vitro, it increases the resistance of collagen type I to collagenases (Kuttan et al., Experientia, 37: 221, 1981), it binds to the extracellular matrix glycoprotein laminin, and it alters the adhesion and morphology of invasive cells to matrix supports containing laminin and/or collagen type IV. Evidence has accumulated, however, that those effects are not due to (+)-catechin itself but to one or more conversion products of the flavonoid in liquid culture media. Known candidates for the active product(s) are : (-)-epicatechin, catechinic acid and quinones. With a preparative HPLC technique we have purified nine fractions from Eagle's minimum essential medium incubated with (+)-catechin for 4 days at 37°C in an atmosphere containing 5% CO_2 in air. A reverse phase C18-column was eluted with a gradient of acetonitrile (10 to 40%) in water. The effect on cell morphology on and adhesion to laminin was confined to one fraction. The chemical composition of this fraction will be discussed, as well as its effect on malignant invasion in vitro and in vivo.

This work was supported by te Koning Boudewijnfonds, the Sportfonds tegen de Kanker and the Fonds voor Geneeskundig Wetenschappelijk Onderzoek (nr 20093) - Brussels, Belgium.

IM 2 INTERCELLULAR COMMUNICATION AND INVASIVENESS OF MALIGNANT CELLS IN A THREEDIMENSIONAL CULTURE SYSTEM

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Intercellular communication via gap junctions allows passage of small ions (electrical signals) or molecules. Using the in vitro invasion assay established by Mareel et al. with cultured embryonic chick heart fragments as target tissue for malignant cells, we concentrated on the question whether the capacity of tumor cells to communicate with each other and with the confronted host cells has any influence on their invasive behaviour. For coupling-competent fibroblastic tumor cells like BICR/M1R-k, C6 and EMT6/Ro heterologous electrical coupling with contacting heart cells could be demonstrated. Injection of Lucifer yellow revealed cell-line-dependent differences in the extent of dye coupling. In the invasion assay, however, all tumor cell lines occupied and progressively replaced the heart tissue in a comparable time span. For the non-coupling epithelial HeLa cells we found a different invasive behaviour, confirming results of Mareel et al.. HeLa cells built a multilayer capsule around the heart fragment and single invading HeLa cells were found only after a significantly longer confrontation period than for the coupling competent cell lines. This different invasion pattern is probably caused by the epithelial organisation of the HeLa cell aggregates. Numerous tight junctions prevent single HeLa cells to separate from the spheroid. Further experiments with the non-coupled fibroblastic cell line L are in progress.

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IM 12 Production and secretion of urokinase and trypsin-like proteinases by mouse lymphoma cells of high or low metastatic capacity.

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The proteinase activities of tumor cells are probably essential for their invasiveness and metastatic potential. We studied plasminogen activator (PA) and trypsin-like proteinases of a low metastasizing (Eb) and a high metastasizing (ESb) lymphoma cell line.

Plasminogen activator: ESb cells produced and secreted PA activity, whereas Eb cells did not. Enzyme production and secretion by ESb cells were maximal in serum-free Iscoves medium and increased with increasing cell density. In the presence of 5% FCS the production and secretion of PA were reduced and independent of the cell density. Within 24 h of culture, ESb cells secrete about the same amount of PA activity into the medium as is contained intracellularly. No PA activity could be detected on the surface of the cells by immunofluorescence and enzyme assays with whole cells. Using polyclonal and monoclonal antibodies the PA of ESb cells was identified as the urokinase type. In western blot experiments following SDS/PAGE of unreduced cell lysates, one main band at about 51 kd and four minor bands at higher molecular weights could be identified.

Trypsin-like proteinases: These enzyme activities were twice as high in Eb than in ESb cell lysates. However, ESb cells secrete five times as much of trypsin-like proteinases than Eb cells do. The amount of secreted activity by ESb cells within 24 h of cell culture is about tenfold higher than the enzyme activity in cell lysates.

TI 11 **An immunohistological investigation of maternal bone-marrow-derived populations in decidua in normal and ectopic human pregnancy**

Judith N. Bulmer, Debra Hollings, Department of Pathology, University of Leeds, LEEDS, LS2 9JT, U.K.

In early human pregnancy fetal extravillous trophoblast invades maternal decidua, myometrium and spiral arteries with no apparent detrimental effect to either mother or fetus. A large population of bone-marrow derived cells has been identified in maternal uterine decidua in pregnancy. These cells have been extensively characterised with monoclonal antibodies in single and double immunoenzymatic techniques applied to acetone-fixed frozen sections or formalin-fixed paraffin-embedded sections. HLA-DR+, leuM3+ macrophages are common throughout pregnancy and are often very closely associated with extravillous trophoblast in decidua basalis. T lineage lymphocytes (OKT3-, DAKO-T11+, DAKO-T2+, OKT10+, MT1+) are abundant in the first trimester but thereafter decline in numbers. In formalin-fixed sections counterstained with haematoxylin and eosin, cytoplasmic granules have been identified in leucocyte-common antigen+, MT1+ cells indicating that the endometrial granulocyte is a T lineage cell.

The relative importance of fetal trophoblast and hormonal milieu on infiltrating maternal leucocytes in decidua has been assessed by studying non-pregnant endometrium as well as the tubal implantation site and intrauterine decidua in ectopic tubal pregnancy. HLA-DR+ macrophages predominate at the tubal implantation site, whereas T cells are rare. In non-pregnant endometrium, macrophages are present throughout the menstrual cycle but T lymphocytes increase in number in the late luteal phase.

The relative importance of decidual macrophages and T lymphocytes in successful pregnancy remains to be established.

FC 12 **Natural human antibody to α-galactose residues inhibits metastasis by MO_4 cells (a mouse malignant fibrosarcoma cell line).**

Vincent Castronovo, Philippe Mahieu, Marc M. Bracke, Jacqueline B. Foidart, Jean-Michel Foidart, Universities of Liege and Ghent (Belgium).

Natural human antibody to α-galactose accounts for 1-2% of total circulating IgG. Alpha-galactose residues present in cell surface glycoproteins are normally masked and unaccessible to this antibody. A good correlation exists between the metastasizing capacity of mice neoplastic cells and their content in exposed cell surface α-galactose (Varani et al., Clin. Exp. Metastasis, 3, 45, 1985). A significant proportion of the galactose groups is located on surface associated laminin. Preincubation of MO_4 cells (a mouse fibrosarcoma cell line) with natural antibody to α-galactose caused an 80% inhibition of attachment to laminin-coated dishes. Prior incubation of MO_4 cells with anti-galactose antibody or with its Fab fragments caused a 50% reduction in the number of metastatic lungs colonies developing in syngenic mice 2 weeks after I.V. tail injections. Finally, immuno-fluorescent and radiolabeling studies confirmed the ability of anti-galactose IgG to bind to mice and human breast adenocarcinoma cells but not to normal human cells.
Altogether our studies indicate that neoplastic human cells contain α-galactosyl residues exposed on their cell surface. Natural circulating anti-galactose antibody specifically binds to these cells and prevents their subsequent attachment in vitro to laminin. It inhibits in vivo lung metastasis. The extent of unmasked α-galactosyl residues in malignant cell surface glycoproteins could therefore contribute to the immune modulation of neoplastic cells growth.

TI 12 **ROLE OF MATRIX MACROMOLECULES IN THE INVASION OF MATERNAL DECIDUA BY TROPHOBLAST.**

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In early pregnancy, cytotrophoblast from the implanted blastocyst invades maternal decidua both in interstitial locations and within spiral arteries. Arterial endothelial cells are replaced by trophoblast which also infiltrates the media of the vessel walls leading to loss of elastic tissue and changes in mechanical properties required for increased blood supply to the placenta. Immunohistochemical studies of decidua from 8-10 weeks' gestation show fibronectin to be widespread in interstitial and vascular areas. Vitronectin is absent from most interstitial locations, but present as a striking spotted distribution within vessels. Laminin is retained in vascular and glandular basement membranes (as before implantation) but also appears as an intense halo around decidual cells. Type V collagen progressively disappears from interstitial locations but is retained in vessel walls.

A new model system has been developed for studying the trophoblast-endothelial cell interaction in vitro. Using confrontation cultures,

we have found that choriocarcinoma (BeWo) cells advance to displace vascular endothelial monolayers (derived from umbilical vein) in a process resembling that seen in decidual vessels. BeWo cells grow in islands with some multi-layering; migration can occur either by sheet outgrowth or escape of single locomotory cells from islands. Thus isolated colonies of 'escaped' trophoblast might be expected within maternal arteries. BeWo cells adhere better to substrate-associated fibronectin than to laminin, collagens I, III, IV or V or fibrinogen. Fibronectin produced by vascular endothelial cells may therefore play a role in trophoblast invasion of vessels. Vitronectin is not produced by vascular cells, but may be recruited from maternal plasma into vascular locations where invasion is occurring.

TI 4 Possible involvement of D-galactose in mouse embryo implantation.

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Blastocysts of all mammals adhere to the luminal epithelium of the uterus regardless whether or not implantation is interstitial. The acquisition of adhesiveness appears to be temporally regulated in the developmental and differentiation process of both the blastocyst and uterine luminal epithelium. Assuming that adhesion of trophoblast to uterine epithelium involves physiochemical interaction between their respective surface coats, an investigation of their surface receptors to lectins was undertaken. During the adhesive stage of implantation changes in the expression of D-galactose (D-Gal) and its N-Acetyl derivative could be detected. Differences involving N-Acetylglucosamine (GlcNAc) were also noted. Since GlcNac is normally present sub-terminal to D-Gal, it was presumed that differential expression of GlcNAc was associated with the differential expression of the D-Gal molecule. D-Gal commonly occupies a position penultimate to terminal sialic acid. To determine whether interference with carbohydrate metabolism, and specifically D-Gal metabolism would result in interference with the implantation process, a series of metabolic inhibitors was introduced into the lumen of one horn of pregnant mice during the peri-implantation period (day 5). Implantation was prevented by α-D-NAcGalactosylaminidase, UDP-Galactose, neuraminidase, and tunicamycin but not by D-Gal, α-D-Galactosidase or β-Galactosidase nor was it affected by administration of physiological saline (20 µl). α-lactalbumin prevented implantation in some mice and retarded embryonic development in others. Implantation was not affected in the uninjected contralateral horn, which served as a control. A decidual cell reaction was stimulated by UDP-Gal only. These data indicate that interference with D-Gal metabolism results in perturbation of the implantation process and that the molecule may be associated with elicitation of the decidual cell reaction. This study was supported by NIH Grant HD-16703.

IM 14 Effect of adriamycin on human tumor cells maintained in continuous organotypic culture.

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In order to find new clinical protocols using adriamycin (ADR) and X rays, we have first tested the drug toxicity on human tumor cells derived from a differenciated lung adenocarcinoma showing a pulmonary surfactant secretion. In our culture model, where the cells are maintained in three dimensions, we have tested the effects on growth, cell necrosis and eventually on regeneration of the tumor nodules characterized by the reapparence of surfactant secretion. For non toxic concentrations (1.10^{-2} µg/ml), a 24 h treatment inhibits more the nodule growth than a 1 h treatment, but, for the toxic doses (1 µg/ml), an opposite effect was observed : at equal concentrations, regeneration of the nodule occured earlier after a 24 h treatment than after a 1 h treatment.

In connection with this, we observed (taking advantage of natural fluorescence of ADR) the presence of ADR in the nucleus after a 1 h treatment, and after a 24 h treatment ADR was located in the cytoplasma.

ADR has some radiosensitizer effect.

TI 6 Preparation of uterine epithelium for trophoblast attachment: changes in apical plasma membrane-bound enzymes

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This study was performed in order to look for cell membrane changes which may prepare the uterine epithelium of the rabbit for attachment of the invasive trophoblast at implantation initiation at 7 d post coitum (p.c.).
Implantation chamber, interblastocyst endometrium as well as uterine epithelium at hCG-induced pseudopregnancy (p. hCG) were compared to distinguish between membrane changes regulated by maternal plasma steroid hormones and those which might be induced locally by blastocyst-derived signals.
Membrane characteristics were evidenced by marker enzymes of the apical plasmalemma of the uterine epithelium. All marker enzymes which could be detected with high activity in the apical plasma membrane were so-called brush-border enzymes: alkaline Phosphatase (aP), Aminopeptidase M (AM), γ-Glutamyltransferase (γ-GT) and Dipeptidylpeptidase IV (DPP IV).
The enzymes tested showed their main activity at 5 d p.c./d p. hCG. The weakest reaction in this series of stages is generally found at 8 d p.c./p. hCG. In interblastocyst segments and in the uterine epithelium of pseudopregnant rabbits there is a continuous decline from 5 d to 8 d p.c./p. hCG which varies in extent and the day of first decline. In the implantation chamber there was a difference between the epithelium surrounding the blastocyst and the epithelium of interblastocyst segments. In contrast to the rest of the epithelium the activity of DPP IV remained high in the cavum epithelium of the para- and obplacental folds and the activity of aP even raised again from 7 to 8 d p.c. indicating a direct local influence of the blastocyst itself on the luminal epithelium. The different stage-specific pattern of enzyme activity indicates a drastic change in the composition of the apical uterine membrane in preparation for adhesion and implantation of the blastocyst. (Supported by DFG grant De 181/9-6)

IM 17 **Correlation of in vitro drug sensitivity with clinical response for patients with brest cancer and malignant melanoma.**

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The inhibition of ^{3}H-uridin uptake by adriamycin in tumor cell suspension measured by liquid scintilation technique ekspressed as a percentage of the total uptake of the control, was taken as a prognostic factor of clinical response. In vitro drug sensitivity was determined for a group of 52 patients with brest cancer and 95 patients with malignant melanoma. The patients with tumors resistant in vitro showed clinical resistance to chemotherapy but longer postoperative recurrence free survival, lover recurrence and mortality rate in contrast to patients with significantly high in vitro sensitivity.

FC 1 **THE INFLUENCE OF CELLULAR INTERACTIONS ON THE CHEMOSENSITIVITY OF MURINE GLIOMAS IN VITRO**

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The relative ineffectiveness of single-agent chemotherapy for malignant glioma must derive from the inherent heterogeneity of these tumours. Using dilution cloning it has been possible to derive six clonal cell lines from the P497 cell line, originally isolated from a spontaneous murine astrocytoma. These clones differ in respect of morphology, growth potential in vitro, karyotype, expression of surface and cytoplasmic markers and tumourigenicity both from each other and from the parental cell line. Using a ^{35}S-methionine uptake assay the sensitivity of these clones has been determined to a panel of cytotoxic drugs. There was wide variation in sensitivity between the clones particularly with respect to the vinca alkaloids, vincristine (VCR) and vindesine (VDS) where there was a 13.7 and 20-fold

difference in sensitivity between least and most sensitive respectively. The differences between least and most sensitive for a panel of other drugs ranged between 2.9 to 4.3-fold. There is now evidence that considerable interaction takes place between cellular sub-populations which modulate the behavior of constituent clones with respect to factors such as chemosensitivity. We have combined VCR or VDS resistant and sensitive clones in vitro and compared the chemosensitivity of the mixed lines and the parental lines. Initial experiments with mixed cultures produced from equal numbers of cells from the F1 (VCR and VDS sensitive) and C12 (insensitive) clones indicate that the combination tends to adopt the chemosensitivity of the more resistant parent. Although these clones had similar population doubling times (23.9 and 22.4h respectively) and plating efficiencies the possibility exists that the most resistant partner became dominant. As the clonal lines are karyotypically distinct (C12 has a modal chromosome number between 60 and 70 and F1 has a modal chromosome number between 90 and 110) experiments are underway to examine the constitution of the mixed culture using flow cytometry.

IM 4 Long term organotypic culture of normal and tumour of human bladder and immunophenotyping

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In vitro maintanance of both normal (N) and tumour (Tm) bladder (B) epithelium is advantageous to study the etiology of bladder cancer. In an organotypic tissue culture (OtC) method 2 mm2 fresh explants (Ex) were placed on cellulose acetate filter (1.2 u) rafts (CAFRs: Millipore) laid on platforms of stainless steel grid (pore size: + 25 mm2) in Falcon culture dishes (25 mm2) containing 5 ml of medium. Waymouth's medium WB, 752/1 was most suitable and contained 10% FCS, hydrocortisone-hemi-succinate (1 µg/ml), L-glutamine (2.5 mM), ascorbic acid (300 µg/ml), FeSO4 (0.45 µg/ml), penicillin-streptomycin (1000 IU/ml). In case of NB, light microscopic (LM) morphology showed that the mucousal architecture of the cultured explants resembled those in vivo and could be maintained indefinitely. Whereas in most cultures of TmEx, cells remained vital upto 125 days in their original LM morphology before being degenerated and leaving the dead stromal mass on CAFRs. Only in 1/10 Tm, the Ex had been maintained in vital state indefinitely. Immunoenzymatic (IE) and immunofluorescence (IF) analysis, using antibody to cytokeratin-18: RGE-53, cytokeratin: RKSE-60, vimentin, desmin (Eurodiagnostics, Holland), T6 (Monosan, Holland), T9 (Ortho), Leu-4, Leu-2a and Leu 3a (Becton Dickinson), showed no changes in cultured NBEx; whereas in case of Tm, Ex showed heterogenic pattern of changes in morphology and antigenic markers during the culture period. The IE analysis of fresh TmEx also showed variable characteristics. Our hypothesis is that the variability in the maintanance of different TmBEx by OtC method might be related to in situ immunestatus and histological gradings of Tm. Such studies combining immunotyping and culture approach will be useful for diagnostics and treatments.

IM 15 Studies on bilharzial cancer using organotypic tissue culture of hamster bladder

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In spite of the epidemiological evidence for an association between bladder cancer and bilharzia (schistosoma infection) in Egypt, other factors such as nitrosamines and synergetic bacterial infection may be required (Hicks et al. BJ Cancer, 42, 730, 1980). Involvement of schistosomiasis mediated immunopathogenesis in the genesis of bladder cancer remains essentially little studied at the in vitro experimental level and this has led to an in vivo/in vitro studies aimed at establishing whether the bilharziasis is a co-factor or a direct carcinogen. In this study, explants (Ex) of hamster bladder (HB) (a laboratory host for schistosoma) have been cultured in Waymouth's medium WB 742/1 containing 10% FCS, hydrocortisone-hemi-succinate, ascorbic acid, FeSO4 and penicillin-streptomycin. In such organotypic culture (OtC) system HBEx could be maintained indefinitely. The light microscopic (LM), electron microscopic (EM) morphology and immunofluorescence (IF) analysis showed that the mucousal architecture of the cultured Ex resembled those in vivo. When such HBEx cultures, treated with either S.mansoni egg hatching product (EHP) in combination with anti-EHP and complement, or ∝-EHP/complement or with antibody or EHP alone, have shown a pronounced urothelial response only when exposed to the first combination, indicating an immunomediated cytotoxicity. The present experimental data suggest that the OtC system in conjunction with functional immunological studies will serve as a valid system for further investigations on the role of such product in the genesis of bladder cancer among Egyptions.

IM 18 Co-cultures of mouse B16 melanoma cells and 3T3 fibroblasts in three dimensions.

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Multicellular spheroids composed of mouse B16 melanoma cells, 3T3 fibroblasts, or these two types mixed together were cultured on agar 1 % in Petri dishes (1). Cells had tight contacts, but an extracellular matrix (ECM) was formed during the culture. These spheroids could be maintained on agar or under agitation at 75 rpm for up to 1 month. Collagens I, III and IV, laminin, and fibronectin were searched with immunocytochemistry (2) and by electron microscopy. Proliferation rate and cell differentiation (pigmentation) were related to the nature of the ECM.
Culture of multicellular spheroids of this type favored cellular differentiation. In B16 and 3T3 mixed cells spheroids, proliferation was lowered, necrosis was noted (essentially in 3T3 cells) and disintegration of spheroids sometimes happened. Immunoreactions for collagens, laminin and fibronectin were positive in ECM but relatively weak as compared to those observed in 3T3 pure spheroids. Laminin was also detected inside 3T3 cells, and in smaller amounts in B16 cells, in pure spheroids.
Works actually in progress concern : comparisons with 2-dimensional cultures, analysis of ECM in B16 melanoma in the mouse and study of B16 conditioned media.

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FC 3 Studies on the biological, hystological and cytogenetic characterization of two sublines of the murine ovarian reticular cell sarcoma M5 showing different sensitivity to cyclophosfamide.

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A subline of the ovarian reticular cell sarcoma (M5076, M5) responsive to cyclophosfamide (CTX) was made resistant to the drug by repeated in vivo treatment followed by transplantation of the regrowing tumor in 16 passages (M5-CTX-16R,R16). In the frame of the studies aiming at characterizing the biological, biochemical and pharmacologycal properties of these sublines, we have observed that the subline R16, which showed at the beginning a slower growth rate with a longer survival time of tumoral mice, has aquired recently biological characteristics perfectly superimposable to those of the parent line. As far as the histological feature are concerned, M5 and R16 show the presence of neoplastic cells in the stroma of the muscle, which resemble an indifferentiated mesenchymal neoplasia with numerous atypical nuclei and caryokinetic figures with large necrotic areas. The cytogenetic examination of the distribution in the chromosomic number of R16 reveals that this subline may be considered a clone of the parent line with a modal class of 35 chromosomes (34-37) versus a class of 34 (31-37) for the M5 line. The modal class of R16 reveals also a number of metacentric chromosomes lower than for M5 (23 vs 25).

FC 14 Reconstituted basement membrane matrix alters fibroblast properties in vitro.

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Extracellular matrices play a significant role in determining the behaviour and differentiation of cells through specific interactions with the cell surface. Although fibroblasts are in contact with basement membrane (BM) during wound healing, in the granulation tissue and in various pathological conditions, little is known about the effect of this interaction. Normal calf skin fibroblasts were grown on a reconstituted BM and their morphology, replicative and biosynthetic activity compared to the same cells in monolayer on plastic. BM components were extracted in urea from Engelbreth-Holm-Swarm tumors. Dialyzed in physiological saline and kept in solution at 4°C, when heated to 37°C they formed a porous gel, termed matrigel. The matrigel was composed of the main macromolecules present in authentic BM : laminin (84%), entactin (11%), type IV collagen (4%), heparan sulfate proteoglycan (1%). The ultrastructural morphology of the gel consisted of a network of thin microfilaments and larger masses of electron dense material. Normal calf skin fibroblasts cultured on matrigel grew in multiple layers and displayed unique ultrastructural features which somewhat resembled contracting myofibroblasts during the active phase of granulation tissue contraction in wound repair. Their cytoplasm contained a conspicuous network of microfilaments and was to a large extent depleted in rough endoplasmic reticulum and Golgi membranes. Culture on matrigel stimulated fibroblast proliferation. A large decrease in collagen synthesis and minor differences in the pattern of newly synthesized collagenous polypeptides were observed when fibroblasts were cultured on matrigel versus plastic. After several days of culture, fibroblasts disorganized their support. Many cytoplasmic vesicles were shown by immunoelectron microscopy to react with an anti-laminin antibody suggesting that fibroblasts might phagocytose laminin. BM components could thus contribute to regulate fibroblast morphology, proliferation, biosynthesis and degradation.

IM 3 Invasion in vitro of human lung tissue by human lung tumor cell lines.

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Human lung tumors exhibit different invasive patterns in situ. To study these processes in detail we have used an in vitro model by confronting tumor cell lines with isolated human lung tissue. Lung tumor cell lines were established from one patient with small cell lung cancer (SCLC) of the oat cell-type with adherent growth in vitro, from a second patient with SCLC of the classical type, growing non-adherently in vitro, from a third with a differentiated metastasizing squamous cell carcinoma, and from a fourth with lung metastases derived from an undifferentiated embryonic rhabdomyosarcoma. These lines showed different morphological characteristics and typical patterns of locomotion. Freshly isolated lung cubes kept in rotation culture regenerated by wound healing of the damaged areas. The regeneration process took about 2 to 3 weeks. Finally the cubes were covered by epithelial-like cells. In this stage they were confronted with the lung cancer cells. All tumor cell lines adhered to the surface of the lung cubes, later on, however, we found a different behavior. By spreading and intensive growth the squamous cell carcinoma and the cells of the metastases of the rhabdomyosarcoma formed a layer coating the lung cubes and, in addition, formed characteristic homotypic round aggregates. The SCLC (oat cell-type) colonized the lung surface as single cells or clusters. The coating of the lung cubes proceeded slowly. The SCLC (classical type) overgrew the lung surface so slowly that a complete coating could not be observed. By means of this model we want to study further steps of invasion and also quantify the invasive behavior of the different tumor cell lines.

IM 1 A double filter in vitro invasion assay

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The invasive ability of a cancer cell is essential for its metastatic potential. To monitor cellular invasiveness, we are developing a double filter assay in which the cells are allowed to penetrate a polycarbonate filter (8 µm pore size) to reach a nitrocellulose filter which is filled by a fibrin gel. After incubation the filters are fixed, separated and stained, and the cells that have migrated into the fibrin matrix of the nitrocellulose filter are counted under the microscope.

The surface of the polycarbonate filter can be coated with proteins, e.g. fibronectin, laminin. Alternatively, endothelial cells can be grown on the filter surface and a basement-membrane-like matrix can be obtained by detergent extraction. Preliminary results with the invasive mouse hybridoma EbF1 demonstrate that the presence of fibronectin and laminin stimulates invasion into the fibrin gel. In the related ESbM cell line however, fibronectin and laminin counteract invasion. These effects are probably due to interference with cell adhesion.

FC 2 Glucocorticoids, cytostasis, and the cell surface

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Methyl prednisolone (MP) and dexamethasone (DX) have a cytostatic effect on cell cultures grown from human glioma, if the cells are maintained at a high cell density. At low cell densities (e.g. during cloning) glucocorticoids are mitogenic. The cytostatic effect is expressed at and above 10^{-7} M in the presence and absence of serum. At 2.5×10^{-9} M, in the absence of serum, the effect is again mitogenic, where glucocorticoid restores the saturation density previously obtained in serum. Glucocorticoid treatment increases cell-cell and cell-substrate adhesion, implying that it may exert its effect via the cell surface. Electrophoresis of iodinated membrane proteins indicates that changes do occur in response to methyl prednisolone although so far no consistent pattern has emerged. Study of glycosaminoglycans, however, show that there is an increase in sulphated and ^{3}H-glucosamine-labelled GAGs in response to both MP and DX. Moreover, the degree of response corresponds approximately with cytostasis, although insufficient data are available as yet to establish a correlation. Preliminary results with immunostaining suggest that laminin may be decreased and fibronectin increased following treatment with MP and DX. The effect of exogenous fibronectin and GAGs are now being explored in an attempt to establish whether a causal relationship exists between the induction of these matrix components and cytostasis.

TI 8 Intracellular and Extracellular Indices of Stromal Cell Differentiation: Possible Relationships to Trophoblast Invasion

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Control of trophoblast invasion is a role assigned to decidual tissue. There is little direct data to explain how decidual cells could fulfill this function. Studies of *in vivo* and *in vitro* rat decidualization have demonstrated non-coordinate (vs. vimentin) expression of the intermediate filament subunit, desmin, is a correlate of hormonally regulated stromal cell differentiation. Vimentin expression serves as a marker of decidual cell growth. Vimentin increases proportionally with the increase in decidual cell protein. Desmin, marginally detectable in undifferentiated stroma, accumulates at a rate greater then the increase in cell protein. At 96 hrs the concentration of decidual cell desmin is equal to or greater than vimentin. The inductive accumulation of the extracellular matrix (ECM) proteins, laminin and entactin, absent from undifferentiated stroma, also serve as markers of decidualization (1,2). Other indices include the down-regulation and reorganization of fibronectin (2), the expression of a decidual luteotrophin (3) and heparan sulfate proteoglycan and chondroitin sulfate proteoglycan (4). These studies suggest that in response to the hormonal interactions that regulate uterine receptivity to the blastocyst the surfaces and subsequently the ECM of epithelial and stromal cells are remodeled. These changes alter cell-cell interactions thereby subserving the specialized attachment and invasive functions of differentiating trophoblast. (Supported by Natl. Inst. Health grants HD-07495, 17147).

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CT 8 **Altered requirement for growth stimulation by extracellular matrix (ECM) in adriamycin-resistant S 180**

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We demonstrated (2) that adriamycin-resistant cells of sarcoma 180 react to serum-free conditions by the appearance of a membrane drug-pumping system and a depression of the thymidine incorporation to 5% in 2 hrs. The wild type cells do not change the pumping behavior and slow down the proliferation only to 50% in 2 hrs. We concluded that the proliferation regulation in adriamycin-resistant cells may be altered.

S 180 wild type and adriamycin-resistant cells (growing in 15, 25, and 50 mg/l adriamycin, respectively) have similar growth rates in RPMI 1640 supplemented with 10% fetal calf serum (FCS). Under serum-free, protein-defined conditions the wild type cells and a low-resistant variant (5 mg/l) are able to grow without major changes. The highly resistant cells reduce their proliferation rate as determined by cell counting by at least 50%. Furthermore, they mostly loose the ability to attach to the plastic substratum; those which attach do not spread and do not proliferate like the FCS-controls. Opposite to the wild type cells they are not autonomous with respect to the production of adhesion- and proliferation- stimulating factors. The production of adhesion promoting molecules is an until unknown feature of the sarcoma 180. We assume that these products belong to the class of ECM constituents. Investigating them we could show that plastic-associated parts of the matrix produced by the wild type cells (freed of cells according to 1) can restore both the adhesivity and the proliferative ability of resistant cells. Both FCS and purified fibronectin applied to plastic surfaces cannot supply for these effects. This may point to the participation of ECM processing mechanisms on the demonstrated phenomenology.

In adriamycin-resistant sarcoma 180 cells, a deficiency with respect to the production and/or processing of the ECM seems to be a resistance-correlated characteristics.

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TI 14 Evaluation of an in vitro model for embryo implantation: Selective receptivity of rabbit endometrium for trophoblast attachment

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Embryo implantation involves trophoblast attachment to and invasion into the endometrium. The initiation is assumed to depend on the coincidence of the invasive phase of the trophoblast with a "receptive state" of endometrium. We have attempted to test in vitro whether this receptivity is selective for the invasive trophoblast only or whether it is of a general type for any invasive cell.

Rabbit endometrial fragments were obtained at day 4 of pseudopregnancy and precultured in order to restore a complete epithelial lining and to induce "receptivity" by progesterone (Eur.J.Cell Biol. 33, Suppl. 5, 17, 1984). They were confronted with day 6.5 blastocysts and kept in co-culture for 2 or 3 days (Eur.J.Cell Biol. 36,Suppl.7,28,1985). It was found essential to successful trophoblast attachment and invasion in vitro that 1) both are kept in a close contact, 2) blastocysts remain expanded, and 3) syncytiotrophoblast differentiates.

Further confrontations were performed with aggregates of mouse fibrosarcoma cells (MO_4) and precultured endometrial fragments. These cells are highly invasive in conventional invasion assays (Mareel et al., Invasion and Metastasis 1, 105-204, 1981). In our studies, however, MO_4 cells were able to invade into "receptive" endometrium only if stromal surface was exposed. Attachment to the epithelium was rather weak and there was no invasion through the epithelial lining. These findings were confirmed by in vivo experiments.

Our results are not consistent with experiments where xenogenic tumor cells invaded into the endometrium of pregnant rats and mice in vivo (Short & Yoshinaga, 1967; Wilson & Potts, 1970). These differences underline the existence of different mechanisms for implantation in rabbits vs. mouse and rat.

Our results suggest a selectivity in the interaction between rabbit endometrium and trophoblast which is not lost in our organ culture model.

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FC 10

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TITLE ROLE OF INTERACTIONS BETWEEN EPITHELIAL CELLS FOR THE METABOLISM OF CARCINOGENS BY HUMAN MAMMARY CELLS

Different types of epithelial cells from rat liver were assayed for culture substrate for initiation and development of culture of human epithelial cells .

The culture system was either feeder layer, either coculture bysimultaneous seeding , either culture with parabiotic leighton tubes .

When the rat liver cells were at a resting state , mammary cells metabolism was assayed . The measure was made by extraction of the metabolites of 7,12 Dimethylbenz(a)anthracene a potent rat mammary carcinogen , and comparison of the level of metabolites obtained .

The results obtained show a triggering effect of contact interaction of cells to cells . The highest level was obtained with seeding of human cells on top a resting layer of rat female adult liver cells .It was possible to shift the type and level of metabolism of male rat liver cells to the subtype of rate female metabolic pattern .

It appears thus the local organization of cell to cell thigh contact plays also a role in the level of metabolism of compounds which need to be activated . The level obtained by coculture is much higher than what could be obtained by adding hormones , but is near what is obtained by effect of conditionned medium by rat liver cells added to human cells growing in their self produced collagenous underlayer .

research supported by Ligue française contre le cancer , Comité de Seine et Marne .

IM 5 Invasive activities of urothelial tumors in vivo and in vitro. Peter A. Jones, Louis Dubeau, Thomas Ahlering and Lynn Allen. Urological Cancer Research Lab, USC Comprehensive Cancer Center, 2025 Zonal Avenue, Los Angeles, CA. 90033

We have studied the in vivo and in vitro properties of a series of human bladder cancer cell lines in the nude mouse and in a serum free defined culture medium. A chemically defined culture medium which allowed for the continuous growth of 3 urothelial cancer cell lines was devised. The culture medium could also be used for the growth of normal urothelial cells for up to 6 passages. The degradative activities of the different neoplastic cell types were compared. All 3 cell types were capable of destroying complex extracellular matrices in culture and the role of specific degradative enzymes such as plasminogen activator and gelatinases was determined. The cell lines were subcloned into populations with high or low expression of plasminogen activator and the abilities of these cells to destroy the extracellular matrices compared. These experiments demonstrated an important role for plasminogen activator in the degradation of some, but not all, of the extracellular matrix components. A nude mouse model to study the metastatic abilities of the cells in vivo was also developed. The bladder tumor cells were injected directly into the urinary bladders of young female nude mice and all the 3 tumor cell lines were capable of growth in this organ. Evidence for metastasis to the lungs was obtained in some cases suggesting that this model may be a useful one for studying the metastatic potentials of cells in vivo. The abilities of subclones of cells isolated in vitro with different proteolytic potentials were compared for their abilities to invade and metastasize. This model in which in vivo cellular behavior from the normal organ environment can be compared with in vitro expression of specific proteases may be important in defining the potential relevance and importance of proteases in metastasis.

TI 16 MECHANISM OF VASCULAR MOBILIZATION INDUCED BY AN ENDOMETRIAL ANGIOGENESIS FACTOR

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In spite of the widespread use of the CAM bioassay for angiogenic activity, detailed descriptions of the actual morphology of the vascular flow circuit in the chick chorioallantoic membrane are scant. Therefore, the three-dimensional structure of the CAM bloodvessels was studied, in order to demonstrate their overall architecture in relation to the basic pattern of flow: arteriole-capillary-venule. A model was constructed based on four different methods of observation: in-vivo stereoscopic observations of the CAM, whole-mount preparations, histological sections, and plastic-cast microinjections.

The architectural design of the CAM circulation comprises a superficial two-dimensional capillary mesh floating over and enveloping a deeper three-dimensional space, which in turn is penetrated from below by larger floating blood vessels. These are branches off the vitelline vessels which emanate from the embryo and constitute the arterial supply and venous drainage for the superficial capillary bed of the CAM proper. Angiogenic activity of human decidual tissue or partially purified UAF (uterine angiogenesis factor) appeared as a typical "spokewheel" configuration at 10X. However, stereoscopic observation at 40X reveals firstly, that these radially organized bloodvessels are located deeper to the plane of the CAM; and secondly, that their tapered tips point in two opposing directions, as is the direction of the flow in them. Some of the blood vessels point towards the source of angiogenic activity – but others point away from it. This finding does not agree with the concept that capillary growth and migration are only directed centripetally. Most important, no tips or sprouts were observed either in the growing CAM or in response to decidual angiogenic factor.

The radial orientation is a consequence of the envisaged model, acted upon by UAF. The main effect of the angiogenic factors seems to be on the expansive growth of the superficial capillary meshwork of the CAM, to which the observed larger blood vessels are attached. The underlying arterioles and venules are thus pulled and stretched out into the observed "spokewheel" format. The physiological significance of this model is that it envisions vascular morphogenesis as a process which operates within the existing framework of circulatory loops.

TI 17 Identification of human trophoblast in culture using monoclonal antibodies.

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A wide variety of techniques are, at present, employed to maintain human trophoblast in culture, but it is unclear how successful these different procedures are. The main reason for this is the lack of a distinctive marker for the identification on trophoblast. Our laboratory has generated three monoclonal antibodies (MonAbs) which could be used for this purpose. Two of these (18B/A5 and 18A/C4) have proved highly efficient in their ability to identify trophoblast, either *in situ* (1,2) or when dissociated from chorionic villi (3). The third antibody (6A/D2) localises to fibroblasts of the villous mesenchyme so this is a useful reagent for the exclusion of non-trophoblast elements. Using these MonAbs, we have managed to identify and to quantitate trophoblast cells in culture (4). This permits us to select the most appropriate methods for the growth of these cells *in vitro*.

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FC 5 Potentiation of the antiproliferative activity by mixtures of recombinant human interferons alpha-2 and gamma on growth of human cancer nodules maintained in continuous organotypic culture.

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Alveolar II pulmonary tumor cells (A549 cells) maintained in continuous tridimensional organotypic culture were used to evaluate the eventual potentiation effect of mixtures of recombinant human interferon alpha-2 and gamma on growth inhibition of the tumor nodules. A continuous 45 day treatment (interferon renewed three times a week) with 10, 10^2 and 10^3 U/ml of interferon gamma or interferon alpha-2 combined with a fixed high dose (10^3 U/ml) of either interferon alpha-2 or interferon gamma resulted in an additive or synergistic growth inhibition according to the dose used. There was a close dose-effect relation, the percentage of inhibition increasing proportionally to the variable interferon doses added to the fixed high dose ; moreover the growth inhibition effect occured earlier with the mixtures than with the interferons used separately. Furthermore, the growth inhibition observed with 2,000 U/ml of the mixture (1,000 U/ml of each interferon) was greater than that induced by 2,000 U/ml of interferon alpha-2 or gamma used alone. A 35 day treatment with interferon alpha-2 1,000 U/ml plus interferon gamma 1,000 U/ml led to a complete growth inhibition and necrosis of the nodules. These data demonstrate that the interferons alpha-2 and gamma cooperate to potentiate the interferon antiproliferative activity.

TI 18 Fine structural changes in the basal lamina of the uterine epithelium in preparation for trophoblast invasion

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Trophoblast invasion appears to stop temporarilly, before progressing into endometrial stroma at the residual basal lamina, which therefore, seems to form some kind of barrier (Schlafke und Enders 1975, 1985). We have studied fine structural changes of the basal lamina in the uterine epithelium as related to implantation in the rabbit. Changes are already observed at day 7 post coitum when implantation starts at the antimesometrial side of the implantation chamber. In this modification the basal lamina appears as a fuzzy structure, missing the lamina lucida. These structural changes are restricted to the epithelial regions where invasion of the trophoblastic knobs starts. At the mesometrial side, part of the basal lamina is converted into amorphous material. This is remarkable since trophoblast attachment and invasion will not commence before one day later. Then, at 8 d p.c., the basal lamina throughout the implantation chamber is transformed into a thinned-out structure; at the mesometrial side it is even partly lacking. Typically, the uterine epithelial cells of the implantation chamber now form numerous cell processes on the basal side which penetrate the vestiges of the basal lamina and extend into the stroma.
By contrast, the blastocyst-free segments of these uteri reveal an intact basal lamina structure with lamina lucida and lamina densa. On day 8 p.c. the latter is replaced by an enormous accumulation of amorphous material (basal lamina material?).
We conclude that the basal lamina of the uterine epithelium undergoes remarkable changes even before trophoblast invasion which might facilitate trophoblast penetration through this specialized type of epithelium.
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FC 6 In Vitro and In Vivo Investigations for the Development of Cytostatic Methylhydrazones

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In in vitro short term (3 hrs) assays, the ß-chloroethyl-methylhydrazones B 1 and B 2 inhibit the uptake of ^{3}H-thymidine by Ehrlich Ascites Carcinoma (EAC) and L 1210 leukemia cells, B 2 being 5 - 10 times more effective than B 1. The growth inhibitory effect of both compounds was also confirmed in long term (7 days) clonal assays using agar-containing glass capillaries, B 2 again being more effective than B 1. In contrast to these differences in vitro, in vivo both substances showed remission to the same degree in EAC- and complete resistance in L 1210-bearing mice. The diverging in vitro/in vivo sensitivities were thought to result from differences in the affinity of the methylhydrazones to the tumor cells: Using short exposure periods (3 hrs) B 1 was more inhibitory than B 2 on both EAC and L 1210 colony growth: i. e. the more hydrophilic B 2 could more easily be washed off. To further test the idea of different cell membrane affinities, the methylhydrazones ZB 1 and P 1 with increasing lipophilic properties were synthesized. In vitro, after both pulse and continuous exposure ZB 1 and P 1 showed enforced inhibitory effects on colony growth. In vivo, ZB 1 and P 1 reduced the tumor weight of EAC-mice, while only P 1 increased the survival time of L 1210-mice. The results suggest that from the combination of in vitro/in vivo-assays pharmakokinetic conclusions can be derived that are valuable for further development of these cytostatics.

TI 19 Harvesting of trophoblasts from mouse ectoplacental cone

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The rationale behind this study, that deals with harvesting and seeding of trophoblast cells from mouse ectoplacental cone (EPC) on day 8 post-coitum (plug day is designated as Day 1), is to identify and characterize (in vitro) the types of trophoblasts. Embryo is prepared under stereo microscope and EPC, rich in trophoblasts, is excised with fine needles. Several EPCs are collected in a centrifuge tube containing HBSS (without Ca^{++} and Mg^{++}) and Trypsin/EDTA mixture (1:250:0.05/0.02% w/v) in equal amount. Hyaluronidaze (1.0 mg/ml), collagenase (0.5 mg/ml), DNase (0.1 mg/ml) and BSA (5% mg/ml) are added into it. The tube is gently rotated in hand (30 min.) and centrifuged (50xg for 1 min.). Cell suspension is washed with complete medium (BME + 10% FCS + BSA) thrice. The dispersed cells are then collected with micropipette and seeded on to the membrane filter. Viability is checked before seeding. Cells are cultured for 24 hr. The membrane filter is treated with ethanol, stained in haematoxylene and examined under the microscope attached with stage micrometer. Three cell types of trophoblasts with different sizes (8.0 to 18.0 µm) are noticed and a correlation has been established with in situ study on mouse EPC.

TI 3 Investigations of estradiol-induced changes in proteins of the mouse uterine epithelial surface.
John E. Morris, Sandra W. Potter, Georgeen Gaza-Bulseco, and Lin Chai. Oregon State University, Corvallis, Oregon 97331, USA

There is a dramatic reduction in exposed negatively charged sugars on the uterine epithelial surface at the time of blastocyst attachment in the mouse. Reports of specific protein induction by steroids in the whole uterus have prompted us to examine relative changes in the glycoproteins and their synthesis at the uterine epithelial surface under the influence of estradiol. Using mild trypsinization conditions, which cleave exposed glycopeptides and proteoglycans without lysing cells, we prepared digests of the uterine epithelial surface of ovariectomized mice with or without priming with estradiol for two days. Uteri were cut into strips and cultured for a 30-min equilibration period and then for 1 hr with [S-35]methionine with or without the hormone continuously present. Trypsinization was carried out under conditions that limited the enzyme to the epithelial surface. Fractions prepared by SDS-polyacrylamide gel electrophoresis were analyzed by silver staining and autoradiography. In some cases, ion exchange or concanavalin A affinity chromatography were used prior to electrophoresis to enrich for surface glycopeptides. Although we have identified several surface glycopeptides by these methods and by monoclonal antibodies against specific oligosaccharides (courtesy of Bruce Fenderson) on transblots, our data do not support the conclusion that estradiol exerts a specific influence on their synthesis or accumulation.

TI 21 Stage-dependent changes in lectin binding patterns in rabbit uterus and blastocyst during the preimplantation period and implantation
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During the preimplantation phase, the rabbit endometrium shows an impressive tissue remodeling and considerable changes in secretory activity. Subsequent implantation of the embryo starts with attachment of the trophoblast to the (otherwise non-adhesive) apical end of the uterine epithelium (1). Lectin probes were used in order to monitor changes in the composition of cell surface coat carbohydrates as related to cell attachment, and changes in intracellular distribution as related to secretory processes. Cryostat sections from rabbit uteri (and embryos where applicable) were taken at the non-pregnant stage and at 3, 5, 7 and 8 days post coitum (d p.c.) and incubated with FITC-conjugated lectins (RCA, PNA, SBA, DBA, WGA, Succinyl-Con A, UEA). Except for UEA, all used lectins showed strong binding to at least some tissues in our material. Binding was generally maximal on blastocyst coverings (except DBA), intermediate in some parts of endometrium (see below), and minimal on the trophoblast. Patterns in the uterine epithelium showed impressive differences with various lectins and in different stages of pregnancy. Considerable contrast in lectin binding site density between deep (SBA) vs. upper parts (RCA, WGA) of uterine crypts and luminal epithelium seems to be correlated with different functional states which are morphologically not very apparent. With PNA, this differential reactivity was found to change to the reverse between 3 and 5 d p.c. At 8 d p.c., a marked loss of WGA, SBA and RCA binding was seen at the uterine epithelium of placental folds. With S-Con A, maximal binding was in the subepithelial endometrial stroma, decreasing at 8 d p.c. Knowledge of these patterns will be used to design experiments on the potential role of the identified carbohydrate groups in embryo implantation. (Supported by Deutsche Forschungsgemeinschaft grant De 181/9-5)
References: (1) Denker, H.-W.: Adv.Anat.Embryol.Cell Biol. 53 Part 5 (1977)

IM 10 COMPARISON OF ADHERENT AND NON-ADHERENT VARIANTS OF HEPATOMA CELLS
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Cell-substratum adhesion is a complex, multistep process, in which several membrane components of different functions are involved. For characterization of those adhesion involved molecules a cell system was established which allowed the determination of molecular alterations associated with the transition of an adherent to a non-adherent phenotype. By propagation of cells isolated directly from the solid Morris hepatoma 7777 an adherent and a non-adherent cell variant have been obtained. To provide a conclusive relation between a molecular alteration and adhesiveness additionally spontaneous revertants were selected from the two directly isolated cell lines. Thus, two adherent and two non-adherent cell lines of the same cellular origin were compared.
Quantitative and qualitative differences in the plasma membrane composition of these cell lines were detected by lectin-affinity chromatography, by immunoprecipitation with antisera raised against whole plasma membranes of liver and hepatoma, and by means of two-dimensional gel electrophoresis. However, it could be assumed that only the expression of proteins in both adherent variants and their loss in both non-adherent cell lines would suggest their involvement in adhesion. Consequently, the altered expression of two membrane glycoproteins both of M_r = 110 k could be correlated with the loss of adhesiveness. One of these glycoproteins was identified as the ectoenzyme dipeptidyl peptidase IV.

TI 7 Raising of antibodies against attaching mouse blastocysts by intrasplenic immunization.

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Implantation involves attachment of the blastocyst onto the uterine surface and a subsequent invasion by the trophoblast into the endometrium. In a normal implantation it is difficult to recover attaching blastocysts, since the various blastocysts in a uterine horn differ with regard to their developmental stages. In an experimentally delayed implantation, however, the blastocysts are developmentally coordinated. This offers a possibility to raise antibodies against trophoblast surface components for studying their characteristics provided that the immunization procedure is sensitive enough to respond to a restricted number of blastocysts. Attempting to produce antibodies against surface components of blastocysts we have chosen to work with an intrasplenic route of administering the antigen (1,2).

The immunization with attaching blastocyst was performed either with irradiated living blastocysts or with blastocysts absorbed on pieces of NC paper. We have obtained both IgM and IgG antibodies and are now checking their reactivity. Also a few rabbits have been immunized by NMRI blastocysts absorbed on NC paper and these polyclonal rabbit antisera are under characterization.

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FC 13 Interactions between adenocarcinoma cells (MCF7) and a laminin rich matrix (Matrigel).

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Neoplastic cells come into contact with basement membrane (BM) and interstitial stroma at several stages during invasion and metastasis. We studied the effect of various matrices on adhesion, spreading and proliferation of MCF7 cells (human invasive breast adenocarcinoma cell line).

When plated on plastic or on a polymeric type I collagen gel used as a model of interstitial matrix, MCF7 cells rapidly attached, spread and grew as monolayer. A model of BM was obtained by heating at 37°C the urea extracted proteins from BM rich Engelbreth-Holm-Swarm tumor which formed a solid gel termed matrigel [laminin (Lam) 85%, entactin 10%, type IV collagen 4%, proteoglycans 1%]. When seeded on matrigel, MCF7 cells attached and formed small clusters. They poorly spread and remained rounded. ^{3}H-thymidine incorporation in the aggregates was about half that in monolayer cultures on plastic or collagen. This pattern was independent of the presence of serum or exogenous fibronectin (Fn) in the culture medium. Similar results were obtained when MCF7 cells were plated on purified Lam coated on the culture dish. However, in such conditions, addition of serum or Fn partly restored the spreading of the cells. MCF7 cells did not adhere to a gel of soft agar and floated as clusters in the medium. By adding minimal concentrations of Lam (0.08%) or matrigel (0.1%) to the agar, the MCF7 cells were allowed to adhere.

Our results indicate that in the presence of type I collagen or plastic, cell-matrix interactions predominate over cell-cell interactions resulting in a monolayer aspect of the culture. In contrast, in the presence of Lam or matrigel, cell-cell interactions predominate over cell-matrix interactions resulting in the formation of aggregates. Such an effect of extracellular matrix protein upon cell-matrix and cell-cell interactions demonstrated here could play significant roles during tumor progression and metastasis.

FC 7 Establishment, some characteristics and differentiation of a human melanoma cell line, BHM-97.

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A new human melanoma cell line, BHM-97, has been established in culture. The tumor tissue was taken from a female patient, 36 yr, dg: melanoma malignum, cutaneous metastasis. Melanoma cells started to grow out of the tumor fragments after 5 months following explantation in a mixture of media RPMI 1640 and Ham F-12 plus 20 per cent newborn calf serum. Cells were growing in monolayer form. Morphology and size of the cells are heterogeneous: the majority of the cells is fibroblast-like, but many transient forms, triangular and epithelial-like, are also present. Numerous binuclear and polynuclear giant cells can be found. Doubling time of the population is about 4 days. The cells contain melanosomes, annulate lamellae and, rarely, multiple rough endoplasmic reticulum cisternae. The cell surface is covered by microvilli. The cell line is hypotetraploid, the modal chromosome number is 80-81. A lot of various markers as well as non-random aberrations were also found. Cells are not capable of growing in soft agar. Induction of xenografts by injecting cells into athymic mice was unsuccessful so far. Cells are very sensitive to the tumor promoter phorbol myristate acetate: 10 ng/ml dose induced intensive dendrit formation, while alpha-difluoromethylornithine and retinoic acid are weak inducers of dendrit formation. Further characterization of this new cell line is in progress. It seems to be suitable for searching and testing new inducers of cell differentiation.

IM 16 Production of angiogenetic factors by tumour cells growing in spheroid or monolayer cultures.
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The question of this investigation was, whether tumour cells cultivated as spheroids release more angiogenetic factors into the medium than growing in monolayer cultures. The stimulation of cell proliferation was measured by ^{3}H-thymidine incorporation into an endothelial cell line derived from rat brain microvessels. The cloned cells show the typical growth characteristics described for capillary endothelial cells (1,2) and produce factor VIII antigen.
Cell proliferation was stimulated by all conditioned media tested compared to unconditioned medium. Media conditioned by spheroid cultures of neuroblastoma and human glioma cells caused a stronger stimulation (about 300% of control) than media conditioned on monolayer cultures with a similar cell number of the same cell lines (150% and 190% of control). With rat glial cells we observed the opposite effect: monolayer medium stimulates cell growth slightly more than spheroid medium.
Concentration of the neuroblastoma medium in an ultrafiltration cell indicated that the molecular weight of the factors lies below 30.000.
Preliminary results show that the factors of monolayer and spheroid medium bind to heparin sepharose (3).
Both the range of the molecular weight and the affinity to heparin suggest that the angiogenetic factor produced by the tumour cells bears some similarity to fibroblast growth factor(s) (4). The production of these factors seems to be increased in spheroid cultures of two of the investigated cell lines.
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TI 1 Interstitial trophoblast invasion of decidua and myometrium in early human pregnancy.
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During the establishment of human placentation cytotrophoblast, proliferating initially from the cytotrophoblast shell and later from the tips of the anchoring villi, invade the uterine tissues. Apart from endovascular trophoblast migration, there is also a marked invasion of the decidual stroma, mainly in the direct environment of the spiral arteries. From 8 weeks on this interstitial cytotrophoblast starts to invade the inner myometrium and seems to fan out during this process. Later, i.e. from 10 weeks myometrial cytotrophoblast starts to fuse to form the characteristic multinuclar trophoblastic giant cells. A correlation was found between the presence of myometrial trophoblast and the appearance of early changes in the myometrial segments of the spiral arteries that precede a second wave of endovascular trophoblast invasion. Therefore a priming role of interstitial trophoblast on spiral arteries is suggested.

IM 9 In vitro migration and malignant phenotype of rat liver epithelial cells.
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The in vitro migration and the malignancy characteristics of non-tumorigenic rat liver epithelial cells (BRL) and a series of tumorigenic derivatives was studied. BRL cells can be induced to migrate by epidermal growth factor (EGF) and the migratory reaction can be modulated (inhibited) by fibronectin [in Vitro Cell.Devel.Biol.21, 245-248 (1985)]. It was therefore of interest to determine if these control mechanisms are modified by neoplastic transformation. From a tumor derived from spontaneously transformed BRL cells, a metastasizing derivative was obtained. From one clone (D5), two sublines (LM1 and IVM/2), were obtained by repeated selection for their ability to form pulmonary metastases after intravenous injection in nude mice. All cells grow in serum-free, hormone-supplemented medium at comparable rates, synthesize fibronectin and laminin and organize the glycoproteins into extracellular matrix structures. All cells are induced to migrate by EGF, but the spontaneous rate of migration of the malignant cells was higher than that of the non-transformed progenitor cells. Fibronectin inhibits migration to a variable degree. After i.v. injection, D5 cells produce few lung metastases after a long latency, whereas all animals treated with LM1 cells die within 3-4 weeks from lung metastases. After s.c. injection all tumor cells behave similarly: the tumors do not produce macroscopically visible metastases in the 3-4 week period. The results indicate that neoplastic transformation does not result in a loss of the mechanisms that control the migration of the non transformed cells. Therefore, the in vitro migration characteristics of the transformed cell lines do not allow a firm conclusion about their malignant phenotype. These conclusions are in agreement with the notion that progression towards a fully malignant phenotype can occur independently for each characteristic. Additional studies, e.g. in vitro invasion, might allow a better prediction on the in vivo malignancy of these cells.

IM 8 Different expression of a 180 protein on murine variants endowed with different metastatic potential

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The expression of a 180 protein present on the cell surface of several murine lung carcinomas and recognized by MoAb 135-13C (1) has been studied on murine variants endowed with different metastatic potential. Recently, it has been reported that MoAb 135-13C modulate the metastatic spreading of the 3LL tumor cells (2) and that 180 protein is highly expressed on highly malignant line 1 carcinoma (1) . Lung carcinoma (3LL) and melanoma (B16) variants and sarcoma (S-180,M-5) tumors maintained in vitro and in vivo have been used. The MoAb 135-13C was used for binding experiments and for immunoprecipitation analysis. Results obtained demonstrate that the MoAb 135-13C binds with higher ability tumor cells endowed with higher capacity to metastasize to the lung and precipitates from the cell lysates of high metastatic variants high amount of 180 protein. Moreover,SDS-PAGE immunoprecipitates from the same cells in presence of an anti-P-tyr antiserum show the same band at 180 kdaltons. Experiments on in vitro lines indicate a possible role of the 180 protein as a receptor for an unknown growth factor. Results are discussed in view of the possible role of this protein in the metastatic process.

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TI 22 **Cytogenetic and immunobiologic characteristics of trophoblastic disease.**

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Early detection of trophoblastic disease has been done by ultrasound or post-molare by beta-clone hCG test.Morphogenetic stages were classified using the conventional techniques into:Hydrovum,Mola hydatidosa partialis and totalis,Mola invasiva and choriocarcinoma.Subclassification was carried out by cytogenetic high resolution banding techniques and by determination of the HLA antigens using modified microlymphocytotoxicity test.The origin of the trophoblastic genome has been identified by chromosomal markers and HLA antigens in trophoblastic and parental tissues. Y chromosome(H-Y antigen) acts as an oncogene.Bipaternal genome,inactive maternal genome and compatibility of partners in HLA Systematy were most often found(1,2,3, 4,5).Prognosis of malignant progression and choice of therapy significantly depend on these parameters.

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FC 8 Analysis of radiosensitizing effect of specific cell cycling blocking agents on M04 cells.

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Recovery from growth inhibition (RGI) in spheroids of malignant mouse embryo cells (M04) after ionizing radiation (IR) was better when they were explanted on glass than when they were kept in suspension culture. We used this explanted culture assay to test the radiosensitizing effect of Vinblastine (VLB) and observed that RGI was significantly inhibited when VLB was added to the culture medium for at least 16 hours before IR (15Gy). Tubolozole-C (1.0 µg/ml), a new synthetic microtubular inhibitor (MTI), showed a similar effect whereas its enantiomer tubulozole-T lacking MTI activity did not affect RGI. Short pretreatments (30 minutes) with MTI leading to complete disassembly of cytoplasmic microtubules (CMTs) and of spindle microtubules (SMTs) did not influence RGI after IR. Since in the latter experiment the majority of cells was in interphase at the time of IR, we hypothesized that the radiosensitizing effect of MTI was due to mitotic block rather than to disappearance of CMTs. Counting of the proportion of mitotic figures and micronucleated cells in the spheroids or in cell cultured on glass 24h after addition of MTI showed that accumulation of cells in M-phase (85% and 79% respectively) correlated with a low RGI (0% to 20%). Treatment of cells with 5-fluoro-uracil (5-FU) (0.5 g/ml) for 24h prior to addition of MTI (and 5-FU) abolished the radiosensitizing effect of MTI. Since 5-FU prevents entry into M-phase this observation confirmed that the radiosensitizing of MTI was due to mitotic block. Since G2-phase of the cell cyclus seems as sensible as M-phase as was shown by different authors, we have tested the epipodophyllotoxines VP16 and VP26. Both have at cytostatic doses, the same RGI as VLB in combination with IR ($p<0.003$) when treated 24h before IR. In conclusion : MTI have an additive effect to IR when added at least 16h prior to IR and G2 phase of cell cyclus seems as sensitive as M-phase when we use G2 blockers such as VP16 and VP26.

IM 11

Effects of retinoic acid on fibrinolytic activity in different cell systems.

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Retinoids have been shown to antagonize several effects of tumor promoters but some similarities have been noted also between the properties reported for tumor promoters and those of retinoids. One of the properties in common between tumor promoters and retinoids (Wilson and Reich, Plasminogen activator in chick fibroblasts: induction of synthesis by retinoic acid; synergism with viral transformation and phorbol ester, Cell, 15, 385, 1978) is to induce plasminogen activator (PA) activity in several cell systems. This work was designed to evaluate the effects of retinoic acid (RA) on the expression of PA and their relation to the changes in growth pattern and virus production. Low concentrations (10^{-6} and 10^{-7}M) of RA inhibited PA synthesis in epithelioid RK13 and IAR 6-7 cells and stimulated PA activity in human embryo fibroblast cells. The inhibition of PA activity in both kinds of epithelioid cells was dependent on the continuous presence of the vitamin in the medium and was accompanied by cell flattening and changes in the growth pattern, including the growth rate and saturation density. Induction of PA activity in human embryo fibroblast cells was not followed by any observable changes in gross morphology or growth rate. Treatment of human embryo fibroblast cells infected with human cytomegalovirus (HCMV) with retinoic acid did result both in the enhancement of virus production and PA activity depression.

IM 17

A new monolayer invasion assay (MIA) to discriminate and isolate metastatic lymphoma cells.

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We have recently developed a monolayer invasion assay (MIA), using murine fibroblastic 10T1/2 cells and interference reflection microscopy. T-cell lymphosarcoma cells of the tumorigenic BW 5147 line, which do not form experimental metastases, did not invade the monolayer, whereas cells of the metastatic derivative BW-O-Li line readily penetrated through the monolayer. A quantitative invasion index could be defined in this model system, based on counting of the underlying cells as seen in phase contrast microscopy.
Due to its simplicity and speed, the MIA should be valuable for the comparison of the invasive capacity of lymphoid cells, and for the quantitative assessment of the effects on invasion of extrinsic factors e.g. drugs and antibodies.
Using this MIA we have studied the invasiveness of twenty BW-related cell lines, prepared by in vivo or in vitro cell fusion with T-cells, by in vivo induction and selection, or by in vitro 5-aza-cytidine treatment. The in vitro invasiveness was qualitatively correlated with the in vivo metastatic potential. The MIA also discriminated between normal non-activated and antigen-activated T-lymphocytes : control spleen T-cells were scored as non-invasive, whereas cells from mixed lymphocyte cultures had a high invasion index.
In addition, using this invasion model system, we were able to isolate a minor subpopulation of invasive cells from a thousandfold excess of non-invasive cells.

FC 9

An in vitro method for rat visceral yolk sac differentiation

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The proliferation and differentiation potentiality of the rat visceral yolk sac is investigated in organ culture. Rat visceral yolk sacs are dissected free from Reichert membrane, amnion and placenta and incubated for 48 h. in a roller bottle system. Subsequently these yolk sacs are put in organ culture for 28 days. Like for the in vivo deplaced visceral yolk sac (1), the proliferation of endoderm and mesoderm and the appearance of poorly differentiated cells as well as trophoblast giant cells are observed. In some cases haemopoiesis and the formation of squamous epithelium is observed (2). Using tritiated thymidine as label, several of these differentiated cells contained the radiolabel, indicating that they derive from the initial proliferating endo- and/or mesodermal cells (3).

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TI 9 Induction of cell coupling via gap junctions in the uterine epithelium of the rabbit prior to trophoblast invasion

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During the preimplantation phase of pregnancy, the endometrium is hormonally conditioned to allow attachment and invasion of the trophoblast. Interestingly, there is also evidence for responses of the uterine epithelium to the presence of the implanting blastocysts. One of these responses is the induction of cell coupling via gap junctions between the uterine epithelial cells.
In the nonpregnant state and in pseudopregnancy (no blastocyst present) the uterine epithelium shows only few gap junctions and a very low degree of coupling as evidenced by freeze-fracture, immunocytochemistry of the gap junctions subunit (26k-protein) and dye-spreading. On contrast, in the implantation chamber an extremly large number of gap junctions and extensive cell coupling is observed adjacent to the blastocyst. The role of the blastocyst in inducing gap junction formation is also shown in unilateral pregnancy produced by tubal ligation.
It will be discussed whether this induction of cell coupling is a precondition for or a defence against trophoblast invasion.
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LATE ABSTRACTS

TI 15 Remodelling of the endometrial vascular bed during trophoblast invasion in the rabbit

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The formation of maternal blood lacunae in the rabbit chorio-allantoic placenta begins on about day 9 post coitum on the mesometrial endometrium and diversifies into three distinct zones until day 10 p.c.: In the zone 1, the large arterial lacunal network originates from the former arterial limbs of subepithelial capillaries and is located at the base of the chorionic sprouts. This network becomes shaped by vessel dilatation and fusion which is an effect of the trophoblast-related degeneration and lysis of the endothelium. The large volume of these lacunae and the syncytial trophoblast ridges oriented perpendicular to the vessel axis may damp the arterial pulse pressure. The large lacunae also favor diffusible materno-fetal substance transfer either by the adjacent thin interhemal membrane as well as by the extended inner lacunal surface being rich of microvilli.
In the zone 2, the small lacunae originate from the former venous limbs of subepithelial capillaries and are located laterally to the chorionic sprouts. The syncytial trophoblast shapes the small lacunae to a real labyrinth by projections and bridges connecting each other to a meshwork. By this, blood flow is slowed down and the lacunal surface is increased so that the system of small lacunae can be expected to be most important for materno-fetal substance exchange.
In the zone 3, venous lacunae, located on the top of chorionic sprouts, progressively develop from venules with an intact endothelium and dilated prelacunae with degenerating endothelium. The endothelium seems to maintain integrity for a long time in spite of the immediate vicinity of trophoblast, but does degenerate when it is undercut and isolated from its substrate by the trophoblast. Although not showing lytic activities at a distance, the advancing trophoblast is clearly phagocytic and takes up debris.

TI 10 Role of laminin and other matrix proteins in trophoblast attachment and in experimental tumor invasion.

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Human cytotrophoblast cells from the implanted blastocyst invade the maternal endometrium and myometrium during the first two trimesters of pregnancy. Interstitial trophoblast infiltrates the maternal decidua while the endovascular trophoblast replaces arterial endothelial cells and smooth muscle cells of arterial media during invasion of the spiral arteries. This results in a dramatic increase in blood supply to the placenta. Such a limited invasion of maternal tissues by trophoblast closely resembles local infiltration by neoplastic cells of host tissues. Trophoblast and malignant cells come indeed into contact with basement membrane (BM) and interstitial stroma at several stages during invasion. Interactions between these cells with their support condition their activities. As demonstrated by immunofluorescence, fibronectin displays an ubiquitous distribution in interstitium and vascular areas. Laminin and type IV collagen are demonstrated in vascular and epithelial glandular BM. Laminin also appears as an intense pericellular halo around decidual cells. These extracellular matrix (ECM) proteins are synthesized by the human fertilized egg from the 4 cells stage. They promote, in vitro, adhesion of trophoblast cells, respectively to type I collagen or type IV collagen. Implantation requires a considerable degradation of the endometrial ECM. A model of BM was obtained by heating at 37°C the urea extracted proteins from BM rich Engelbreth Holm Swarm (EHS) tumor which formed a solid gel termed matrigel [laminin 85%, entactin 10%, collagen IV 4%, proteoglycans 1%]. When seeded on matrigel, malignant trophoblast cells (Bewo) readily attached and formed clusters. These cells synthesized laminin, fibronectin and type IV collagen. They degraded the matrigel within a few days. Type IV collagenase activity was detected in the supernatant medium but not in cultures on plastic or polymeric type I collagen. This enzyme degrades BM collagen and its activity is proportional to the invasive potential of malignant cells. Induction of type IV (but not type I) collagenase activity could be achieved by addition of laminin to Bewo cells cultured on plastic. These studies therefore indicate that invasive trophoblast cells adhere to the ECM using fibronectin or laminin. This latter protein also induces type IV collagenase activity. Cells could thus penetrate the vascular and epithelial BM of maternal endometrium and infiltrate the interstitium. Such an effect of extracellular matrix protein upon cell-matrix interaction could play significant roles during trophoblast progression and embryo implantation.

TI 20 Scanning electron microscopic study of serotonin-induced disruption of implantation in the rat.

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Serotonin (5-hydroxytryptamine: 5-HT), administered on the day after initiation of blastocyst implantation, promptly terminates pregnancy (1). The disruption of nidation is associated with protracted concomitant reductions in blood flow to implantation sites and in availability of oxygen within the uterine lumen (2). In order to determine the time-course and character of the morphological changes associated from 5-HT induced disruption of blastocyst attachment, implantation sites were examined by scanning electron microscopy in control vs. 5-HT treated rats. Pregnant rats (day 1 = sperm) received an sc injection of saline or 5-hydroxytryptamine creatinine sulfate: 20 mg/kg at noon, day 6 post coitum. At selected times thereafter, Evan's Blue dye was injected iv to render implantation sites visible. At 15 minutes post dye injection, uteri were removed and immersed in Karnovsky's fixative. Implantation chambers were exposed by bisecting uterine segments along the mesometrial border. Serotonin effects on implantation site morphology were apparent within 1 hour of treatment and resulted in total blastocyst resorption within 2 h. Effects were apparent in the epithelium in contact with the blastocyst, progressed rapidly throughout the implantation chamber and ultimately spread to the immediately adjacent epithelium of the uterine lumen. The zone of degeneration was clearly delimited and restricted to the region of implantation. Epithelium between implantation sites showed no sign of alteration. Restriction of necrosis to the nidatory epithelium is consistent with data suggesting that 5-HT acts on the vasculature of the implantation chamber.

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